

Contemporary Endocrinology
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Endocrinology of Physical Activity and Sport

Second Edition

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Endocrinology of Physical Activity and Sport

Second Edition

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PREFACE

The preface to a book should give an account of its genesis. The first edition of the present publication, *Sports Endocrinology* (Michelle P. Warren and Naama Constantini, editors, 2000), offered one of the first incursions into a novel topic. It answered a recognized need and was well received by the scientific community. Since then 12 years have elapsed and certain changes (title of the book, editorship, authorship of new chapters) have imposed themselves. The focus of the work however has remained the same: an insightful discussion of the key elements of endocrinology as they relate to physical activity, exercise, and sport. Including essential topics such as endocrine assessment methodology, the study of endocrine systems that relate to exercise performance, growth, development, and health is among the issues explored—not least aspects of the practice of doping which continues to be an affront to all athletes who compete honestly.

The editors are profoundly grateful to the contributors that have made this volume what it is. Their scholarship, scientific devotion, and professionalism not only reflect the present state of knowledge but will undoubtedly serve as a stimulus for further advances in a constantly challenging subject.

Jerusalem, Israel
Chapel Hill, NC, USA

Naama Constantini
Anthony C. Hackney

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Methodological Considerations in Exercise Endocrinology

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INTRODUCTION
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INTRODUCTION

Over the last several decades an increasing number of exercise science investigations have begun to incorporate measurements of endocrine function (e.g., hormones, cytokines) into their research designs and protocols (1, 2). This approach has allowed for a heightened level of investigation into research examination of the physiological mechanisms associated with clinical and performance-related conditions found in individuals involved in exercise training.

Some exercise science investigations, however, have not always controlled certain critical factors (e.g., time of day for blood sampling) that can influence many of the hormones associated with the human endocrine system. This lack of investigative control has often resulted in the resulting research findings to be inconsistent, contradictory, and sometimes extremely difficult to interpret. This insufficient control of biological experimental factors appears to be due in part to limited knowledge by exercise science researchers in the area of clinical endocrine methodology and techniques.

Experts suggest that the factors that influence hormonal measurements, and contribute to variance in experimental outcomes, can be categorized from two potential sources: factors affecting physiological variation (i.e., affiliated with the physiological function status of the subject) and factors affecting procedural-analytical variation

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Table 1
The following are abbreviations commonly used for various hormones seen in exercise science and sport medicine endocrinological research (see reference (4))

<i>Name</i>	<i>Abbreviations</i>
Adrenocorticotrophic hormone	ACTH
Aldosterone	ALD
Antidiuretic hormone	ADH
Atrial natriuretic peptide	ANP
Arginine vasopressin	AVP
β -Endorphin	β -END
Catecholamines	Cats
Corticotropin-releasing hormone	CRH
Cortisol	CORT
Epinephrine	EPI
Estradiol- β -17	E ₂
Follicle-stimulating hormone	FSH
Glucagon	GLU
Gonadotropin-releasing hormone	GnRH
Growth hormone	GH
Growth hormone-releasing hormone	GHRH
Insulin	IN
Insulin-like growth factor (1)	IGF ₁
Leptin	LP
Luteinizing hormone	LH
Norepinephrine	NOR
Progesterone	P
Prolactin	PRL
Reverse triiodothyronine	rT ₃
Testosterone	TEST
Thyrotropin-releasing hormone	TRH
Thyroid-stimulating hormone	TSH
Thyroxine	T ₄
Triiodothyronine	T ₃

(i.e., determined by the investigators conducting research) (1, 3). Regardless of the source of variance, subject or investigator derived, if it is not controlled or accounted for appropriately, the resulting hormonal measurements obtained can be compromised and thus call into question the scientific validity of a research study.

The intent of this chapter is to provide background information for exercise science researchers on those physiological-procedural-analytical factors that can potentially affect endocrine measurements. The intent is for this material to serve as an introductory “basic coverage” on this topic in hopes of improving the quality of research in exercise endocrinology.

The field of endocrinology uses numerous abbreviations for the many of the hormones that exist. To aid those researchers unfamiliar with this lexicon, Table 1 lists those abbreviations for the most common hormones associated with the area of exercise science (4).

PHYSIOLOGICAL FACTORS

As mentioned, factors that can influence hormonal measurements can be categorized into two broad areas: “physiological” and “procedural-analytical.” The physiological factors are those that are determined to be connected in some way to a biological function or status of the subject at the time of the collection of the specimen (e.g., blood) to be analyzed. These are factors that can be viewed as pertaining to endogenous aspects.

Sex

It appears that until the onset of puberty there is little difference between males and females in their resting hormonal profile. Once puberty is reached though, there is increased androgenic steroid hormone production in the male, and the female starts the characteristic menstrual cycle pulsatile release of gonadotropin and sex steroid hormones (5–7). Additionally, at puberty, resting leptin (an adipocyte cytokine; a low molecular weight protein that has endocrine-like actions on select physiological process such as the immune system (8)) levels tend to become increased in females, as compared to those in males (9). In adulthood, the differences that begin to manifest at puberty tend to remain until females reach the postmenopausal period and males reach andropause (8, 9).

There are some sex-specific differences in the hormonal responses to exercise in males and females. These include an earlier and greater rise in testosterone in males during exercise as compared to females, and a greater pre-exercise growth hormone response in females. Furthermore, the magnitude of the sex steroid hormonal response to exercise in females is influenced by the status and phase of their menstrual cycle (10, 11). Interestingly, the menstrual cycle hormones can influence other hormones and their response to exercise (e.g., increased estradiol- β -17 \rightarrow increases growth hormone levels) (10–12). (See later discussion concerning the menstrual cycle in this chapter.) On the other hand, some hormones show little or no differences in response to exercise between the sex (e.g., water balance hormones such as aldosterone and arginine vasopressin) (5, 10, 11).

Due to these potential differences in outcomes due to sex, the researcher should be cautious when using subject populations involving a mixture of males and females in their studies. To avoid confounding results, researchers need to be certain that the hormonal outcomes they are measuring are not influenced by sex.

Age

If subjects are not matched for age and maturity level, whenever possible, variance in the outcomes can be potentially increased. For example, a prepubertal and postpubertal child (of the same gender) will not typically display the exact same hormonal exercise responses or relationships (13, 14). This is illustrated by the well-documented increase in insulin resistance which is observed as an adolescent goes through puberty (15).

This concern should also be extended to the other end of the age spectrum. That is, a postmenopausal female or andropausal male could have drastically different hormonal responses when compared to a relative prepausal individual. For example, growth hormone and testosterone typically decrease with age while cortisol and insulin resistance increase (16–18).

These types of age-related differences can exist at rest, in response to exercise, and even after completing an exercise training program. For this reason, it is important to match subjects in research studies by chronological age and/or maturation level in order to increase the homogeneity of the responses and decrease interindividual variability, obviously, that is, unless the researcher is trying to study age-related changes among groups of individuals. (3).

Ethnicity and Race

A variety of different humoral constituents are known to vary between people of different races and ethnic groups (1, 3). However, only a few hormonal differences have been identified. For example, resting parathyroid hormone levels tend to be higher in Black compared to Caucasian individuals (19). Caucasian females tend to have higher levels of estrogens than Asian females (1, 20). Evidence also suggests that reproductive hormone levels during gestational periods may vary greatly across several races and ethnic groups (Caucasians, Blacks, Latinos, Asians, and Indians) (20–23). Findings of greater resting insulin and degree of insulin resistance in certain Native American tribes (e.g., Pima Indians) have also been reported; however these differences may in fact be more related to obesity issues in these individuals (24).

Hormonal responses to exercise and exercise training related to race and ethnicity have not been well studied, and the limited available findings do not suggest drastically different response outcomes. Further research is necessary and warranted in this area (1, 23, 24).

Body Composition

The level of adiposity of the body can greatly influence the release of certain cytokines by adipose tissue (3, 8, 9). These cytokines in turn can have autocrine-, paracrine-, and endocrine-like actions and influence aspects of metabolism, reproductive, and inflammatory function (2, 3, 8, 9). Additionally, several of these cytokines have been directly linked to the promotion of increased hormonal levels (e.g., increased interleukin-6 → increased cortisol) (8). This situation becomes compounded as adiposity reaches the level of obesity and subsequently affects many hormones to a far greater degree. For example, insulin and leptin levels tend to be appreciably elevated at rest in many obese persons (25–29).

As levels of adiposity increase (from normal levels moving towards becoming obese), the hormonal response to exercise and exercise training can change considerably from that of a normal-weight person. As an illustration, in obese persons, catecholamine and growth hormone response to exercise becomes blunted (29). Cortisol responses to exercise seem to become elevated in some overweight-obese individuals; in contrast, some cortisol responses have been shown to be blunted and reduced (28, 29). Exercise training often allows a loss of body mass, in particular fat mass, which helps to normalize these hormones with levels observed in normal-weight people (29–33).

To insure that varying levels of body composition of subjects will not confound hormonal outcomes, investigators need to match their subjects for adiposity as closely as possible and not just use body weight matching as criterion. Exactly how close of a match is

needed is not known, but grouping normal-weight, overweight (body mass index (BMI) ≥ 25.0 – <30.0 kg/m²), and obese (BMI ≥ 30.0 kg/m²) individuals into the same subject group can most certainly complicate, and add variance to some hormonal outcomes (1, 29).

Mental Health

Select mental health conditions and states are associated with high levels of anxiety and apprehension (e.g., posttraumatic stress disorder), which can lead to enhanced activity of the sympathetic nervous system and hypothalamic-pituitary-adrenal axis (34–36). Subsequently, resting levels of circulating catecholamines, adrenocorticotropic hormone, β -endorphin, and cortisol can be elevated. In contrast, persons who are experiencing depression can have low arousal levels, and the above-mentioned hormones could be suppressed. Moreover, depression is sometimes accompanied by low activity levels in the hypothalamic-pituitary-thyroid axis (i.e., low thyrotropin-releasing hormone, thyroid-stimulating hormone, thyroxine, and triiodothyronine) creating a euthyroid sick syndrome response (34–36). These alterations in resting hormonal levels can in turn result in altered hormonal responses to exercise and exercise training in individuals who have high levels of anxiety (37–39). In some cases this can result in heightened responses (excessive) or diminished responses. (37–39) Evaluating the mental health status, via the completion of a screening questionnaire by a subject, can serve as an excellent tool to determine if a potential emotional or psychological problem exists which could confound hormonal measurements. A variety of such screening tools are available, and the reader is directed to several excellent references for overviews of this topic (40, 41). Importantly, it is highly advisable that any such determination be performed by a trained, qualified individual.

Menstrual Cycle

Menstrual status (eumenorrheic vs. oligomenorrheic vs. amenorrheic) and cycle phase (follicular, ovulation, luteal) in females can produce basal changes in key reproductive hormones such as estradiol- β -17, progesterone, luteinizing hormone, and follicle-stimulating hormone. These changes can be large and dramatic within select individuals. For example, the ovulatory and luteal phases result in increases in all of the aforementioned hormones above what is seen in the follicular phase (e.g., 2–10-fold greater in eumenorrheic female) (42). These typical changes are depicted in Fig. 1. As noted earlier, select reproductive hormones (sex steroids) at rest can influence certain other nonreproductive hormones and nonreproductive physiological function such as estradiol- β -17 enhancing growth hormone release (11, 43, 44).

The menstrual status and cycle phase hormonal influences can carry over to have an impact on exercise and exercise training responses, too. Consequently, researchers may need to conduct exercise testing with females of similar menstrual status and/or in similar phases of their cycle. This precaution is also applicable to females who are using oral contraceptives, which can mimic some hormonal fluctuations similar to cycle phase changes (44, 45). The precise impact of oral contraceptive (OC) depends exactly upon the type of OC used (mono-, bi-, or triphasic) and the dosage of the active estrogen and progestin agents in the pharmaceuticals.

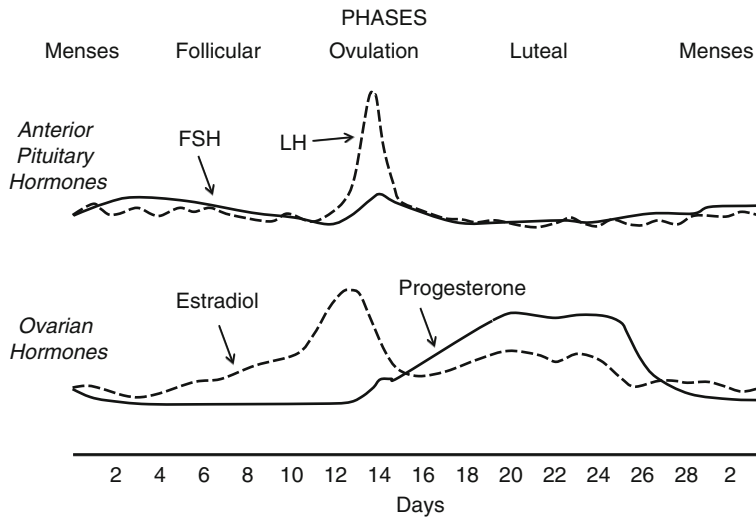


Fig. 1. Typical hormone changes (arbitrary scaling for concentration changes) in hormones associated with the menstrual cycle in amenorrheic women.

Circadian Rhythms

Over the course of a 24-h period many hormonal levels will fluctuate and display circadian variations. In some cases these variances are due to pulse generator aspects, which is the spontaneous release of select hypothalamic hormonal releasing factors/hormones (46) within the endocrine regulatory axis. In other cases, variances are related to humoral stimuli, changes brought on by subject behavior or environmental factors, and these humoral stimuli influence hormonal release (47, 48). Circadian hormones can display dramatic changes in levels due to their rhythm patterns, cortisol being a prime example. Morning cortisol levels are typically twice that of those found later in the day (49–51). Table 2 provides some reference on the circadian pattern seen in some key hormones.

These fluctuations and circadian variations need to be addressed when conducting exercise research. Studies demonstrate that the magnitude of exercise responses may not be similar at different times of the day, even if the exercise intensity and duration are held constant (1, 49). Investigators should plan accordingly so as to more carefully control and replicate the time of day in which research evaluations are conducted and hormonal specimen collected (52, 53).

PROCEDURAL-ANALYTICAL FACTORS

The second category of factors influencing hormonal measurements is made up of those factors that have procedural or analytical aspects to them. These factors are determined, selected, or in some way controlled for (i.e., potentially) by the investigators conducting, or the subject involved with the research (1). These factors can be viewed as exogenous relative to their influence.

Table 2
Hormones that display discernable circadian patterns. The arrows indicate a relative direction for changes in concentration levels

<i>Hormone</i>	<i>AM concentration</i>	<i>PM concentration</i>	<i>Remarks</i>
ACTH	↑ Early AM	↓	Highest levels may be during sleep
Aldosterone	↓	↑	Highest levels may be during sleep
Cortisol	↑↑	↓↓	Influenced by food intake; highest levels may be during sleep
Growth hormone	↑ Early AM	↓	Only slight differences; highest levels may be during sleep
LH-FSH	↓↑	↓↑	Pulsatility or release and menstrual cycle phase override circadian pattern
Melatonin	↑ Early AM	↓	Highest levels may be during sleep
Parathyroid hormone	↓	↑	Highest levels may be during sleep
Prolactin	↑ Early AM ↓ Late AM	↑	Highest levels may be during sleep
Testosterone	↑	↓	Lessens with age

Arrows indicate an increase (↑) or decrease (↓) in hormone concentration

Ambient Environment

When conducting research investigations it is important to remember that excessive exposure to hot or cold ambient temperatures can stimulate the release of various hormones, e.g., those involved in water balance (aldosterone) or energy substrate mobilization (cortisol) (37, 54, 55). Even elevated ambient relative humidity (water vapor) can induce this effect, primarily due to a compromised heat dissipation through reduced evaporative efficiency adding to the body core temperature (55). These effects can be further augmented if hypoxemia is induced along with temperature extremes, as can occur when moving to higher elevations and being exposed to high altitudes (56–58).

Many of the exercise and exercise training hormonal responses are tremendously impacted by environmental factors. In particular, catecholamines, growth hormone, aldosterone, arginine vasopressin, adrenocorticotrophic hormone, and cortisol are all susceptible to changes in environmental conditions and show highly exacerbated responses in varying conditions (1, 37, 54, 55).

To minimize these influences it is critical to conduct exercise testing in controlled, standardized conditions such as in a laboratory. On the other hand, if conducting field research (where environmental standardization can be impossible), then it is important to measure/record environmental factors and convey them in any subsequent reporting of the data in the literature.

Nutrition

The nutritional status and practices of a research subject, including composition of diet, caloric intake, and timing of meals, can greatly impact the hormones associated with energy substrate mobilization and utilization (e.g., insulin, glucagon, epinephrine, growth hormone, insulin-like growth factor, cortisol) (1, 59, 60). The exact nature of the effect (augmented or attenuation) depends on the interaction of the nutritional factors just mentioned and how severely the alterations are from the normal nutritional regimes of the subject (1, 29, 34).

The hormones noted above are critical during exercise to insure that energy metabolism meets the demands of exercise. Thus, altered dietary practices and nutrition status of a subject can change energy substrate (glycogen) storage and availability (60–62). This in turn can cause the hormonal response to exercise to vary to some degree. For example, Galbo and associates demonstrated that the glucagon, epinephrine, growth hormone, and cortisol response to exercise were greater when a low-carbohydrate, high-fat diet is consumed (i.e., 4 days of consumption) compared to a normal mixed diet (59).

Normally in clinical settings it is recommended that subjects be fasted prior to blood hormonal evaluations (e.g., 8 h). It is not always practical, however, for athletes to comply with such request due to their high demand for adequate caloric intake to maintain energy balance, anabolism, and muscle glycogen reserves. Therefore, a modified fasted approach may be necessary for this special population such as only a 4–6-h fast. Even with the constraints of working around an athlete's special needs, it is still advisable that exercise investigators try to control and standardize the dietary practices of their subjects as much as possible to mitigate the effects of differing diet between subjects, and within an individual subject's diet, if a repeated measures research design is being used (34, 59).

NUTRIENT TIMING

The concept of nutrient timing is a relatively new component to the field of exercise science and is arguably one of the most important aspects to account for when designing a study and evaluating results (63). The evaluation of timing food consumption has been shown to influence muscle morphology outcomes directly and indirectly by stimulating hormone secretion (64). As coined by Dr. John Ivy, the nutrient timing system accounts for three phases: the energy phase, anabolic phase, and growth phase (63). Additional consideration should be given to the pre-exercise phase, which can largely influence the endocrine response during and after exercise. Although most research protocols hold diet constant, considerations for what the subject consumes before and after, ad libitum, may have substantial influences on acute and chronic adaptations, in part due to the stimulation of hormones.

Pre-exercise: Cortisol levels, which help to maintain the integrity of the immune system, are strongly influenced by glucose availability (65–67). Additionally, acute carbohydrate intake can stimulate an increase in insulin and glucose levels, sparing muscle glycogen as well as reducing cortisol levels. Acute consumption of a glucose-electrolyte solution (GES) prior to exercise has been shown to significantly reduce cortisol levels, when compared to water. Allowing a subject to consume a carbohydrate drink before testing, independent of amount (i.e., 25 g vs. 200 g), may significantly maintain glucose

and cortisol levels post-exercise, as well as stabilizing the neutrophil to lymphocyte ratio (67). Pre-exercise vitamin consumption, or an antioxidant enhanced beverage, will protect against acute tissue damage augmenting exercise adaptations, and results demonstrate a chronic effect on maintaining immune system markers (68).

Anabolic Phase (During): Carbohydrate supplementation during exercise has also been associated to a blunted cortisol, growth hormone, and cytokine response, while also maintaining glucose levels and insulin stability (69, 70). There is additional evidence demonstrating reduced T cell and NK cell levels with carbohydrate feeding during exercise (69). Acute, uncontrolled feedings should be accounted for when establishing a study design as well as potential confounders when interpreting immune function results. Protein consumption during exercise blunts protein degradation and has a sparing effect on muscle glycogen (64).

Post-exercise (Growth Phase): Immediate post-workout fuel consumption has the potential to highly influence muscle machinery by utilizing the anabolic characteristics of insulin. Additionally, an increase in insulin post-exercise can enhance muscle glycogen resynthesis, and is enhanced with a protein/carbohydrate combination (64, 71). A carbohydrate-amino acid supplement influenced testosterone and cortisol levels 120 min after intake and exercise (72). However, longer duration post-exercise consumption (i.e., 8–9 h) has demonstrated no hormonal influence (73). Intake of carbohydrates + protein + vitamins post-exercise has been shown to reduce free radicals and maintain immune function. This may be a consideration for researchers evaluating exercise and immunology characteristics, as well as various aspect of overtraining.

MEAL FREQUENCY AND PATTERNING

Meal frequency and overall caloric consumption may also influence markers, such as C-reactive protein, fasting plasma glucose, insulin, as well as total cholesterol (74, 75). In as much, investigators may consider questioning participants about food consumption patterns or utilize a food frequency questionnaire.

DISORDERED EATING

The eating disorder “anorexia nervosa” is a special concern relative to nutrition status due to its profound effect on the endocrine system (1, 45, 76). Anorexics tend to have lower resting luteinizing hormone, follicle-stimulating hormone, and estradiol- β -17 levels (76). Anorexia also affects the pituitary-thyroid-glandular axis. Specifically, the condition is associated with suppression of triiodothyronine, somewhat decreased thyroxine, elevation of reverse triiodothyronine, and, occasionally, decreases in thyroid-stimulating hormone (76). Such a thyroidal state is referred to as the “euthyroid sick syndrome” and can accompany severe body weight loss (3, 45, 76). There is also an effect on the adrenocortical axis, with higher levels of cortisol due to an increased liberation of corticotropin-releasing hormone (76). Growth hormone is also increased, although insulin-like growth factor-1 levels (which facilitate the physiological actions of growth hormone) are suppressed in the anorexia condition (76). Due to the psychological aspects of the anorexia nervosa (see references (77, 78)), this condition could, in the context of the organization of this chapter, be further discussed with mental health issues. Thus this factor could also be considered of a biological nature, and consequently has powerful effects on a multitude of endocrine measurements.

Stress-Sleep

Emotional stress and/or sleep deprivation are each known to affect certain hormones within the endocrine system. For example, emotionally distraught individuals will typically have elevated basal catecholamine, growth hormone, cortisol, and prolactin levels (1, 79–81). Those hormones with circadian patterns (see Circadian Rhythm section; e.g., luteinizing hormone, follicle-stimulating hormone, adrenocorticotropic hormone, cortisol) can be shifted in their characteristic pattern-rhythm by disruption of sleep cycles (36, 39, 79–83).

These types of factors (i.e., stress, sleep deprivation) can also influence the hormonal response to exercise and exercise training. Investigators must attempt to control these factors whenever possible. In fact, it is advisable to have a pre-exercise questionnaire completed by a subject to monitor and evaluate the level of these factors, and if a pre-determined status is not obtained, then hormonal measures and exercise testing should be rescheduled.

As a footnote to this issue, many investigations in the exercise area use college students as research subjects. Such students can have high levels of emotional stress due to their education demands (e.g., examination periods, projects being due, oral reports). Care should be taken to not utilize student subjects when there are in high emotion stress periods as a multitude of hormones can potentially have very atypical values and responses (36).

Physical Activity

The proximity in time between exercise sessions can affect the hormonal profiles of individuals (84, 85). If inadequate amounts of time have elapsed (lack of recovery), some hormonal responses at rest, or in the subsequent exercise testing, can be attenuated and others augmented. Furthermore, the magnitude of this effect can be influenced by the exertion required of the prior exercise (e.g., high-intensity intervals require longer recovery).

If possible, researchers may require a 24-h recovery prior to a subject reporting to the laboratory for testing. However, subjects who are athletes may find it difficult to reduce their training or miss a workout session for experimental purposes in research studies. A modified approach may be necessary, such as only a 12- or 8-h recovery period because this could somewhat prevent stress and anxiety (which as noted can affect the endocrine system) in the athlete since they would be missing less training time (1, 85–87).

A powerful influence on resting and exercise hormonal response of a subject is the exercise training status—that is, trained vs. sedentary. The more “trained” a subject is, typically the greater the effect on the neuroendocrine system. Many hormones show attenuated resting and submaximal exercise responses in trained individuals, although some can actually be augmented (e.g., testosterone in resistance-trained individuals) in response to submaximal and maximal exercise (2, 88–92). An extensive dialogue on the influence of exercise training on hormonal profiles at rest and in response to exercise is beyond the scope of this chapter, but the reader is directed to references (2, 3) for more in-depth discussions.

Subject Posture-Position

There are changes in the plasma volume component of the blood as a subject changes position. Standing upright results in a reduction of plasma volume compared to a recumbent position (93). These shifts in the plasma fluid are in response to gravitation effects as well as alterations in capillary filtration and osmotic pressures (93). Large molecular size hormones, or ones bound to large weight carrier proteins, could be trapped in the vascular spaces; this means that a loss of plasma fluid would increase the concentration of these hormones (hemoconcentration). Conversely, a gain of plasma fluid would decrease the concentration of these hormones (hemodilution) (37, 94). These adjustments in fluid volume to move in or out of the vascular space due to posture shifts typically require approximately 10–30 min (93, 94).

In exercise research situations where blood is drawn to assess hormones, it is recommended that the condition of specimen collection related to the subject's position be controlled and reported in publications. This type of information is most certainly necessary if a postural change is occurring for a 10-min or greater duration (51, 94).

Specimen Collection

Suitable precautions must be taken in the collection and storage of blood specimens to insure they are viable for later hormonal analysis. In clinical and exercise-related blood work, venous blood is the specimen usually utilized. If the blood specimen is being obtained by venipuncture, it is important to not have the tourniquet on the subject's arm too long (~1min or longer). Greater lengths of time can result in fluid movement from the vascular bed due to increased hydrostatic pressures (94). Once collected, the blood sample should be centrifuged at ~ 4°C in order to separate the plasma (collection tube contains anticoagulant) or allowed to clot (collection tube is sterile) then centrifuged for serum. If centrifugation cannot be done immediately, then the blood sample should be placed on ice, but it is more prudent to centrifuge without delay. Once separated, the plasma/serum should be aliquoted and stored at a temperature of –20 to –80°C until later analysis. Care should be given to ensure certain plasma/serum is stored in airtight cryofreeze tubes (screw-cap type is recommended), which allow for a longer storage period. It is also advisable to split up specimens into several aliquots if multiple hormonal analyses are going to be conducted. Once a sample is thawed, it has a relatively short “shelf life” in a refrigerator, and repeated unthawing and refreezing cycles can degrade certain hormonal constituents and compromise the validity of the analysis (95–97). Care should be taken to insure that the assay procedures employed are specific for plasma or serum, as in some cases these cannot be used interchangeably in the assay (e.g., adrenocorticotrophic hormone is measured in plasma). Furthermore, an examination of the research literature may be necessary to determine if one form of blood component is more popular or prevalently used in research.

In blood specimens, either plasma or serum is utilized for biochemical analysis, but some hormonal measures can also be made in urine and salivary samples. In general plasma and serum give very similar values for hormonal analytes, and seldom is one considered better than the other in blood analysis (96). Be aware however, specific assay

procedures do, in some situations, have a preferred blood fluid for analysis. Thus it is critical for the researcher to know what each hormonal assay requires as the analyte and then plan accordingly. This type of information is provided by the manufacturer of the analytical supplies-components used in the assay procedures.

With respect to urine and saliva, they are attractive as specimens to collect because of their noninvasive nature. They do, however, have certain drawbacks. Urine analysis tends to be limited primarily to steroid-based hormones, and there is usually a need to collect 24-h urine specimen. The collection of 24-h urine specimens can be a tedious and demanding process for the subject. Also, urine measurements may not always be reflective of “real-time” hormonal status either, as urine can sit in the bladder for hours before being voided. Saliva allows for easier sampling procedure and can reflect hormonal status in a more real-time fashion. However, saliva also primarily only allows for steroid hormonal assessments (i.e., constituents that can cross from the blood into the salivary gland) (98). Furthermore, saliva is limited to free hormonal concentrations as the protein-bound constituents typically cannot pass through the salivary gland due to their large molecular size. Research does suggest that the blood and saliva levels of hormones can mirror each other in their relative changes, but not perfectly, so correlation coefficient of only 0.7–0.8 is typically found (1, 95, 98). Researchers must determine if these limitations preclude the use of these biological fluids in their studies (95, 98, 99).

Analytical Assays

A variety of biochemical analytical methods (i.e., “assays”) exist for measuring hormones in biological specimens. Chromatographic, receptor, and immunological assays are all available. Perhaps the most prevalent contemporary technique in use is immunological assays, which have variations such as chemiluminescence immunoassay (CLIA), radioimmunoassays (RIA), enzyme immunoassays (EIA), enzyme-linked immunoassays (ELISA), and electrochemiluminescence immunoassays (ECLIA) (100–102). Each of these techniques has its strengths and weaknesses, and the discussion of each is beyond the scope of this chapter, but the reader is directed to references (103–105) for more background and explanation about this subject.

Researchers should always know the particular aspects of the hormonal assay techniques they plan to use in their studies. Specifically, it is important they be aware of the precision of the assay (“how accurate is it?”), sensitivity of the assay (“how small of a change can it detect?”), and the specificity of the assay (“how much cross-reactivity is there with similar looking chemical structures in the specimen?”). Ideally the researcher wants the most precise, highly sensitive, and specific assay they can obtain, but cost considerations can impact decision-making in these matters. It is advisable for the researcher to report precision, sensitivity, and cross-reactivity values in publications to allow readers to determine the quality of the analytical techniques and procedures of the assays that were used. Additionally, it is desirable to report in subsequent publications the coefficient of variation (CV) “within” an assay and “between” an assay for each respective hormone measured. This will allow the reader to determine how well the analytical technical procedures were carried out (105, 106). One step to mitigate the potential between-assay CV is to collect and analyze your biological samples in batches of specimens and not as isolated specimens on a day-by-day basis. However, caution is

necessary here as batches that are too large can influence your outcome by creating “end of run effects” within the assay. That is, running such a large number of samples in a single batch that the precision of the technician performing the assay may be compromised (i.e., procedural fatigue), or the kinetics of the specific assay may be influenced by the length of time it takes to pipette the various components in assay (i.e., in adding the chemical reagents to the first sample tubes vs. the last tubes; too much time has transpired, resulting in different lengths of time for chemical reactions to take place within the specimen tubes) (105, 106).

Data Transformations

Before conducting statistical analysis on hormonal data measured within the assays, it may be necessary to transform the data. Two of the most common endocrine transformations usually seen in literature are (1) expressing the data as a percent change from some precondition (i.e., before exercise), basal value; and (2) conducting a logarithmic conversion of the data. The first is typically done to account for relative changes in hormonal concentrations when absolute magnitude of change may be misleading. For example, a cortisol change from 276 to 331 nmol/L is highly different from a 55–110 nmol/L (20% vs. 200%) even though the absolute magnitude is identical. A 200% increase in the hormonal concentration may have many more profound physiological effects than the smaller percentage. In the second form of transformation, logarithmic transformation is normally performed due to a large degree of variance in the subject data resulting in a nonnormal distribution. This can be due to sample size issues, variance with the analytical technique, or the physiological nature of the hormone being studied. Despite of the transformation used, it is vital that the researcher report to the reader in the publication if and how the data were manipulated prior to conducting the statistical analysis (and what was the rationale for performing the transformation) (100, 107).

A third data transformation that is less frequently used is the area under-the-curve (AUC) procedure. This is carried out when there are serial specimen samples (repeated measures design) from a subject. These serial values are plotted, and then an integration of the area under the plotted responses curve is determined, thus collapsing numerous data values into one response and potentially eliminating some of the variability associated with having many hormonal measurements (108). This approach is favored by some researchers; their rationale is the overall response of the hormone, and gland in question can be better quantified. Nonetheless, the procedure can be influenced by the number of serial samples collected to determine the response curve as well as the circadian rhythm of the hormonal release. The latter point results in the need for highly variable hormones (pulsatile) to be assessed using more frequent specimen sampling because misleading results can occur if the sampling is too infrequent (109).

Statistical Analysis

The statistical analytical procedures applied to any research study data are dictated by the design of that study. Most research in the exercise area tends to employ parametric analysis (e.g., *t*-test, one-way ANOVA, Pearson correlation). These analytical

procedures work well with endocrine data, provided that the underlying assumptions for their use are not violated (see reference (110) for details). Furthermore, many North American journals prefer this form of analysis due to the robust nature of the techniques and the reduced likelihood of making a type I error (indicating findings are significant when they are in fact, not). Nevertheless, nonparametric analysis (e.g., Wilcoxon signed-rank test, Mann–Whitney U test, Friedman test) can be equally applicable for endocrine use when study designs are not excessively complex and sample sizes are relatively small (110). It is important, however, to recognize the likelihood of increasing the occurrence of a type I error with small sample sizes. Regardless of whether parametric or nonparametric analyses are used, it is vital that the researcher report in a publication of their work what the specific statistical analysis being used is and what the rationale was for their usage (109–112).

Once assays are performed and statistical results are obtained the researcher needs to try and understand their data in order to interpret the magnitude of treatment outcomes and physiological effects. In this interpretative process many researchers focus intently only upon obtaining statistical significance, usually a probability level less than 0.05 ($p < 0.05$). Obtaining such significance is important, however, a key question that has to be addressed in the data is the issue of “statistical significance” vs. “practical (clinical) significance” for the hormonal findings. To address that question, the researcher must think about and take into account the smallest clinically important positive and negative response value levels of the effect being researched, that is, the smallest change value levels that matter. Studies can be statistically significant yet largely insignificant clinically. It is important to note that large sample sizes can produce a statistically significant result even though there might be limited or no practical importance associated with the findings (113).

To this end, effect sizes (ES) are becoming an increasingly important index used to quantify the degree of practical significance of study results (see reference (114) for explanation to calculating the ES statistic). Once computed, the ES statistic can be a useful indicator of the practical-clinical importance of research results because it can be operationally defined; that is, it is possible to give the observed ES ratings such as “negligible-trivial,” “moderate,” or “important-very large.” (115) Such ratings allow the researcher to discern the form and quantity of significance they have obtained in their study findings. In addition, the ES statistic has two advantages over traditional statistical significance testing: (a) it is independent of the size of the sample, and (b) it is a scale-free index. Thus, ES can be uniformly interpreted in different studies regardless of the sample size and the original scales of the variables being examined (114).

SUMMARY AND CONCLUSION

To conclude, over the last several decades, exercise researchers have steadily increased the number of studies conducted which have examined hormones and the endocrine system. Unfortunately, not all investigators working in this area of research are entirely aware of the factors that must be accounted for, and controlled, in order to insure that valid and accurate data are obtained. This chapter reviewed some of the key physiological and procedural-analytical factors that can confound endocrine data and add variance to hormonal findings, and those steps to be taken to reduce the confounding

factors. Implementation of these steps can greatly aid the researcher in the interpretation and understanding of their endocrine data and in turn make their research more scientifically sound.

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2

Exercise and Endogenous Opiates

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INTRODUCTION (ENDOGENOUS OPIATES)

Endogenous opiate-like substances were first discovered in the mid-1970s, when opioid receptors were identified and located within the brain and hypothalamus (80). This led to the discovery that endogenous opioid-like molecules, enkephalins (41) and endorphins (6, 62), were produced within the CNS. Subsequently another class of opiate-like molecules known as dynorphins was identified as being synthesized within the body (33). Endogenous opiates therefore fall into three major classes of substances: *endorphins*, a peptide of 31 amino acids long; *enkephalins*, smaller peptide molecules of five amino acids in length (denoted either as leu- or met-, based on the terminal carboxyl amino acid of the peptide); and *dynorphins*, located in the posterior lobe of the pituitary gland (54) and gastrointestinal tract (35) with a 13 amino acid length.

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Enkephalins were first noted in areas of the brain and parts of the endocrine system. The original studies noted that both endorphins and enkephalins were important regulators of pain (3, 62). However, more recent studies have identified that enkephalins play an important role not only with pain regulation but with certain behaviors, cardiac function, cellular growth, immunity, and ischemic tolerance. Various tissues (heart, smooth and skeletal muscle, kidney, and intestines) in animals and humans have recently been shown to have proenkephalin expression (14). Recently, inflammatory cells were shown to produce and release these opiates, and endorphins seem to be involved not only in immune function (51, 52, 72), pain modulation (93), and the exercise pressor response (43, 73, 97) but also in metabolic control (43, 50, 66, 101). Therefore, there are numerous challenges to be clarified concerning the role of these endogenous opiates, on these processes as they relate to exercise. This is especially true dealing with the control of cellular functions not only under normal conditions but when acute and chronic exercise stress is imposed.

Beta-endorphins (β E) were first identified within specific regions of the brain and the hypothalamus. The release of β E into the peripheral circulation was first ascribed to the release from the anterior pituitary gland after being activated by several factors within the hypothalamus. These factors activate the anterior pituitary gland to synthesize the parent molecule pro-opiomelanocortin (POMC) which can be cleaved into various active components, one of them being β E. β E is therefore an important neurotransmitter within the brain and a neurohormone outside the central nervous system when released from the anterior pituitary gland into the circulation, to act on receptors on numerous target tissues throughout the body.

The molecule POMC, the precursor polypeptide for several factors that arise from the hypothalamus and the paraventricular nucleus (PVN) in the brain, can be stimulated to shorter active peptides. POMC has a section towards the C terminus known as β -lipotropin (1–89 amino acids) that ultimately is cleaved to β -lipotropin (1–56) and β E (59–89). Both β E and β -lipotropin molecules help to mobilize lipid molecules from adipose tissue. Originally the assays that were developed to measure these molecules did not effectively differentiate between β -lipotropin and β E, which were thus denoted as having both β -lipotropin/ β E activities. β E is also known to act as a neurotransmitter within the brain.

There is limited information related to exercise and brain β E modulation (39, 83, 90). β E immunoactivity in cerebrospinal fluid (CSF) of spontaneously hypertensive rats was shown to be significantly higher (about twofold) in runners (5–6 weeks) than in controls (39). This latter study also reported that CSF β E was elevated up to 48 h after cessation of voluntary wheel running. It was suggested that this β E effect may be at least partially responsible for the beneficial effect of exercise on controlling blood pressure (39). β E immunoactivity taken from CSF in dogs was shown to increase with low-intensity exercise but not with high-intensity exercise (83). In contrast, circulating β E immunoactivity increased in these dogs at both intensities of exercise (83). This indicates that the β E level within the brain is not reflected by the amount of β E within the circulation. Rat brain receptor binding of [3 H]diprenorphine, a β E analog, was not significantly elevated 1 h following a swim but was shown to increase in several regions (5 of 6 rat brain 1 areas) 2 h after the exercise (90). It is unclear if this was related to changes in β E concentration or a change in the availability of the receptor. When naloxone (a receptor antagonist for β E)

was injected into the brain ventricles after 5 weeks of exercise training, the increase in the pain threshold that occurred with the exercise was abolished (94). This suggests that the opioids were involved in elevating pain threshold in response to exercise training in these rats. Clearly more work is needed in this area. The specific areas of the brain that might be involved with β E and pain regulation in response to different types of exercise still needs further investigation.

β E within the circulation has been implicated in a number of processes including immune function, pain modulation, and assisting in glucose and lipid homeostasis. The major function of these endogenous opiate-like molecules was first identified as modulators of pain and euphoria based on the receptors they activated. As a result of this, the phenomena known as “runner’s high,” “second wind,” and “exercise dependency” were postulated to be related to this endogenous activity.

This chapter will summarize what is currently known about the stimulation of these endogenous opiates in response to exercise or physical activity. The influence of an acute bout of exercise on the β E response will be presented first as these studies were the impetus of the original research. The influence of training on β E will then be presented. The influence of an acute bout of exercise on enkephalins will then be presented. This will be followed by the influence of training on enkephalins. The physiological mechanisms responsible for the activation and the secretion of these substances will be discussed when known and related to functional outcomes when possible.

INFLUENCE OF ACUTE EXERCISE ON β E LEVELS

The initial studies that were conducted to examine the impact of exercise on endogenous β E levels utilized various modes of exercise. The original articles examined various activities such as running at various distances to determine if blood β E level was elevated (10, 12, 13, 100). These studies noted elevated β E after the activities. This led to more controlled studies utilizing incremental graded exercise tests within laboratories to ascertain the β E response (31, 36, 37, 69, 75, 84). These studies suggest that blood β E can increase from 1.5 to 7-fold following these graded exercise tests. The large variation in the β E response was in part attributed to procedural methods for the exercise tests as well as methods to determine β E.

AEROBIC EXERCISE AT VARIOUS WORK INTENSITIES RELATED TO % VO_2 MAX (AEROBIC CAPACITY)

Several studies determined if there was an intensity of exercise effect on the blood β E level. McMurray et al. (68) was one of the first studies to examine the β E response to a specific exercise intensity. Donovan and Andrew (16) noted that β E did not increase after 8 min of cycling at 25% and 50% VO_2 max but increased after 75% VO_2 max after similar duration. They also noted a greater increase in β E at 95% VO_2 max. Goldfarb et al. in that same year examined several intensities of exercise (60, 70, and 80% VO_2 max cycling exercise) to determine if there was a critical intensity needed to induce circulating β E increases (29). β E concentration increased in the two higher exercise intensities but not at 60% VO_2 max. The time course of β E changes at these exercise intensities up to 30 min of exercise was examined with β E increases occurring

sooner with the highest exercise intensity (by 5 min). Research comparing 60% VO_2 max and 80% VO_2 max as well as self-paced running for 30 min noted only an increase after the 60% run (20); however they utilized βE /B-lipotrophin immunoreactivity. A run at 60% VO_2 max for 60 min noted no change in βE (61). Exercise at 80% VO_2 max for 30 min with or without naloxone increased βE with a greater increase with naloxone (1). These studies taken together suggest that circulating βE can increase with an appropriate minimal intensity of exercise (>60% VO_2 max) but this is not always the case. Later it was reported that gender did not influence the βE response to either 60 or 80% VO_2 max (32, 36, 85).

It was noted that menstrual cycle had minimal effects on the exercise βE response in women (28, 32). The time course information also suggested that higher intensities of exercise would result in βE increases more rapidly (20, 22, 31, 36). However, other factors might have differed which could have contributed to the discrepancy in the literature such as nutritional status of the individuals, time of day, immune function, and training status. Farrell et al. utilized well-trained endurance athletes and noted that βE +/ β -lipotropin levels only increased at 92% VO_2 max whereas lower intensities did not elicit significant increases (20).

Instead of a critical intensity related to one's aerobic capacity, other studies related the increase in circulating βE to the lactate threshold (89). They plotted the change in lactate with increased work intensity and compared the βE response. Incremental increases in exercise intensity elevated circulating βE levels and showed a similar pattern of change as blood lactate. However, it should be noted that these similar changes are only for short-duration incremental exercise. For activities with longer duration, the βE increase does not always coincide with lactate changes (29). In addition, other factors such as diet, training status, and immune function can influence the βE response.

HIGH-INTENSITY BOUTS WITH AN ANAEROBIC COMPONENT

Short bouts of highly intensive exercise (anaerobic exercise), consisting of various types of exercise from a few seconds up to several minutes duration, can induce an increase of βE . A few studies have reported that βE concentration in the circulation can increase about 2–4-fold above resting with these high-intensity anaerobic exercise bouts (22, 69, 84, 89). It was also noted that there was a positive relationship between lactate and the βE response to the exercise (89). These authors also noted a significant increase in blood catecholamines with these exercises that correlated with the maximal lactate concentrations.

Resistance exercise as a stimulus to augment circulating βE concentration in humans has only a limited number of published studies. Conflicting results have been reported and this may be related to differences in subjects, type of exercise intensity, workload volume, and time of measurement. Typically the resistance exercise was related to the person's 1-repetition maximum (1-RM), i.e., the maximum weight that can be lifted or pushed/pulled by a subject with maximal effort. Often the load is referenced as a percentage of this 1-RM. Circulating βE level increased in response to high total workloads (55). These authors noted that the total work, rest to work ratio, and total force needed most likely influenced the βE response. An increase in βE in 28 elite male weight lifters was also reported after a moderate- to high-intensity workload (57). An increase in βE

level was also reported after three sets of work at 85% 1-RM in females but only was significantly elevated (3.7-fold) when the women were in a negative energy balance (102). An increased $\beta\text{E}/\beta$ lipotropin level was reported in response to weight lifting in five males (18).

In contrast, low-volume resistance exercise did not result in any change in βE levels (55). Furthermore, blood βE level based on immunoreactivity decreased after exercise compared to at rest in ten male and ten female college-aged students who performed three sets of eight repetitions at 80% 1-RM on four exercises (81). This same group had reported earlier that resistance-trained subjects ($N=6$) showed no change in blood βE level compared to baseline after three sets of eight repetitions at 80% 1-RM (82). Both resistance exercise and treadmill exercise were reported to significantly increase circulating $\beta\text{E}/\text{B}$ -lipotropin immunoreactivity (18). Unfortunately the intensity and volume of exercise was not available. McGowan et al. however noted a decrease in βE concentration after exercise at 80% 1-RM in 20 college-aged subjects (both males and females) (67). It appears that resistance exercise of sufficient intensity and volume (workload) can result in a transient βE increase within the circulation in both men and women but this finding is sometimes equivocal.

INFLUENCE OF TRAINING ON BETA-ENDORPHIN LEVELS

The training status of the individual probably should influence the response to exercise for a number of reasons. One reason is related to the relative intensity of the exercise. Well-trained athletes can typically perform at a greater absolute workload and usually would exercise at a higher relative workload compared to an untrained individual. Therefore, when comparing the βE response one should compare the absolute workload and the relative intensity. In addition, other factors might influence the secretion of βE such as the diet or immune function which can be influenced by training. Typically one would expect a downregulation on the secretion of βE to a similar absolute workload. However, there could be an upregulation of the capacity of the hypothalamic–pituitary–adrenal (HPA) axis in trained individuals.

INFLUENCE OF ENDURANCE TRAINING

Resting levels of βE in endurance-trained individuals were reported to be lower (65) or unchanged (30, 36, 37, 40). The studies that reported no changes were mostly cross-sectional studies. In contrast, the studies that reported lower levels used an endurance training program and compared the βE level before and after the training program at rest. In contrast, Heitkamp reported that women who trained three times per week for 30 min per day at their individualized lactate threshold did not have changes to their resting βE (36). Harber and associates compared normal 10 eumenorrheic sedentary, 11 eumenorrheic-trained, and 11 amenorrheic-trained women and reported that βE varied considerably but there was no menstrual cycle effect at rest on βE (34). They also noted that resting βE levels were higher in the trained women compared to the sedentary women. Goldfarb et al. reported a trend for lower βE levels during the luteal phase of the menstrual cycle compared with the follicular phase, but this did not reach significance (32). They also noted no significant difference in resting levels of βE comparing men and women.

Therefore, there is currently no consensus in the literature as to the effect of endurance training on resting β E levels.

The findings for β E during exercise are in slightly better agreement. One early study reported a higher β E concentration after 4 months of aerobic training six times per week (10). They reported that the β E level was higher cycling at 85% max heart rate (HR) than before training. This occurred after 2 months of training with no further changes through the rest of the training. It should be noted however that to elicit a similar 85% max HR, the subjects worked at a greater absolute workload.

Most of the other studies have reported no detectable differences in trained and untrained state regardless if it was a cross-sectional design (30) or longitudinal design (7, 19, 36, 37, 40). Goldfarb et al. compared untrained ($N=6$) and trained ($N=6$) cyclists who cycled for 30 min at 60, 70, and 80% VO_2 max with subjects randomly assigned in a counterbalanced order (30). There was no difference in the β E concentration for the trained and untrained at similar relative workloads despite higher absolute workloads for the trained. Both untrained and trained groups responded with higher β E concentrations for the 70 and 80% workloads compared to rest and the 60% VO_2 max. Heitkamp et al. reported that after training the β E response was comparable but was obtained at higher absolute workloads for the trained subjects (36). They also reported that after training the recovery β E was lower suggesting faster removal of the β E. Howlett et al. also reported that there was no difference in β E concentration after endurance training at maximal workloads but met-enkephalin concentration was reduced after 4 months of training (40). Bullen et al. reported greater peak β E/ β -lipotrophin after exercise after 8 weeks of cycling training in seven women (7). Engfred noted there were similar β E increases after 5 weeks of cycling training at 70% VO_2 max to cycling to exhaustion (20). A 12% increase in VO_2 max occurred after 5 weeks of training so that the workload to elicit the cycling after training was at a higher absolute workload. In conclusion, it appears that that the blood β E concentration will be similar to before training if the workload is at the same relative intensity of aerobic capacity. This would require a higher absolute workload for the trained individual.

INFLUENCE OF RESISTANCE TRAINING ON CIRCULATING β E

Unfortunately there are few studies that have examined the influence of resistance training and circulating β E. There are no published studies found which indicate that β E concentration would change at rest or at any specific workload or a percentage (%) of one's maximal capacity with resistance training. Fry and coworkers reported similar β E concentration after both 4 and 9 weeks of resistance training to baseline levels (25). It is important to note that most of the resistance research typically utilized resistance trained subjects. As noted above, higher total work volume with resistance exercise resulted in greater increases in circulating β E (56).

β E AND IMMUNE SYSTEM

β E within the circulation has been implicated in a number of processes including modulation of immune function, pain modulation, blood pressure regulation, and assisting in glucose homeostasis. β E receptors have been identified in many locations within

the body including nerves, adipose tissue, pancreas, and skeletal muscle. However, the exact role(s) β E may have on these tissues is still being elucidated.

The influence of β E on immune function has been investigated *in vitro* but has not been adequately investigated *in vivo*. β E (both rat and human) was shown to stimulate T lymphocyte proliferation (38). The data suggests that β E mode of action was not through a mu opioid receptor. It was shown that synthetic β E could bind to non-opioid receptors on T lymphocytes and this binding was not blocked by naloxone or met-enkephalin (74).

β E was shown *in vitro* to stimulate rat spleen lymphocytes in a dose-dependent manner by enhancing the proliferative response to several mitogens (27). This binding was not blocked by naloxone. β E enhanced the proliferative response of splenocytes on T-cells from adult male F344 rats (98). In addition, naloxone was not effective in blocking the β E effect. β E stimulated the proliferative effect on human T lymphocytes using the mitogen concanavalin A (76). This β E-stimulated mitogen response demonstrated a bell-shaped curve indicating that too high a dose would actually inhibit the response. It was noted that this response may change with time, dose, or mitogen used (70). These authors also noted that the inhibition of the immune response to cortisol may be partially reversed by β E. Therefore, the activation of β E may inhibit suppression of the immune response by acting on cortisol actions *in vivo*.

β E was noted to enhance human natural killer cell function *in vitro* in a dose-dependent manner but was inhibited by naloxone (53). This suggests that the mode of action on natural killer cells appears to be different than the enhancement of T lymphocyte function. The β E levels effect on natural killer cell activity (NKCA) and amount was examined after exercise (26). Naltrexone treatment given 60 min before a run at 65% VO_2 max which elevated blood β E levels at 90 and 120 min did not alter the exercise response in NKCA or counts. These authors suggested that β E may work independent of the mu receptor action to assist NKCA (49). Chronic exercise (wheel running for 5 weeks) in spontaneously hypertensive rats enhanced NKCA. The β E levels in CSF increased after the running and enhanced lymphoma cell clearance from the lungs. The delta-receptor antagonist naltrindole significantly but not completely inhibited the enhanced NKCA after 5 weeks of exercise. Neither α nor β receptor antagonists influenced the NKCA. These authors suggested that the endurance training mediated central receptor-mediated adaptations. However, if β E levels in the periphery were given subcutaneously, this did not alter NKCA *in vivo* (49). In contrast, NKCA after central injection of a delta opioid receptor agonist was enhanced (2). In addition, a single injection of a mu agonist into the intracerebral ventricle reduced NKCA. Furthermore, a single morphine injection into the periaqueductal area suppressed NKCA (103). This suggests that central mediated β E levels may act to modulate NKCA via both delta and mu receptors. Clearly more research with human models is needed but this may be difficult as most of these actions appear to be centrally mediated.

Additional modes of action of β E on the immune response include mononuclear cell chemotaxis (78, 99), immunoglobulin migration (88, 99), and lymphokine production (99). Macrophages showed migration to β E levels injected into the cerebral ventricles in rats (99). Human neutrophils demonstrated enhanced migration to β receptors when β E was infused and this response was blocked by prior incubation with naloxone. Analogs of opioids appear to have different responses when injected into the cerebral ventricles (88). Some may stimulate macrophages and others may influence neutrophils.

The chemotaxis response appears to be dose dependent (78). High doses of β E levels (10^{-3} M) inhibited the chemotaxis response whereas low concentrations stimulated upregulation of neutrophils. Since physiological β E concentration is below the high-dose level utilized even when elevated by exercise or other stressors, it is likely that β E at these low levels provide a stimulatory effect on this aspect of the immune system.

It has been suggested that the opioid peptides such as β E and the enkephalins have a similar structural component of interleukin-2 (48). Interleukin-2 and other interleukins are involved in the inflammatory response and are targets of β E levels and cortisol. It is highly likely that both β E levels and cortisol influence the immune response by interacting with interleukins (104). The inhibitory response may act at a number of levels including the attenuation of the production of both interleukin-1 and interleukin-6 in a dose-dependent manner.

It appears that β E levels may act on a number of immune factors both centrally and in the periphery and may act through both opioid and non-opioid receptors. Additionally, β E action may work through direct inhibition of cortisol.

Both β E and cortisol influence immune function with β E generally enhancing immune function and cortisol acting as an immunosuppressant. The interplay of β E and cortisol in regulating immune function in response to both acute and chronic exercise requires more research to clarify their contributions. Adaptation effects to training also need further study. In addition, nutritional factors (i.e., carbohydrate level) have not been adequately examined in relation to both β E and cortisol influence on the immune response and with exercise. A recent study reported β E increased to a similar level after cycling to exhaustion at 90% VO_2 max after cycling for 60 min at 65% VO_2 max independent of a high or a low glycemic diet or placebo prior to the exercise (44).

ENDOGENOUS OPIOIDS AND PAIN PERCEPTION

There are numerous citations that have implicated endogenous opioids and pain perception. A good number of these have suggested that endogenous opioids are involved in the processes of myocardial ischemia and or angina (46, 95). It was reported that endorphins could modulate adenosine-provoked angina pectoris-like pain in a dose-dependent manner in seven healthy subjects (95). In contrast, met-enkephalin had no apparent effect on the pain. There may be a gender difference as angina pectoris pain induced by adenosine was attenuated by β E in males (both healthy and with coronary artery disease) but β E infusion did not modulate the pain nor did naloxone in females (87). Increased plasma concentrations of β E were shown to alter peripheral pain threshold but did not alter angina threshold in patients with stable angina pectoris (46). Therefore, peripheral pain may be influenced by β E, and the β E level may in part manifest some alteration in pain threshold. However, it is more likely that peripheral nerves which contain β E and/or immunocytes which release β E are involved with altering pain perception and reduction of damage (72).

Several studies have reported that exercise can modulate pain perception and this has been attributed to endogenous opioids. Both acute and chronic exercise were reported to significantly enhance mu opioid receptor (MOR) expression in the hippocampal formation (15). However, acute and chronic exercise had no significant effect on MOR expression in trained rats. Immunohistochemical techniques showed a higher number of

MOR-positive cells after acute exercise compared to a control group. These authors noted that both acute and chronic exercise modulate MOR expression in the hippocampus region of rats. Higher pain thresholds for pain were reported in individuals who exercised for both finger and dental pulp stimulations (17). Plasma β E levels increased after exercise to exhaustion as did cortisol and catecholamines but pain threshold level changes did not correlate with plasma β E. Furthermore, naloxone failed to affect pain thresholds, despite the fact that with naloxone and exercise, β E levels increased to a greater extent. These authors suggested that the pain-related changes with exercise were not directly related to plasma β E. Janal et al. reported that after a 6.3 mile run at 85% VO_2 max, hypoanalgesic effects to thermal, ischemic, and cold-pressor pain occurred, together with enhanced mood (45). In this study, naloxone infusion partially inhibited some of the pain and mood effects with the exercise. This suggests that that exercise can modulate pain and it appears it is related to β E but may not be directly related to the plasma β E concentration.

Perception of pain in trained ($N=17$) men after a run (12 min for maximal distance) with either placebo or with naloxone was examined (79). After the exercise β E levels increased in a similar manner for both trials, but pain level was greater with the naloxone treatment. These authors concluded that the perception of pain associated with exhaustive exercise may be related to endogenous opiates but this had no effect on performance. Low-intensity exercise was noted to reverse muscle pain in rats and this was blocked by naloxone (3). Microinjections of opiates into the periaqueductal gray matter in the brain of rats attenuated pain symptoms (91). It was noted that systemic and supraspinal opiates could suppress pain in rats (62). These studies suggest that pain can be altered by opiates and that exercise can modify pain; however the alteration in pain does not appear to be related to circulating β E.

Neuropathy-induced mechanical hypersensitivity occurred in wild-type mice subjected to a chronic constriction injury of the sciatic nerve (60). It was reported that T lymphocytes infiltrating the injury site (11% of total immune cells) released β E. Corticotropin-releasing factor (CRF) was applied at the injured nerve site and fully reversed the hypersensitivity. These authors noted that the T lymphocytes which contain β E are crucial for not only immune function but also altered pain with peripheral nerves.

It is now clear that β E are located in parts of the immune system, and can act both centrally and peripherally to help modulate pain. It is unclear how these different areas in the body respond to both acute and chronic exercise, but it appears that β E are involved. Part of the modulation of pain perception is clearly related to MOR within the brain, and more research is needed to understand the effects of both acute and chronic exercise on these receptors. In addition, circulating β E may increase, but this may not always be related to pain modification, and naloxone may not always block this effect. Therefore, the peripheral mediated β E effect on pain thresholds may not be related to the MOR in the periphery.

β E AND GLUCOREGULATION

The opioid system has been implicated in the control of blood glucose concentration during rest (23, 86) and exercise (24, 43, 44). β E and opiate receptors have been isolated from sites that are involved in glucoregulation (103). Additionally, it has been

reported that β E appears to play a role in metabolic regulation during exercise or muscle contraction (50). A bolus injection of β E followed by intravenous infusion of β E in rats raised β E levels 6–7-fold and resulted in higher plasma glucose levels at 60 and 90 min of exercise compared to saline infusion (24). Lower insulin and higher glucagon levels were evident compared to saline infused rats at these times. Additionally β E exerts an effect on insulin and glucagon at rest (23, 77) in humans and animals. β E infusion without a bolus infusion of β E compared to saline infusion enhanced glucose homeostasis and exacerbated the glucagon rise in rats that were exercised (43). This study reported that β E infusion independent of a β E bolus during exercise can attenuate the blood glucose decline and increase the glucagon response to exercise. Additionally, β E infusion alone did not alter insulin, catecholamines, corticosterone, or FFA's response during exercise. It appears that β E infusion alone at a level to increase β E at 2.5-fold greater than normal levels does not inhibit insulin whereas, if the β E level increased to greater than 2.5-fold (infusion and/or increase by exercise), inhibition of insulin occurs to help maintain blood glucose.

INFLUENCE OF ACUTE EXERCISE ON ENKEPHALINS

There is some evidence that exercise can increase enkephalin concentration and or opioid receptor numbers within the brain (11, 15). These alterations in the brain have been linked to changes in mood state (45), the exercise blood pressure control (4, 42, 47, 73), cardiac ischemia and angina (95), pain (94, 95), and immune function (8). However, it should be understood that some of the actions of these opioid molecules may manifest themselves in other compartments such as vascular control. Research is unfolding as to the actions of these enkephalins and enkephalin-like molecules. For example, proenkephalin peptide F which is primarily released from the adrenal gland and co-released with epinephrine has immune-modulating functions (8, 51, 96).

Met-enkephalin levels were reported to be unchanged after a Nordic ski race determined in both highly trained ($n=11$, active for 150 km/week with greater than 3 years' experience) or recreationally trained ($n=6$, active for 20 km/week with no competitive experience) skiers (71). The distance covered was 75.7 km and subjects were allowed to have water and food ad libitum. Met-enkephalin concentration in plasma was determined at rest prior to a graded treadmill exercise to exhaustion and after a run of 87.2 km in these same individuals (5 min post). The basal level of enkephalin was 171.7 ± 7.16 fmol/mL and increased after the treadmill exercise to 265.8 ± 9.88 fmol/mL and further increased after the run to 378.3 ± 15.16 fmol/mL. These authors suggested the increase in met-enkephalin in plasma may be related to intensity and duration of exercise (93). These same authors tested unfit ($n=24$) and fit ($n=23$) subjects utilizing a progressive intensity treadmill run to exhaustion with 4 min stages of at least five stages. Met-enkephalin concentration in plasma was lower for the unfit compared to the fit (126.3 ± 5.3 fmol/mL vs. 156.7 ± 6.9 fmol/mL). Both groups demonstrated increased plasma met-enkephalin after the treadmill exercise with the fit group showing a greater response (unfit = 180.4 ± 5.3 fmol/mL vs. fit = 278 ± 6.58 fmol/mL) (92). In contrast, Boone et al. reported that met-enkephalin was no different in trained and untrained subjects following 4 min of exercise at 70% VO_2 max and 2 min at 120%

VO₂ max (5). These authors noted that cryptic met-enkephalin (activated) was elevated similarly in both groups after the 70% VO₂ max and returned to baseline levels at the higher workload.

The response to exercise in met-enkephalin concentration in the plasma from trained and untrained subjects was reported to be similar (47). Subjects were rested for at least 15 min prior to a resting blood sample and then performed a graded treadmill protocol to maximum, and then another blood sample was obtained. There was no difference in the met-enkephalin concentration in plasma, red cells, cytoplasm, or ghosts comparing the pre- to postexercise in either the trained or untrained. However, the degradation rate was slower in the trained group to the untrained group independent of time (pre- and postexercise). The authors suggested this may facilitate opioid responses and could provide added tolerance for the trained subjects.

One of the early works in this area examined leu-enkephalin activity in plasma both before and after a competitive run (21). Experience runners (9 males and 5 females) gave a resting blood sample and after (2.5–8 min) the 10 mile road race. The resting value of leu-enkephalin was 22.2 ± 13.7 pmol/mL and increased ($p > 0.05$) to 26.05 ± 21.5 pmol/mL which was modest at best. The change in leu-enkephalin was inconsistent and variable and did not relate to time of completion as some runners showed increases, others decreased with most showing very little change.

In conclusion, the influence of exercise in met-enkephalin is variable and appears to depend on the type of assay used. There is inconsistency in the results, as some studies suggest enhanced levels and others no change. This may be related to fitness level, but again, there are not enough studies to suggest that aerobic capacity or fitness level consistently influence the met-enkephalin level. There appears to be a lack of research with leu-enkephalin, with one study suggesting modest changes after a 10 mile run but with large variations in the individual response of study participants.

There is limited information on exercise training programs with enkephalins. Chen et al. examined the acute and chronic exercise training effects on leu-enkephalin in the caudate-putamen of rat brains and compared the levels to sedentary control rats (11). The trained rats exercised on a motorized treadmill for 5 weeks with a progressive increase in time and speed and ran 5–7 days per week ultimately running for 25 min/day at 35 m/min. This latter group was then either exercised or rested and then sacrificed. The staining of leu-enkephalin was primarily in the PVN and the caudate-putamen region (CPR). The acute exercise increased staining in the CPR region and remained elevated in this region for up to 180 min postexercise but gradually decreased over time after exercise (11). These results suggest that there is a central mediated enkephalin response influencing the brain and that acute exercise increased enkephalin in this brain region. Unfortunately this study did not have a sedentary acute exercise group, to determine if the endurance training had any influence.

There is also some information that has dealt with the influence of exercise on the proenkephalin peptide F that is typically released from the adrenal gland (medulla) and is often co-released with epinephrine (64). The influence of intensity of exercise and training was examined in college-aged students (59). The trained subjects were middle-distance runners ($n=10$) and the untrained individuals ($n=10$) were not in any formal activity for two years. The subjects had their VO₂ max determined on a cycle ergometer and then returned to do several 8 min stages which elicited 28, 53, and 84% VO₂ max

and then went to VO_2 max. Blood was obtained at the end of each workload and during recovery. Peptide F levels at rest were twice as high in the trained group compared to the untrained but were very low (<0.1 pmol/mL). Neither group demonstrated any significant change in peptide F at the 28% workload with both groups having similar levels. At 54% workload the trained group showed a significant increase and at higher work intensities the peptide F level stayed fairly constant (0.4 pmol/mL). In contrast, the untrained group did not demonstrate as rapid an increase and only reached a similar level of peptide F at 100% VO_2 max. However, the peptide F levels continued to increase at 5 min into the recovery and then started to decline. It is interesting to note that the epinephrine level for both groups showed a similar response. This suggests that the alterations in peptide F level may be related to other factors than its release.

The effect of fitness and intensity of exercise was examined in women to see if peptide F levels might be altered differently in women (96). Women who were endurance trained (>3 times per week, 30–45 min per session) were compared to inactive women. They were exercised on a cycle ergometer at 60% (15 min) and 80% VO_2 max (15 min) during the early phase of the follicular phase of the menstrual cycle. Blood was collected at rest and 10 min into each intensity and 5 min into recovery. The authors reported that only the fit women demonstrated a significant increase in peptide F at the 80% intensity workload. However, this increase was very modest from 0.046 to 0.056 pmol/mL. In contrast, the untrained women showed a greater epinephrine level compared to the fit women. This again suggests dissociation in the amount of epinephrine and peptide F within the circulation.

The influence of menstrual cycle on peptide F to maximal exercise was reported in ($N=8$) eumenorrheic women (58). There appears to be a slight but nonsignificant (0.06) effect of menstrual cycle on plasma peptide F level at rest. In addition, there was no exercise main effect on plasma peptide F levels. These results suggest there may be fluctuations in peptide F levels over time as well as over the course of the menstrual cycle. This suggests that the minor changes in the previous study with much lower peptide F levels may be an anomaly. Clearly, more research studies are needed as there is inconsistency in the results with exercise on peptide F levels. Furthermore, many of the variables that might influence baseline peptide F levels should be considered.

The roles of peptide F are not well defined at this time. It is clear that it is co-released from the adrenal medulla, but its actions and implications with exercise have not been elucidated. Peptide F appears to have an important role in the immune system. Therefore, peptide F and its possible exercise-modulated effect on immune function should be further investigated.

SUMMARY

In conclusion, exercise of sufficient intensity and duration may influence the endogenous opioids, but what is measured in the circulation does not necessarily reflect what happens within the brain. Numerous factors such as sex, menstrual cycle, diet, plasma volume, carbohydrate level, and inflammation can influence the endogenous opioids. Furthermore, immune function and neural control can clearly alter endogenous opioid activity. Finally, a greater understanding of the influence within the brain needs to be established.

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3

The Effect of Exercise on the Hypothalamic–Pituitary–Adrenal Axis

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OVERVIEW

This chapter focuses on the relationship between the HPA axis and physical exercise. The first section provides an overview of the HPA axis as well as some of the major factors regulating its physiological and pathophysiological effects both in the central nervous system and in periphery. The following sections review the effects of endurance and resistance training, the intensity during a specific physical activity, elite training in athletes, overtraining, level of fitness and aging, memory, defeat fear, and cognitive functions on the activation of the HPA axis. The potential outcomes of the HPA axis on the control of voluntary physical activity and aggressiveness in sports are also discussed.

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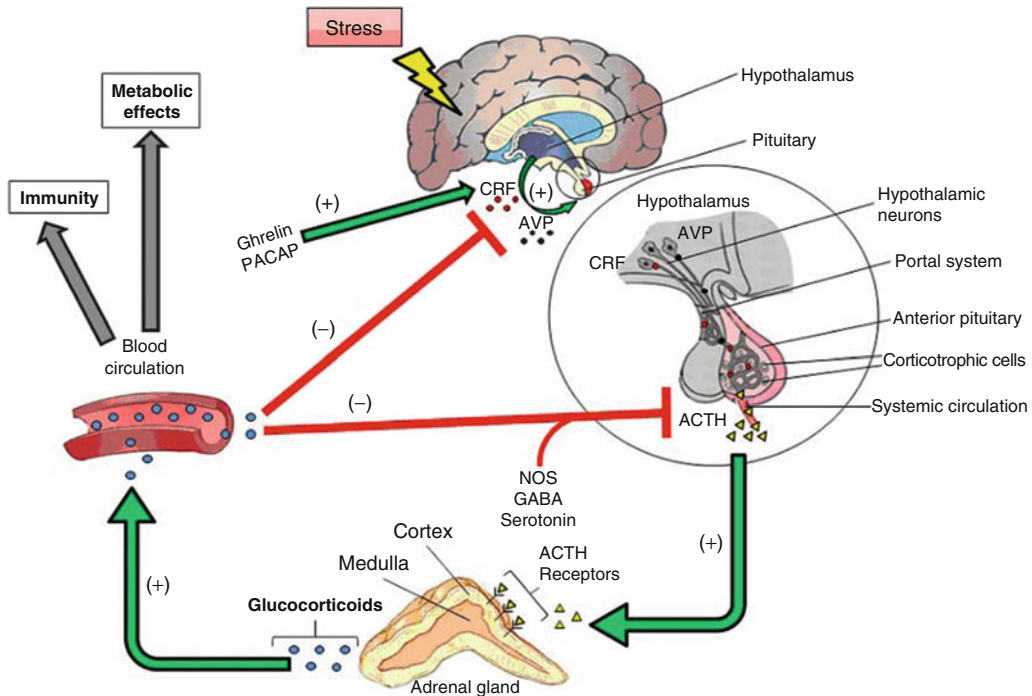


Fig. 1. Summarization of the mediators of HPA axis activity.

THE HPA AXIS

As summarized in Fig. 1, the HPA axis consists of three structurally independent components including the hypothalamus, the anterior pituitary, and the adrenal cortex. These structures are intimately interacting through the release of neuroendocrine messengers. In the medial parvocellular and the magnocellular parts of the paraventricular nucleus of the hypothalamus (PVH), corticotrophin-releasing factor (CRF, a 41-amino acid peptide) and arginine vasopressin (AVP, expressed in approximately half of the CRF neurons) are synthesized (1). CRF neurons project to the exterior layer of the median eminence and release CRF into the portal circulation until they subsequently reach corticotroph cells from the anterior pituitary to stimulate the secretion of adrenocorticotrophic hormone (ACTH). In turn, ACTH is released and transported via the general circulation to activate the adrenal secretion of glucocorticoids. Importantly, it is known that glucocorticoids negatively control pituitary corticotrophs and PVH CRF neurons through direct or hippocampus-mediated feedback inhibition mechanisms (2, 3).

In mammals, the CRF system is not limited to PVH CRF neurons. The system also comprises two CRF-receptor types (CRF-R1 and CRF-R2) (4), a CRF-binding protein (5), and endogenous CRF-receptor ligands that include mammalian peptides CRF (6), urocortin (UCN) (7), UCN II (8, 9), and UCN III (9, 10). In the brain, the broad distribution of CRFergic cells, UCNergic neurons, and CRF receptors is compatible with the main functions attributed to the CRF system (11). Central administration of CRF evokes autonomic responses (12, 13), general arousal (14), as well as anxiety-like behaviors (13, 15).

Furthermore, central CRF injections also activate the sympathetic while inhibiting the parasympathetic branches of the autonomic nervous system by stimulating cardiorespiratory functions (16) and reducing the activity of the digestive system (17). Because of their selectivity for CRF-R2, UCN II and UCN III (10) (also referred to stresscopin in humans) have been described as “stress-coping” peptides capable of exerting anxiolytic effects (9).

AVP is a 9-amino acid peptide with a disulfide bridge that is mainly secreted from the magnocellular cells of the supraoptic nucleus and the PVH, and transported to the circulation to exert its effects on kidneys and blood vessels (18, 19). In addition, AVP’s expression is also reported in the parvocellular neurons of the bed nucleus of the stria terminalis, the medial amygdala, the suprachiasmatic nucleus, and the PVH (20–22). Three major types of AVP receptors are known: AVPR1a, AVPR1b, and AVPR2 (23, 24). The activation of AVPR1b in the anterior pituitary stimulates the release of ACTH (25) while AVPR1a and AVPR2 are mainly expressed in the kidneys and blood vessels (26).

ACTH is a 39-aa peptide derived from the proteolytic cleavage of the proopiomelanocortin (POMC) gene (27–29). The expression of ACTH is modulated positively by CRF and AVP, naloxone, interleukin (IL)-1, IL-6, and leukemia inhibitory factor (LIF), but negatively by glucocorticoids (30–34). However, other factors such as pituitary adenylate cyclase-activating peptide (PACAP), catecholamines, ghrelin, nitric oxide synthase (NOS), DOPA (dihydroxyphenylalanine), serotonin, and GABA (gamma-aminobutyric acid) are also suspected to influence ACTH secretion through still ill-defined mechanisms (35–37). ACTH is released in a pulsatile manner and has been shown to be regulated through a calcium-dependent mechanism (38). It is subsequently transported in the circulation to activate the melanocortin type 2 receptor (MC2R) from the adrenal glands (39, 40) and, ultimately, stimulate species-specific glucocorticoid (either cortisol in human, nonhuman primates, pigs and dogs, or corticosterone in laboratory rodents such as rats and mice) synthesis and secretion (41). In a matter of seconds to minutes, the release of glucocorticoids from adrenal glands will activate glucocorticoid receptors (GR), stimulate Annexin 1 (ANXA1) production and, consequently, block CRF-induced ACTH secretion (42, 43). It is however been suggested that the level of complexity of the direct and indirect mechanisms through which glucocorticoids exert their repressive effects on the HPA is much higher than what was anticipated during the 1980s (1).

THE HPA AXIS AND EXERCISE

Endurance Training

The effect of endurance training on the activation of the HPA axis was investigated in animal and human models. Pigs on a high-fat diet submitted to an endurance training program displayed a 60% increase in ACTH following a stress challenge; this effect was associated to a 56% decrease in serum free fatty acids (FFA) without any change in body composition and insulin sensitivity (44). In another study, in rats confined to a cage that allowed voluntary wheel running, corticosterone responses to various stimulatory challenges of the HPA axis were shown to be significantly higher than in untrained animals (45, 46). Interestingly, this enhanced adrenal sensitivity to ACTH was completely restored to normal following 5–8 weeks of training. In an ovine

model, ACTH levels were found to rise in response to exercise, even though the animals had been previously submitted to a CRF infusion (47). The latter suggests that ACTH release could be stimulated by another factor than CRF and the authors suggested AVP as a plausible candidate.

In human studies, the activation of the HPA axis in response to physical activity has been abundantly reported (48, 49). For instance, individuals submitted to chronic endurance training display higher hair cortisol levels (50). In endurance-trained men, after a day without physical exercise, ACTH and cortisol concentrations were similar to those of untrained controls (51). For most of these athletes, dexamethasone was not found to influence the activity of the HPA axis; however, in contrast to untrained subjects, a subsequent administration of CRF was shown to increase circulating cortisol concentration. On the other hand, obese adolescents submitted to a chronic physical activity program displayed a marginal decrease in glucocorticoid sensitivity and increased levels of glucocorticoid receptor- α (GR- α) expression in blood mononuclear cells (52).

The influence of an acute bout of endurance exercise on HPA axis activity has also been investigated. In response to a challenge comprising exercise and heat exhaustion, trained individuals displayed significantly higher cortisol levels than untrained subjects, but ACTH concentrations were not different (53). Interestingly, in response to the same challenge in trained and untrained individuals, ACTH, norepinephrine, and dehydroepiandrosterone-sulfate (DHEA-S) levels are significantly increased while growth hormone (GH), aldosterone, and epinephrine concentrations are initially elevated but reach a plateau. In athletes submitted to a strenuous exercise, CRF and cortisol responses to HPA activation are not blunted by physiological endogenous hypercortisolism; this suggests that pituitary sensitivity is decreased in response to the feedback inhibition induced by cortisol (54). Acute physical activity has been reported to influence the HPA axis activity; however, the relevance of considering other physiological conditions should not be neglected. In fasting subjects submitted to physical exhaustion, ACTH and cortisol levels significantly increased in hypoglycemic conditions, but this effect was abolished when pretest glycemia levels were maintained (55). This also suggests the relevance of further examining the HPA axis activation in response to blood glucose levels under 3.3 mM.

While above-mentioned information indicates that HPA axis activity is modulated by chronic and acute training, it is also important to evaluate the effect of a recuperation phase. In runners, it has been observed that cortisol and ACTH levels are significantly lower 2 days following a marathon, while whole body 11- β hydroxysteroid dehydrogenase 1 (11 β -HSD-1) activity and ghrelin levels are upregulated (56). Also, the suppression of cortisol in response to a dexamethasone challenge is strongly increased after 6 weeks of reduced training.

Resistance Training

Although the effect of endurance training on the activation of the HPA axis has been abundantly studied, fewer studies have evaluated the effect of resistance training. Resistance training can be defined as any exercise program using one or multiple training strategies (own body mass, free weights, or diverse exercising machines) to enhance health, fitness, and performance (57). In healthy untrained men submitted to acute

resistance training, cortisol concentrations are not modulated (58). However, in the same subjects, catecholamines, lactate, TNF α , IL-2, and epidermal growth factor (EGF) levels increase, while monocyte chemoattractant protein-1 (MCP-1) concentrations decrease, indicating the activation of the catecholaminergic system as well as a mild inflammatory response. Furthermore, a positive correlation was observed between the concentrations of cortisol and TNF α . In competitive athletes performing in power disciplines, an isokinetic exercise induces a higher increase in ACTH, cortisol, and lactate with respect to endurance athletes but no difference was observed during recovery (59). This information suggests that the HPA axis activation might be influenced by the type of training and the intensity at which it is performed.

INTENSITY OF PHYSICAL ACTIVITY AND HPA AXIS ACTIVATION

It is abundantly reported that the HPA axis is activated in response to physical activity, but few groups have investigated the influence of different levels of intensity. In moderately trained men, increased levels of aerobic physical exercise elevated cortisol and decreased ACTH concentrations at moderate to high intensities but the opposite effect was observed at low aerobic intensity (60). In trained athletes submitted to a prolonged high intensity exercise, increased plasma concentrations of cortisol, ACTH, CRF, and AVP are observed (61). It has also been reported that the rise in osmolality observed during exercise correlates with increases in plasma AVP. Furthermore, for a given type of physical activity, high intensity and prolonged duration respectively increase AVP and CRF levels.

HIGHLY TRAINED AND ELITE ATHLETES

Overall, the increased activity of the HPA axis in highly trained athletes could have important implications on their somatic and mental health. In highly trained athletes, the morning surge in ACTH and cortisol is observed earlier, and ACTH levels are significantly higher than in normal individuals (62). In addition, the stimulation of CRF and ACTH release is more pronounced in highly trained athletes than in untrained individuals following the administration of the nonselective opioid receptor antagonist naloxone (34). Interestingly, while moderate and high intensity exercises increase cortisol levels during daytime, the opposite effect is observed later at night in endurance athletes, suggesting an acute stimulation of the HPA axis combined to a subsequent reduction of its activity (63). Altered HPA axis functions have also been observed in elite athletes. For instance, artistic gymnasts competing at the European Championships display higher salivary cortisol concentrations and more important levels of psychological stress than controls (64). In addition, higher psychological stress and saliva cortisol levels were also observed in female vs. male athletes. In elite junior soccer players, nonfunctional overreaching performances were associated with higher scores of depression and anger, whereas resting GH and ACTH concentrations after maximal effort were diminished (65). These observations could be attributed to the decreased expression of GR- α mRNA in highly trained individuals and to lower increases in atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) levels in response to exercise (66–71). These elements suggest

the influence of the HPA axis on stress and emotional status. Ultimately, these factors could also have a major impact on sportive performances in elite athletes.

Corticosteroids are commonly used as anti-inflammatory agents in injured athletes. In these athletes, adrenal insufficiency is observed for up to 14 days in response to single intra- or periarticular injections of corticosteroid (72). This suggests the relevance of evaluating HPA axis functions in injured athletes and reporting any adverse symptoms to their physician.

OVERTRAINING

The available information regarding altered HPA axis functions in athletes suggests the relevance of considering potentially pathological conditions such as overtraining. In overtrained Standardbred racehorses, cortisol levels were significantly below normal (73, 74). In different populations of athletes, several alternative methods such as a CRF stimulation test (evaluation of basal ACTH concentrations and GH pulsatility), free testosterone over cortisol concentrations, low basal cortisol levels, as well as HPA responses to two standardized exercise tests were proposed for the diagnostic of overtraining (49, 75, 76). For instance, in response to two acute bouts of exercise, increased prolactin (PRL) levels and decreased ACTH concentrations are reported in overtrained athletes (77–79). These effects could be mediated by the repetitive occurrence of muscle and skeletal trauma resulting in local inflammation and, consequently, in a systemic inflammatory response as well as depleted performances (49).

LEVEL OF FITNESS AND AGING

In a recent review, Garrido reported that genetic factors and increased levels of stress experienced in early life and adulthood could enhance the vulnerability towards neurodegeneration during aging (80). He also suggested that cognitive-demanding tasks combined to physical exercise could prevent the detrimental effects of stress on neurological functions during aging. In a series of reports, Traustadottir et al. observed that older unfit women display higher cortisol responses and ACTH levels than their young unfit and older fit counterparts when submitted to stress (81). In addition, the same group reported that a bout of physical activity at high intensity induced a comparable cortisol response while older unfit women displayed a delayed reduction in ACTH levels when compared to older fit and younger women (82). This therefore indicates that both age and the level of fitness may influence mechanisms controlling the regulation of HPA axis functions.

MEMORY, DEFEAT, FEAR, AND COGNITIVE FUNCTIONS

Rodent models have been used to investigate the effect of the HPA axis on memory, defeat, and fear. In rats administered with metyrapone (a corticosterone synthesis disruptor), impaired traces of fear conditioning have been observed (83). A number of studies also evaluated the effects of CRF on defeat conditioning as well as on memory. The central administration of anti-sauvagine-30 (a CRF-R2 receptor antagonist) reduced defeat conditioning in Syrian hamsters (84). However this effect was not observed in

response to neither metyrapone nor CP-154,526 (a CRF-R1 antagonist) administrations in the lateral ventricle of rats increased spatial memory through a β -adrenergic-dependent mechanism (85). Also, central administrations of NBI30775 (CRF-R1 antagonist) prevents stress-induced hippocampal dendritic spine loss while restoring stress-impaired cognitive functions (86). This suggests that stress-induced central effects are mediated through the activation of CRF-R1.

VOLUNTARY PHYSICAL ACTIVITY AND AGGRESSIVENESS

Although detrimental effects of the activation of the HPA axis are often reported in response to exercise or training, one can also take into consideration its relevance in the regulation of the will to initiate a physical activity. For instance, mice predisposed for high voluntary wheel running display corticosterone levels approximately twice those of their “normal wheel runner” counterparts (87). In addition, single nucleotide polymorphisms in glucocorticoid (NR3C1) or AVP (AVPR1b) receptors were both found to be associated with increased aggressive behavior in swine (88). This underlines the potential influence of the HPA axis on the initiation of voluntary physical activity as well as the control of the aggressiveness necessary for sport performance.

CONCLUSION

Obesity and consequent metabolic dysfunctions have reached epidemic levels in North American populations. Among the various approaches recommended nowadays to address obesity, physical exercise has become an important element of a healthy lifestyle for a growing number of individuals. As one consequence, it has wrongly become common belief that more strenuous exercise will bring about faster and better results, with the result that a significant number of ill-prepared, athletes with an inappropriate training program have to prematurely interrupt or slow down with their physical training program for various clinical reasons. Our current knowledge of the relationship between the HPA axis and physical exercise reviewed above clearly highlights the importance of adequate preparation for exercise. Also, data indicate that complex molecular and cellular mechanisms occur in highly trained athletes, but not in normal individuals. As a whole, the above information suggests that the HPA axis importantly influences stress-induced functions and that the intensity of HPA axis activation is intimately related to the type of training and the intensity at which it is performed. In brief, activation of the HPA axis introduces a number of peptides such as ACTH and CRF and increases cortisol levels in the systemic circulation, inducing a stress response that readies the individual to meet an emergency. Therefore, the short-term benefits of HPA axis arousal are largely positive and were required for primitive human survival, but the consequences of its long-term arousal that result from prolonged stimulation through intense exercise are mostly deleterious, bringing about, among other inconveniences, fatigue, myalgia, sleep disturbance, and circadian dysrhythmia in normal untrained individuals. Overall, this indicates the detrimental influence of prolonged activation of the HPA axis fostered by intense athletic performance.

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4

Impact of Chronic Training on Pituitary Hormone Secretion in the Human

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INTRODUCTION

The impact of chronic training on pituitary function is best understood by a basic appraisal of the neuroendocrine physiology of any given individual axis and the more complex interactive pathophysiology among axes (1–12). Interaxes interactions have received relatively little attention. Even evaluating a single neuroendocrine axis in its dynamic state is a complicated challenge, given combined feedforward and feedback activities among the key control loci within any given axis (13, 14). For example, in the case of the growth hormone (GH) and insulin-like growth factor 1 (IGF-1) axis, hypothalamic GH-releasing hormone (GHRH) secreted by arcuate nuclei stimulates pituitary GH secretion acutely, whereas the somatostatinergic system originating in the paraven-

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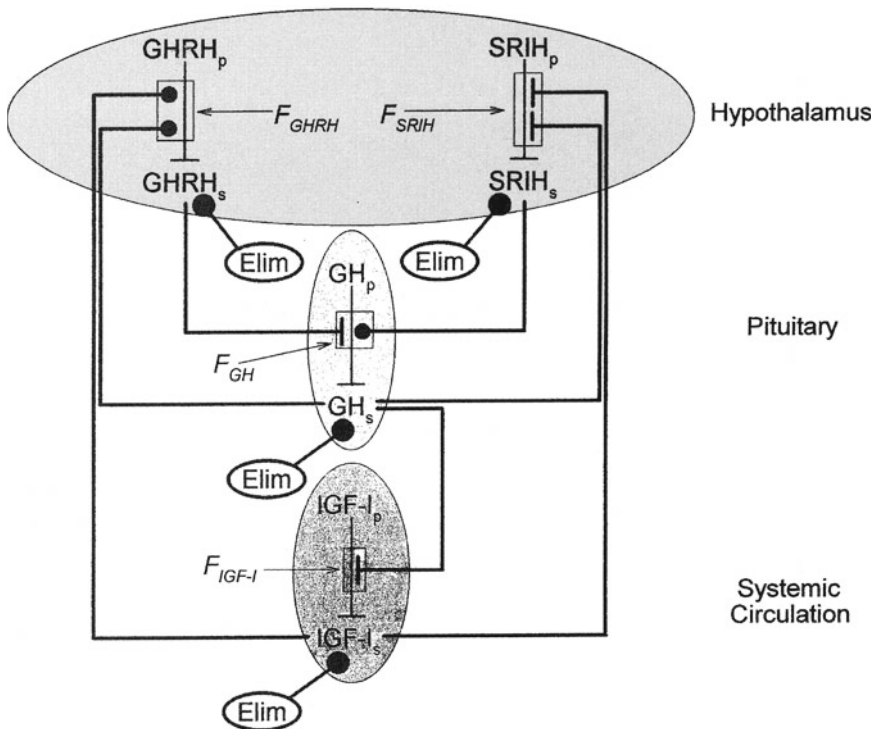


Fig. 1. Network feedback and feedforward linkages within the basic GHRH–somatostatin/GH–IGF-1 axis. Somatostatin is abbreviated here as SRIH. “Elim” denotes metabolic elimination; “F” defines selective input functions, e.g., F_{GHRH} indicates relevant input into GHRH neurons via SRIH and other neuromodulators; F_{SRIH} defines input into SRIH neurons by GHRH and other neurotransmitters; and subscripts “p” and “s” represent particular (tissue or secretory-granule contained) and secreted hormone or peptide, respectively. Some lines denote negative feedback (or feedforward) onto the target node marked by a dot, whereas other lines mark a positive effector pathway terminating with a bar. The interconnected dynamic system shown is simplified from a larger family of interrelated parameters anticipated within the full GH–IGF-1 axis. Additional possible secretagogue input via a putative GHRP-like ligand family is not illustrated, although GHRP receptors (*see text*) are expressed in the hypothalamus and pituitary gland. No endogenous GHRP-receptor ligand(s) has (have) been isolated definitively. Adapted with permission from Straume et al. (14).

tricular nuclei opposes GHRH action (15). These two neuronal inputs are reciprocally interconnected by intrahypothalamic synapses and common impinging neuromodulator pathways (14). In addition, secreted GH feeds back on brain GH receptors, stimulating somatostatin secretion and possibly inhibiting GHRH release. Available GH secreted into the bloodstream triggers IGF-1 production in various target tissues, and circulating IGF-1 is capable of inhibiting pituitary GH secretion indirectly and directly (*see Fig. 1*). Such feedforward (GHRH’s driving GH secretion) and feedback (GH’s inhibiting its own secretion, IGF-1’s inhibiting GH secretion, and so forth) dynamic control mechanisms in principle can be modified by the effects of exercise at one or more levels within the axis. Moreover, multiple determinants modulate neuroendocrine responses to training, such as the body composition of the individual, concurrent stress and/or weight loss, gender, diet and energy balance, concomitant drug or hormone use, age, puberty, pregnancy, and/or lactational status (16–18).

Here, we will examine the neuroendocrine determinants of pituitary responses to exercise training, explore some of the confounding issues (e.g., species differences, varying modes of neurohormone secretion, within- and between-axis regulation, and so on), and explore the overall notion of neuroendocrine axes as feedback and feedforward control systems capable of within-axis as well as between-axes interactions. Finally, metabolic mechanisms, although likely multifactorial, will be examined briefly, and their clinical implications underscored.

MULTIPLE DETERMINANTS OF PITUITARY RESPONSES TO EXERCISE TRAINING

Among other determinants of neuroendocrine responses to exercise training is the acuteness vs. chronicity of the training or exercise stimulus (2, 5, 11, 12, 19–22). In particular, numerous studies demonstrate that acute exercise induces a variety of short-term changes in multiple hypothalamo–pituitary axes, including the nearly immediate secretion of GH and adrenocorticotrophic hormone (ACTH), β -endorphin and cortisol, whereas the results of chronic training are not necessarily identical (20, 21, 23–30). Moreover, stress or acute exercise imposed in an untrained individual will elicit endocrine responses potentially distinct from those observed in a highly physically trained subject (3, 8, 9, 11, 31–40). Thus, many studies are confounded in part by the nature of the prior or concomitant training regimen, its duration, and its intensity. Finally, extreme physical exertion, “overreaching,” often evokes neuroendocrine disturbances that are not typical of either short-term submaximal exertion or chronic training (5, 9, 41–43).

Neuroendocrine axes are exquisitely sensitive to nutrient intake, body composition, and total (and percentage) body fat (44–51). Recent studies of the GH axis document unequivocally that percentage body fat, and in particular visceral (intra-abdominal) fat accumulation (52), negatively influences pulsatile GH secretion by suppressing the mass of GH secreted per burst and shortening the half-life of GH in the circulation (44, 45, 53–56). The reciprocal relationship between visceral fat mass and GH secretion is illustrated in Fig. 2. Impaired GH secretion and more rapid GH removal jointly serve to reduce 24-h pulsatile serum GH concentrations in otherwise healthy but relatively more (viscerally) obese individuals. In contrast, acute weight loss or nutrient deprivation potently stimulates GH secretion in the human (while suppressing it in the rat) by 3–10-fold, with augmentation in both men and women of GH secretory pulse amplitude and mass and, to a lesser degree, burst frequency (47, 57, 58). Consequently, nutrition, body weight, and body composition are prime determinants of pituitary (GH) secretory activity, which likely condition responses to exercise (59). In addition, in men, as well as more recently recognized in women, body mass index (relative obesity) is a negative correlate of LH pulse amplitude (49, 60) and of the serum testosterone concentration in middle-aged men (49).

Gender distinctions also strongly influence the secretory output of several neuroendocrine axes. Foremost, the gonadotropin-releasing hormone–luteinizing hormone (GnRH–LH) follicle-stimulating hormone (FSH)–sex steroid axes in men and women exhibit clarion differences, particularly at the level of so-called positive feedback, which is mechanistically required to achieve a preovulatory LH surge in women (61). The GH–IGF-1 axis is also strongly sexually dimorphic in the human (as well as in the rat, as reviewed earlier (15)). For example, in healthy premenopausal men and women, GH

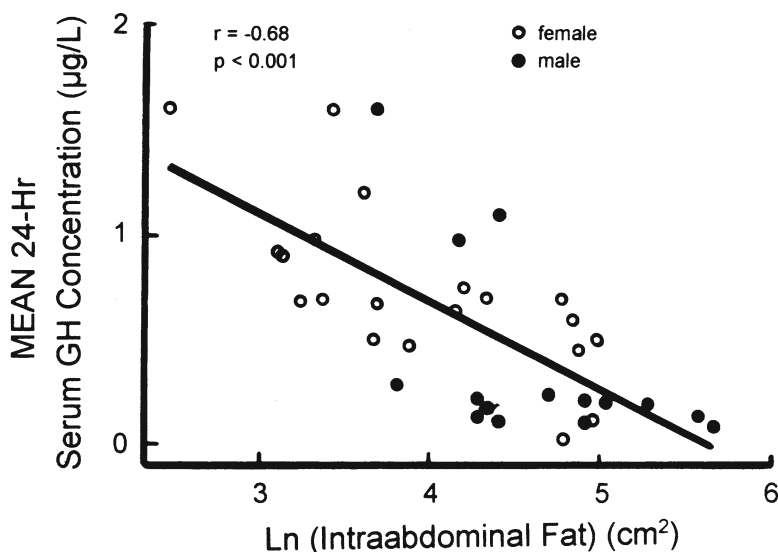


Fig. 2. Negative relationship between 24-h mean serum GH concentration and intra-abdominal (visceral) fat mass, as determined by computerized axial tomographic scanning of the abdomen, in a cohort of healthy middle-aged men and women. GH concentrations were determined by 20-min blood sampling for 24 h and subsequent assay by immunofluorometry. The *solid circles* denote male subjects, and the *open circles* females. The *regression line* shows a strongly negative relationship between the natural logarithm of intra-abdominal adiposity and daily GH secretory activity in both men and women. In multiple linear regression analyses, intra-abdominal fat mass accounted for the majority of the variability in integrated serum GH concentrations, exceeding that owing to age and gender in this population. Redrawn with permission from Vahl et al. (56).

secretion differs quantitatively by way of a nearly twofold greater mean (24-h) serum GH concentration, higher plasma IGF-1 level, greater mass of GH secreted per burst, and a more disorderly pattern of GH release in women compared to men (62). In addition, the individual negative impact of age, body mass index, or percentage body fat on GH secretion is 1.5–2-fold more evident in men than women (48); the positive effect of physical conditioning (increased VO_2 max) on GH release is also more prominent in the male (48) (Fig. 3). The tissue responses to GH also may be sex-specific in part, since estrogen can antagonize GH-driven IGF-1 production by the liver (15). Consequently, gender must be identified as a major determinant of neuroendocrine responses in the GH–IGF-1 axis. Exercise-stimulated GH secretion may be less gender-dependent (63).

A lesser gender difference is observed for the corticotropin-releasing hormone (CRH)–arginine vasopressin (AVP)/ACTH–cortisol axis, where in the female, relatively increased expression of the CRH gene and increased adrenal responsiveness to ACTH are proposed (64). However, the orderliness of individual 24-h ACTH and cortisol release (approximate entropy) or their relative synchrony (crossentropy) in men and women is similar (65).

Another significant confounding influence on neuroendocrine axes is age. For example, in the case of the LH–testosterone axis in men, there is progressive deterioration of LH or testosterone’s individual orderliness of release over 24 h and of LH–testosterone coupling or synchrony, when assessed by either crosscorrelation analysis (indicating diminished

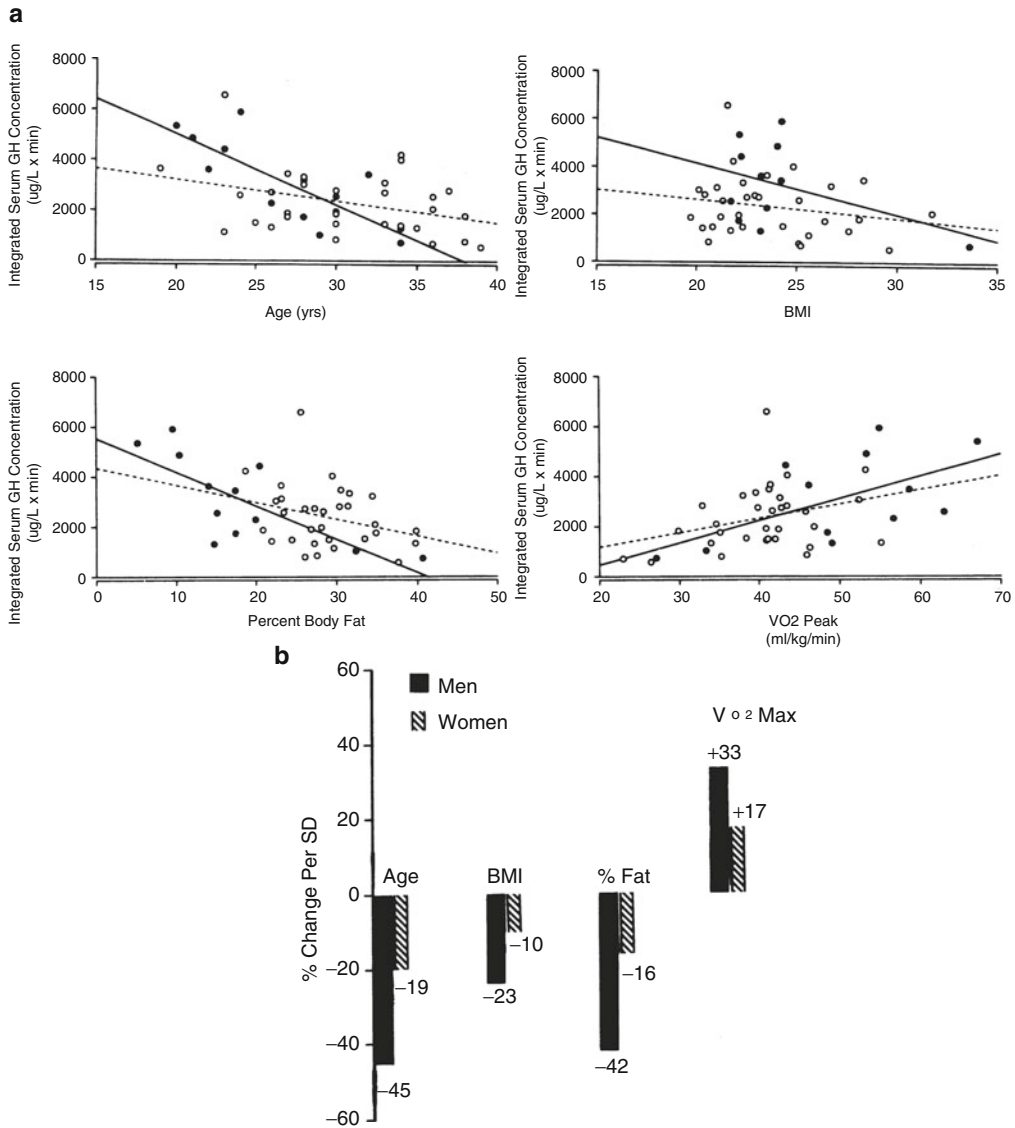


Fig. 3. (a) Impact of gender on the effects of age, adiposity as measured by body mass index (BMI) or percentage body fat, and physical fitness as quantitated by maximal oxygen consumption (VO_2 max peak or max) on integrated (24-h) serum GH concentrations in normal men (filled circles, $N=12$) and women (open circles, $N=32$). Linear regression plots are given for each sex. The solid lines denote regression in men, and the interrupted lines depict women's data. **(b)** Approximately twofold greater impact of age, BMI, percentage body fat, and VO_2 max on 240-h mean serum GH concentrations in men than women. Data are means \pm SEM expressed as standardized regression coefficients for the regression lines in (a). The gender-specific standardized regression coefficient is the slope of the linear relationship (given as a percentage) adjusted per unit standard deviation (SD) of the male or female group as pertinent. Redrawn with permission from Weltman et al. (48).

feedforward control) (66) or crossapproximate entropy (indicating decreased pattern synchrony within the reproductive axis' feedback system) (67). The regularity of GH or ACTH/cortisol release also deteriorates with age in men and women (65, 68). In addition, in both men and women, there are marked quantitative decreases in overall GH axis secretory activity, with a progressive fall in plasma IGF-1 and daily GH secretion rates with aging, especially in men compared to women of premenopausal age (44, 45, 48, 54).

Concurrent drug and/or hormone use can also markedly modify several pituitary-target tissue axes. For example, prescribed or self-use of anabolic steroids will profoundly suppress LH and FSH release and reduce levels of endogenous sex steroids, while potentially stimulating the GH-IGF-1 axis (if aromatizable androgens are employed) (13, 63, 69–71). Likewise, the use of birth control pills in young women stimulates GH secretion significantly, and may produce some alterations in body composition (72). At puberty, when sex-steroid hormone secretion changes more dramatically (73, 74), the individual's GH-IGF-1 and/or GnRH-LH axis may be uniquely susceptible to the impact of exercise training (at least prior to pubertal onset), resulting in a significant delay in sexual maturation and adolescence and possibly reduced predicted adult height (75) (see Chap. 17; First edition).

We infer that an array of important factors, such as exercise intensity and duration, its acuteness vs. chronicity, associated weight loss and/or stress (discussed further below), diet and energy balance, body composition, gender, age, and maturational status (e.g., prepubertal vs. pubertal) may all codetermine the neuroendocrine and pituitary responses to a stress perturbation, such as exercise.

OTHER CONFOUNDING ISSUES

One confounding issue experimentally in evaluating the impact of acute or chronic physical training on pituitary function is species differences. For example, in the rat, physical exertion reduces GH secretion (15), whereas in the human acute and chronic exercise both increase GH secretion significantly, the former within 15–30 min and the latter following sustained exercise at an intensity above the individual lactate threshold (15, 20, 21, 24, 76, 77). Indeed, chronic physical training in women results in a doubling of the 24-h mean serum GH level even on days when exercise is not undertaken (21) (see Fig. 4 (20)). Consequently, many experiments carried out in the rodent do not find applicability, especially for the GH-IGF-1 axis, to human studies. Moreover, the gender differences in the GH axis in the rat and human are readily distinguishable mechanistically in the two species, with a greater mean amplitude (and mass) of GH secretory bursts in women than men (but the converse occurs in the rat) (62). A similarity in the two species is a more disorderly pattern of GH release in the female (78).

Further complicating interpretation and analysis of pituitary secretion are the multi-fold temporal modes of physiological pituitary-hormone release:

1. Pulsatile.
2. Nyctohemeral or circadian.
3. Entropic, or moment-to-moment variations in the orderliness of secretion (67, 79–81).

Pulsatile hormone secretion typically mirrors episodic neural input that acts via intermittent secretagog delivery to a responsive pituitary cell population in the absence of

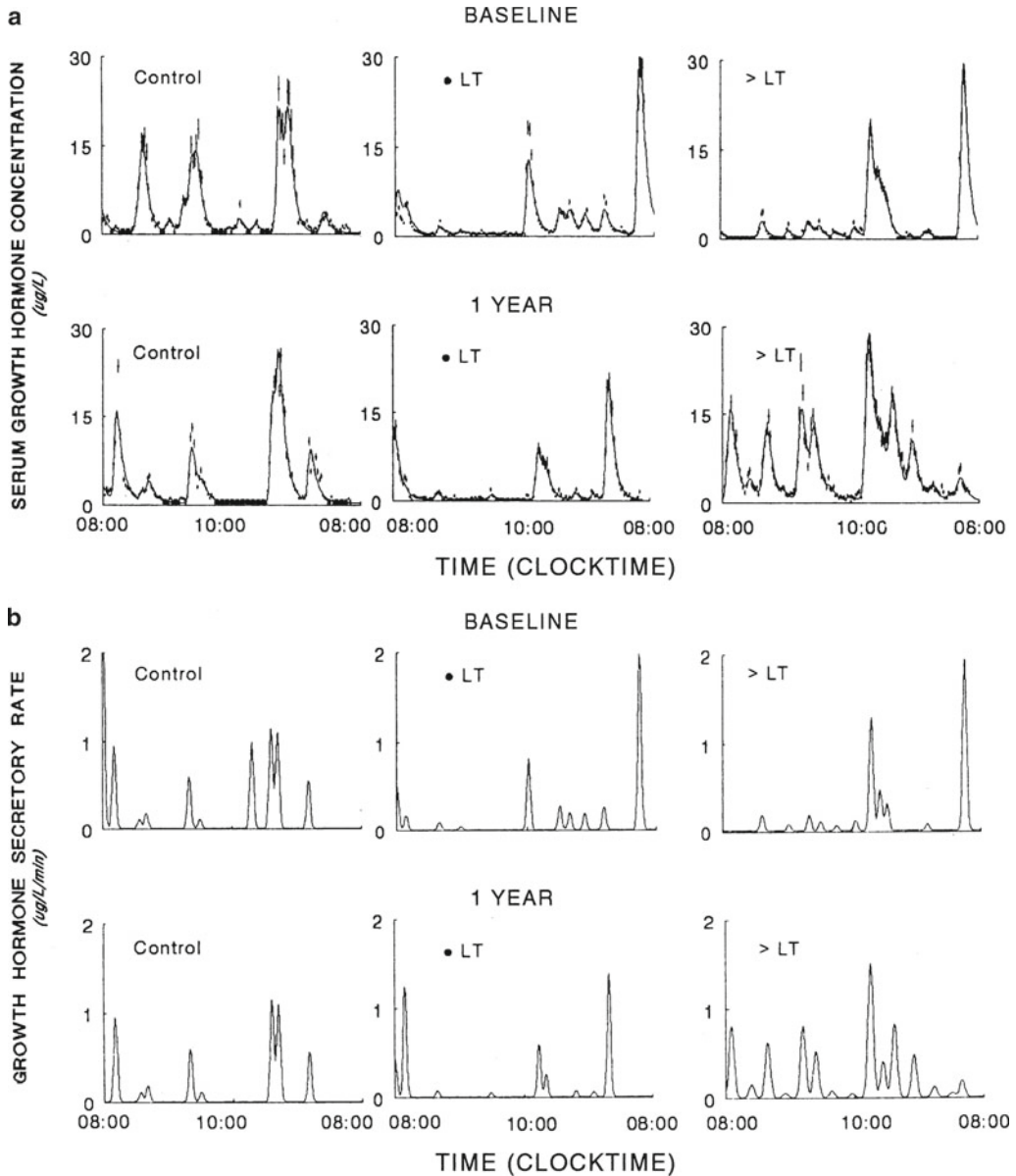


Fig. 4. Twenty-four hour serum GH concentration or secretion profiles in three different premenopausal women each studied twice: control (*left*; no exercise training, sedentary volunteer); before (*baseline*) and after 1 year of exercise training below (*middle panel*), or at or above (*right panel*) the individually determined lactate threshold (LT). Adapted with permission from ref. (20).

significant inhibitory input concurrently. Indeed, a pulse of pituitary hormone secretion can be viewed as a collection of secretory rates, centered about some moment in time. This concept is illustrated in Fig. 5.

In contrast to the foregoing episodic (pulsatile) secretory mode are less rapid, 24-h variations in serum hormone concentrations, which are well established for ACTH, LH,

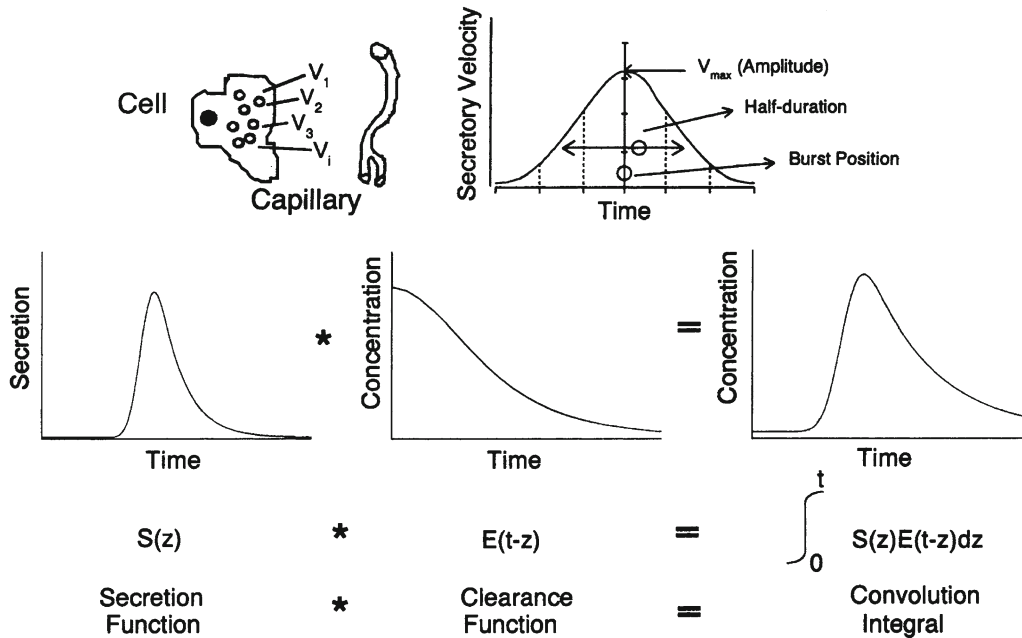


Fig. 5. Schematized illustration of a model-specific deconvolution concept implemented to quantitate (GH) secretion. The *upper* landscape depicts an intuitive formulation of a hormone secretory burst, as arising from (multi-)cellular discharge of individual hormone molecules more or less in concert temporally, each at its own particular secretory rate (velocity). A secretory burst (or pulse) is visualized as an array of such molecular secretory velocities centered about some moment in time, and dispersed around this center with a finite standard duration (SD) or half-width. The burst event may or may not be symmetric over time. The *lower* landscape with the algebraic subheads shows the mathematical notion, whereby a plasma hormone concentration peak (*far right*) is viewed as developing from a burst-like secretory process (*far left*) and a finite hormone-specific removal rate (half-life of elimination). The so-called convolution (intertwining or interaction) of the simultaneous secretory and elimination functions creates a resultant (skewed) plasma concentration pulse. Deconvolution analysis consists of mathematically estimating the constituent underlying secretory features (and/or associated half-life), given (a series of) blood hormone concentration peaks as the starting point. A variety of model-independent (waveform-invariant) deconvolution strategies can also be applied, if a priori knowledge of the pertinent (biexponential) hormone elimination rate process is available. Adapted with permission from ref. (15).

GH, thyroid-stimulating hormone (TSH), prolactin, cortisol, and so forth (82). These nyctohemeral (night–day) variations constitute only a small part of the total variation in daily neurohormone release. True circadian rhythms are so-called free-running with a periodicity of 24 h, temperature-compensated, and susceptible to Zeitgebers or specific phase-entraining cues (83). Not all human 24-h neuroendocrine rhythms conform to this definition, which would denote true (suprachiasmatic nucleus-driven) circadian activity. Based on sleep-reversal studies, and so forth, circadian rhythmicity clearly does exist for ACTH/cortisol release in the human and GH secretion (approx 50% of the 24-h GH rhythm is sleep- and activity-entrained, and 50% is circadian) (15, 84).

Neurohormone release also exhibits features of minute-to-minute patterning, serial orderliness, or relative regularity, which can be quantified by an approximate entropy

statistic (67, 78). Higher values of approximate entropy denote greater disorderliness of hormone release, and are a feature of female GH secretory patterns (compared to male), healthy aging of the human insulin, GH, LH, and ACTH/cortisol axes (54, 65, 67, 78, 85, 86), as well as aldosteronomas (87), tumoral pituitary hormone secretion (acromegaly, Cushing's disease, and prolactinomas (65, 88)), and insulin release in type II diabetes mellitus (89, 90). Thus, entropy measures can identify secretory disturbances complementary to pulsatile or circadian variations.

The complex mode of pituitary hormone secretion imposes the need for appropriately rigorous sampling intensity and duration to capture the pulsatile, circadian, and entropic features, followed by application of relevant analytical tools appropriately validated under those conditions of study. Such technical issues have been reviewed recently (80, 91–93).

Further confounding in the literature arises because biochemically measurable endocrine changes do not always imply definite biological or clinical sequelae. For example, studies of the thyrotropin-releasing factor (TRH)–TSH–thyroidal axis have revealed numerous biochemically measurable changes during acute or chronic exercise, but their clinical sequelae are not known (94). Similarly, in relation to the male reproductive axis, a variety of pituitary–gonadal changes are well established in response to chronic exercise, such as diminished LH pulse frequency at least in a subset of men, and relatively decreased spermatogenesis (e.g., a 30–50% decline in sperm number). However, clinical signs and symptoms of androgen deficiency rarely, if ever, occur, and male infertility is not known to be associated with chronic physical training (5, 32–35, 43, 95–100). Finally, multiple hormones are produced by the anterior pituitary gland, and, as discussed further below, the corresponding individual axes may evince significant interactions.

NEUROENDOCRINE AXES AS FEEDBACK AND FEEDFORWARD CONTROL SYSTEMS

As intimated in the Introduction, neuroendocrine axes should be viewed as dynamic feedforward and feedback control systems. The term feedforward defines the ability of a secreted agonist to act on a remote or proximal tissue and evoke a typically sigmoidal (e.g., log-logistic) dose–response curve, e.g., as anticipated for GHRH's acting on somatotrope cells in the anterior pituitary gland, GnRH's acting on gonadotrope cells, and so forth (15, 101). Conversely, feedback denotes the ability of a secreted product from a target tissue to inhibit the production of the agonistic signal, e.g., testosterone feeds back on hypothalamic GnRH secretion in the male, IGF-1 feeds back on pituitary somatotrope secretion of GH, l-thyroxine feeds back on TSH secretion at the pituitary and hypothalamic levels, and so forth. As highlighted in Figs. 1 and 6, both the GHRH–somatostatin/GH–IGF-1 axis (14), and the GnRH–LH/FSH/sex steroid (101) axes should be viewed as complex feedback and feedforward control systems (13, 14, 79, 101–103). This concept is physiologically critical, since most pathophysiological stimuli impinge on several points within the feedback control system, thus impacting on the overall dynamics. Such system-level responses cannot be observed readily when separated components are studied individually. Similarly, the stress-responsive ACTH–adrenal axis comprises CRH–AVP/ACTH–cortisol, with corresponding feedforward and interactive feedback mechanisms inherent (3, 40, 104).

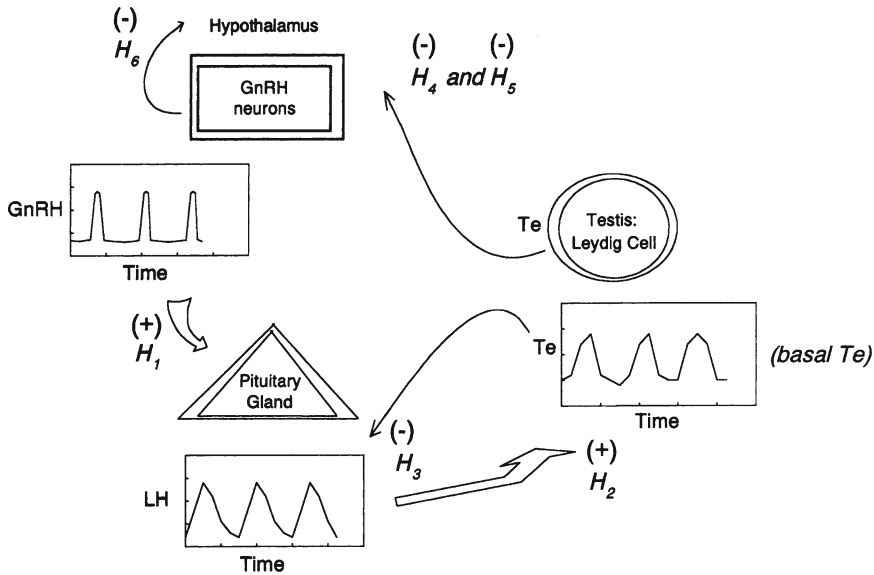


Fig. 6. Schematic illustration of the time-delayed negative feedback (-) and positive feedforward (+) within the human male GnRH-LH-testosterone (Te) axis. The *broad arrows* indicate feedforward (+) stimulus-secretion linkages, and the *narrow arrows* denote feedback (-) inhibition. The “H” functions are developed further in ref. (101), and serve to define the dose-response relationships at each feedback interface within the axis. Adapted with permission from ref. (101).

An important notion in future studies of chronic exercise effects on the pituitary will be to limit isolation of individual components of the axis, and rather study the overall axis dynamics. Technology, such as approximate entropy (67, 105) and network analysis (14, 101), for accomplishing the latter is just beginning to emerge. To date, the vast majority of published literature (as discussed throughout this volume) has enunciated changes at individual control points, which unfortunately subdivides the feedback system artificially, and limits insights into its interactive properties, which function from minute to minute and day to day.

INTERACTIONS AMONG NEUROENDOCRINE AXES

Foremost among the challenges to be addressed in investigative and clinical neuroendocrine pathophysiology are the nature and mechanisms of interaction between two, or among three or more, neuroendocrine axes. For example, in relation to chronic exercise or other stressors in experimental animals, alterations occur not only in hypothalamic GHRH and somatostatin gene expression, but also in the GnRH neuronal ensemble and neuropeptide Y (NPY)- and CRH-secreting neurons (104, 106). In conjunction with concurrent changes in dietary intake, activity of TRH neurons in the hypothalamus may also be suppressed (reviewed in ref. (107)). Relevantly, these multiple neuronal pathways are directed by corresponding families of neurotransmitters (e.g., norepinephrine, serotonin, acetylcholine, and so forth), as well as various potent neuromodulators (e.g., NPY, galanin, and so on). Thus, a major focus in understanding the whole-body neuroendocrine responses of an intact organism to chronic exercise training must eventually

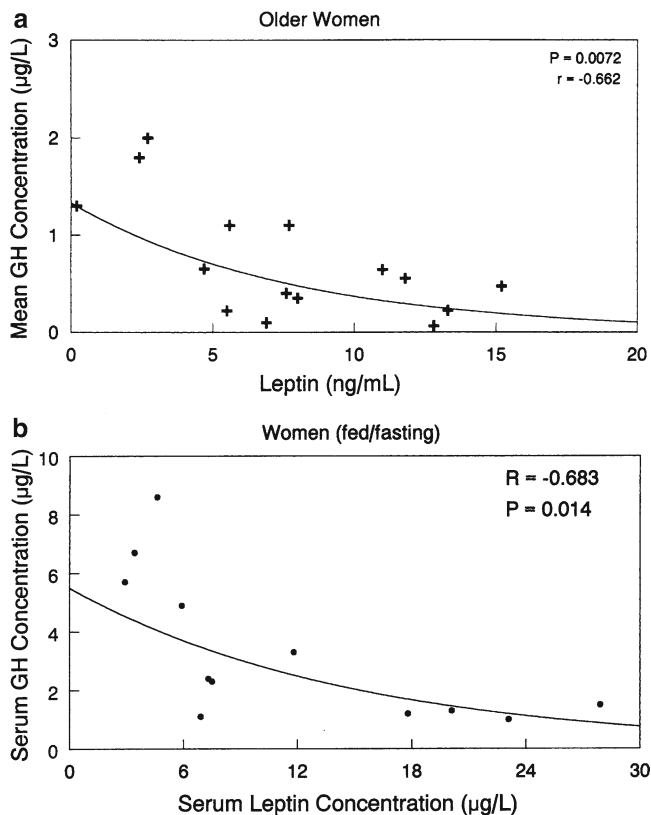


Fig. 7. (a) Inverse log-linear relationship between fasting serum leptin concentrations and integrated 24-h serum GH concentrations in 15 healthy postmenopausal women (55). (b) Similar inverse (exponential) regression between serum leptin and GH output in young women fed or fasted (58). *P* and *r* values for the linear regressions are shown. Adapted with permission.

include the articulation of not only individual neuronal pathway changes, but also their collective and interconnected alterations owing to common neuromodulatory inputs. For example, infusion of leptin, the product of the *ob* gene in adipocytes, is capable of: rescuing suppressed hypothalamic TRH secretion in fasting; relieving inhibited GnRH gene expression in certain stress models; and stimulating GH secretion in the fasted male rat (presumptively by reducing hypothalamic somatostatin gene expression). Thereby, leptin may integrate a complex response pattern via concerted hypothalamic actions that supervise diverse pituitary hormone secretory activities (107–109). However, in the human, leptin levels correlate inversely (rather than directly, as in the rat) with GH axis secretory activity, as illustrated in Fig. 7 (55).

METABOLIC MECHANISMS

The exact metabolic mechanisms that subservise hypothalamo–pituitary responses to exercise training are not known. Among those extensively considered are free fatty acids, which clearly can inhibit GH secretion (15). On the other hand, any direct role of free fatty acids in modifying the GnRH–LH–gonadal axis is not evident.

Similarly, both insulin and free IGF-1 can inhibit GH secretion directly at the anterior pituitary level, and indirectly via hypothalamic effects under several conditions in certain species (15).

Moreover, prolonged nutrient and/or glucose deprivation can arrest puberty in the immature sheep, and modify hypothalamic peptide secretion (e.g., stimulate CRH and/or AVP, while inhibiting GnRH, secretion) (104). In contrast, carbohydrate ingestion during exercise in one study in the human seemed to increase cortisol and decrease gonadotropin release (110), whereas maintenance of euglycemia in another study abolished exercise-induced ACTH–cortisol release in nearly exhaustively exercised volunteers (111). Finally, as intimated above, the peptide leptin can modify somatostatin, GnRH, TRH, and NPY gene expression, among other hypothalamic responses to the stress of fasting (55, 58). Overall, we postulate that such multifactorial metabolic cues and the sex steroid milieu significantly codetermine neuroendocrine responses to exercise training (112–114). In addition, under the most severe exercise stimulus, overall “final-common-pathway” stressor responses may prevail, such as secretion of reproductively inhibitory CRH and endogenous opioids, with consequent suppression of GnRH–LH secretion and conversely (in a species-specific manner) stress-driven alterations in the GH–IGF-1 axis (10, 15, 38, 115–123).

IMPLICATIONS

Among other implications of chronic training are favorable nonendocrine adaptations of hemodynamic and cardiovascular function. These changes are likely to be important in long-term health risk. Moreover, body compositional changes, motivated in part by the above neuroendocrine alterations, would be predicted to have a propitious impact on population-wide morbidity and mortality (12, 117). In contrast, alterations in bone density accompanying chronic exercise have bipotential implications, e.g., with putatively increased fracture risk owing to sex steroid deprivation (amenorrhea) and possibly reduced total (height) growth potential (75), and, conversely, variably decreased fracture risk owing to increased bone density associated with the stress–strain mechanism of enhanced bone apposition accompanying sustained physical training (22, 124–126). However, other confounding factors, such as concurrent estrogen status, activity of the GH–IGF-1 axis, ethnicity, and gender, can also modify bone density and fracture risk. For example, we recently observed that black men and women show increased bone mass over their Caucasian counterparts, but that only in men is the higher bone density in blacks associated with correspondingly increased GH secretion (127). The mechanisms underlying such ethnic differences are also not yet understood, nor are possible ethnic differences in endocrine responsiveness to exercise stress well investigated.

SUMMARY

The impact of chronic exercise training on the neuroendocrine control of the anterior pituitary gland, and its feedback and feedforward inputs, is complex. Multiple determinants influence adaptive hypothalamo–pituitary secretory responses to physical stress, namely, training intensity and duration, including overreaching exercise, concurrent

weight loss, diet and energy balance, other associated stressors (both psychological and physical), body composition, gender, age, the sex steroid milieu, and developmental/maturational status. Confounding variables include interspecies differences, the complexity of neurohormone secretion (pulsatile, circadian, and entropic rhythms), the difficulty in interpreting earlier cross-sectional studies (with possible ascertainment bias) compared to longitudinal data, and the distinction between biochemical changes in and clinically significant sequelae of neurohormonal alterations with exercise. We emphasize that measurable pituitary responses to exercise should be viewed as part of a feedforward and feedback control system, as exemplified for the GH-IGF-1, GnRH-LH, CRH-AVP-ACTH, and other axes, with yet additional between-axes interactions. Although the final metabolic mechanisms that direct neuroendocrine changes in chronic training are not known definitively (e.g., free fatty acids, insulin, IGF-1, glucose, sex hormones, leptin, and/or others), their nature is likely multifactorial. In response to extremely strenuous exercise, stress-like neuroendocrine reactivity may predominate, whereas with appropriately modulated exercise intensity and volume, favorable clinical benefits, such as augmented GH secretion, cardiovascular conditioning, improved sense of well-being, and preserved reproductive function and bone density, likely ensue.

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5

Exercise and the GH–IGF-I Axis

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INTRODUCTION

Physical activity plays an important role in tissue growth and anabolism, but the mechanisms that link exercise patterns with tissue development are not completely understood (1). The exercise-related anabolic effects are age and maturity dependent. Spontaneous levels of physical activity are considerably higher during childhood, and concurrently a substantial increase in muscle mass and strength occurs during adolescence. The combination of rapid growth, high levels of physical activity, and the natural puberty-related increase in anabolic hormones (growth hormone (GH), insulin-like growth factor-I (IGF-I), and sex steroids) suggests the possibility of integrated mechanisms linking exercise with anabolic responses. In contrast, unsupervised participation of young athletes in intense competitive training, especially if associated with inadequate caloric intake, might lead to reduced growth potential (2).

Training efficiency depends on exercise intensity, volume, duration, and frequency, and on the athlete's ability to tolerate it. An imbalance between the training load and the individual's tolerance may lead to under or overtraining. As a consequence, many efforts are made to develop objective means to quantify the fine balance between training load and the athlete's tolerance. The endocrine system, by modulation of anabolic and catabolic processes, plays a major role in the physiological adaptation to exercise training (3). For example, changes in the testosterone/cortisol ratio, as an indicator of the anabolic–catabolic balance, have been used, with limited success, to determine the

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physiological strain of training (4). In recent years, changes in circulating components of the GH–IGF-I axis, a system of growth mediators that control somatic and tissue growth (5), have been used to quantify the effects of training (6). Interestingly, exercise is also associated with remarkable changes in inflammatory cytokines, and the exercise-related response of these markers can be also used to gauge exercise load (7–9). Therefore, it was suggested that evaluation of changes in these antagonistic circulating mediators may assist in quantifying the effects of different types of single and prolonged exercise training and recovery modalities.

In this chapter we will demonstrate the effects of exercise on components of the GH–IGF-I axis, with an emphasis on the unique relationships between the exercise-related anabolic response and exercise-associated change in inflammatory mediators. More importantly, the major goal of this chapter is to share our experience and introduce to elite competitive athletes, coaches, and their medical staff how exercise-induced changes in components of the GH–IGF-I axis and inflammatory mediators may be used to evaluate the training load during different training stages throughout the competitive season and to assist in the preparation for competition in “a real life” setting.

THE GH–IGF-I AXIS

The GH–IGF-I axis is composed of hormones, growth factors, binding proteins (BP), and receptors that regulate essential life processes such as growth and development, metabolic and reparative processes, and aging. Therefore, the understanding of the axis must consider each individual component and the interaction between them, under both physiologic and pathological conditions. The axis starts at the level of the central nervous system where several neurotransmitters (catecholamines, serotonin, cholinergic agents, etc.) stimulate the hypothalamus to synthesize growth hormone-releasing hormone (GHRH) and somatostatin (SMS). GHRH stimulates the anterior pituitary to synthesize and secrete GH, while SMS inhibits GH secretion.

Growth hormone is the major product of the axis. One of the GH most important functions is the stimulation of hepatic IGF-I synthesis. However, some effects of GH on metabolism, body composition, and tissue differentiation are IGF-I independent. GH exerts direct feedback mechanism on the two hypothalamic hormones that control its secretion. Tissue GH bioactivity result from interaction between GH and the GH receptor. The GH receptor is composed of intracellular and extracellular transmembrane domains. The extracellular domain is identical in structure to GH binding protein (GHBP) (10); therefore, a unique feature of this axis is that GH receptor number and activity can be determined easily by measurements of circulating GHBP levels.

IGF-I is one of the insulin-related peptides. IGF-I can act as a hormone, and these effects are GH dependent, but the majority of its actions occur due to paracrine or autocrine secretion and regulation, which are only partially GH dependent. IGF-I is responsible for most, but not all, anabolic and growth-related effects of GH. IGF-I stimulates SMS secretion, and inhibits GH by a negative feedback mechanism (11). It is not clear whether this feedback mechanism is caused by circulating or local central IGF-I.

The bulk of circulating IGF-I is bound to several IGF-BPs. The most important circulating BP is IGF-BP-3, which is synthesized mainly in the liver. When bound to IGF-I, the IGF-BP complexes with an acid-labile subunit to form a circulating ternary complex

that carries most of the IGF-I in the serum. Some IGFBPs are GH dependent (e.g., IGFBP-3), but others, like IGFBP-1 and IGFBP-2, are insulin dependent (being high when insulin level is low). The interaction between IGF-I and its BPs is even more complicated since some BPs stimulate (e.g., IGFBP-5), while others inhibit (e.g., IGFBP-4) IGF-I anabolic effects (12).

The effects of IGF-I result from its interaction with two different receptors. Type I receptor has tyrosine–kinase activity, and mediates most of IGF-I effects. This receptor exhibits similarities to the insulin receptor and, therefore, may bind also insulin which has known anabolic effects as well. The type II receptor is identical to the mannose-6-phosphate receptor, and binds IGF-II as well.

Some hormones in the GH–IGF-I axis (i.e., GHRH, SMS, and GH) have a pulsatile secretion pattern, and it has been shown that the pulsatility of GH secretion is significantly important for accelerated growth rate (13). In contrast, IGF-I and IGFBPs level are relatively stable throughout the day.

In addition to the important effect on growth, GH and IGF-I have a marked effect on body composition. Both hormones stimulate increases in muscle mass and bone mineral density, and reduce fat distribution. Several components of the axis are age dependent. Both GHRH, GH, GHBP, IGF-I, and IGFBP-3 reach their peak circulating levels during puberty (14), and decrease with aging (15). These changes are partially sex hormone mediated. Nutritional state has also a remarkable influence on the GH–IGF-I axis. For example, fasting and malnutrition increase GH secretion, but despite elevated GH, IGF-I levels are reduced due to lower level of GH receptors (16). All these factors must be taken in account when studying the effect of exercise on the GH–IGF-I axis and its components.

ASSESSMENT OF THE TRAINING LOAD

One of the unique features of exercise is that it leads to a simultaneous increase of antagonistic mediators. On the one hand, exercise stimulates anabolic components of the GH–IGF-I axis. For example, exercise-induced increase of IGF-I, one of the most important anabolic factors, occurred following very short supramaximal exercise (i.e., 90 s) (17), 10 min following the onset of endurance exercise (18, 19), and in exercise below and above the lactic/anaerobic threshold (LAT) (18). Exercise also lead to an increase in urinary IGF-I (20). It is believed that the increase in these anabolic factors plays a key role in exercise training-induced muscle hypertrophy.

On the other hand, exercise elevates catabolic pro-inflammatory cytokines such as Interleukin (IL)-6, IL-1, and tumor necrosis factor- α (TNF- α) (7, 8). IL-6, but not other inflammatory mediators, increases even following relatively moderate intensity exercise (21) suggesting that it is the most exercise-sensitive inflammatory cytokine. The major source for the exercise-related IL-6 increase is believed to be the skeletal muscle (22). Yet, IL-6 increases during exercise both with and without evidence of muscle damage. It is believed that IL-6 plays an important mediatory role in the inflammatory response needed for exercise-associated subclinical muscular and/or soft tissue damage repair that may occur even following moderate intensity training (23, 24). To make it even more complex, exercise may lead to a simultaneous increase of catabolic hormones like cortisol as well, and also to an increase in the anti-inflammatory cytokine IL-1ra.

It is now clear that the exercise-associated increase in IGF-I is not related only to the classic mechanism of increased hepatic IGF release due to exercise-induced secretion of GH, and that GH-independent, more rapid changes in IGF-I distribution occur (25). It was believed that the source for the increase in circulating growth factors and inflammatory cytokines is release from the exercising muscle. To test this, we used a trial of unilateral repeated wrist flexion against relatively high resistance, with simultaneous blood samples collection from the basilic vein of the exercising (representing local release) and resting arm (representing systemic response). Results showed a bilateral, simultaneous increase in both the growth factors and the inflammatory cytokines, suggesting that the local exercising muscle was not the only source for their increase (26, 27). The rapid exercise-induced increase in these cytokines must reflect circulatory distribution changes due to release from more available marginal pools.

The concomitant antagonistic exercise response emphasizes probably the importance of optimal adaptation to the stimulation of exercise. The very fine balance between the anabolic and inflammatory/catabolic response to exercise will determine the effectiveness of exercise training and the health consequences of exercise (see Fig. 1). If the anabolic response is stronger than the catabolic response, exercise will probably lead ultimately to increased muscle mass and improved fitness. A dominant catabolic response, especially if it persists for long duration, may lead to overtraining. Therefore, changes in the anabolic–catabolic hormonal balance and in circulating inflammatory cytokines can be used by athletes and/or their coaches to gauge the training intensity in individual and team sports. The response of these mediators to different types of sports, training sessions, or training protocols can be used as an objective tool to monitor the training load and to better plan training cycles throughout the competitive season (see Fig. 2).

OPTIMIZING DIFFERENT TYPES OF TRAINING MODALITIES

Aerobic Training

The vast majority of the current knowledge regarding the importance of the GH response to exercise is based on studies of the effect of aerobic-type exercise (28, 29). It was reported that when exercise is performed at the same absolute intensity, the GH response was greater in *less fit* subjects (30). However, when subjects with various fitness levels perform exercise at the same *absolute*, rather than *relative*, intensity, some individuals exercise below, while others exercise above, their LAT. This is important since circulating GH levels increase only in response to aerobic exercise intensity above the LAT, and because exercise loads of 75–90% of the maximal aerobic power yielded greater GH increase than milder loads. Therefore, results of studies in which the GH response to exercise was tested at an *absolute* work rate demonstrate simply that as individuals become fitter, the stress associated with exercise at an absolute work rate is reduced. The obvious implication for athletes is that as they become fitter, a more intense exercise should be performed to stimulate GH secretion. This is consistent with the common coaching modality of training cycles with increased intensity throughout the training season.

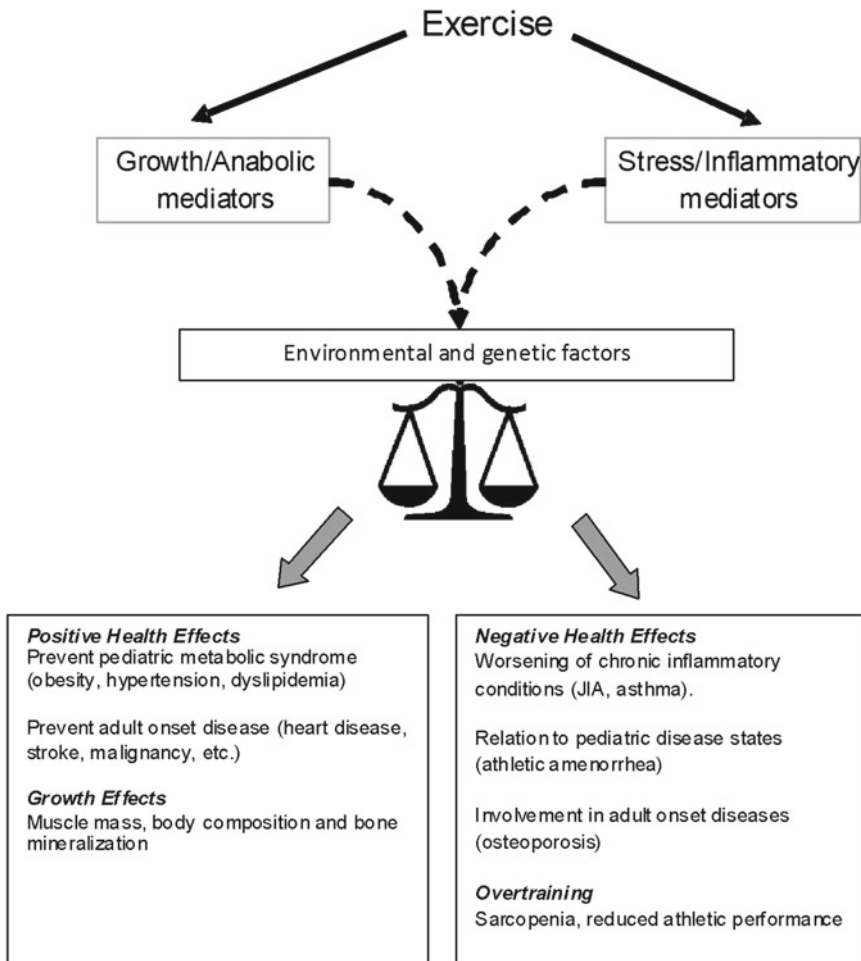


Fig. 1. The balance between exercise-associated increase in growth/anabolic factors and inflammatory/stress mediators.

The duration of aerobic exercise for the stimulation of GH secretion should be at least 10 min (31). The exercise-induced GH peak occurs 25–30 min after the start of exercise (slightly earlier in females compared to males), irrespective to its duration (6, 18, 32). Thus, when the exercise task is brief (e.g., 10 min), GH peak is reached after the cessation of exercise, while when exercise is long (e.g., 60 min), GH peak is reached while the individual is still exercising. The important possible implication for athletes is that brief training sessions can be good enough to stimulate the GH-IGF-I axis and to achieve “training effect.”

Pituitary refractoriness, a time in which the normal pituitary gland will not respond sufficiently to any stimulus for GH release, could also influence the GH response to exercise. The GH response to exercise was inhibited if a spontaneous, early morning, GH pulse had occurred within 1 h prior to the exercise test (32). A refractory period of at least 1 h was also shown following exercise-induced GH secretion (i.e., the subsequent GH response to exercise was attenuated) (33). GH auto-inhibition, exercise-induced

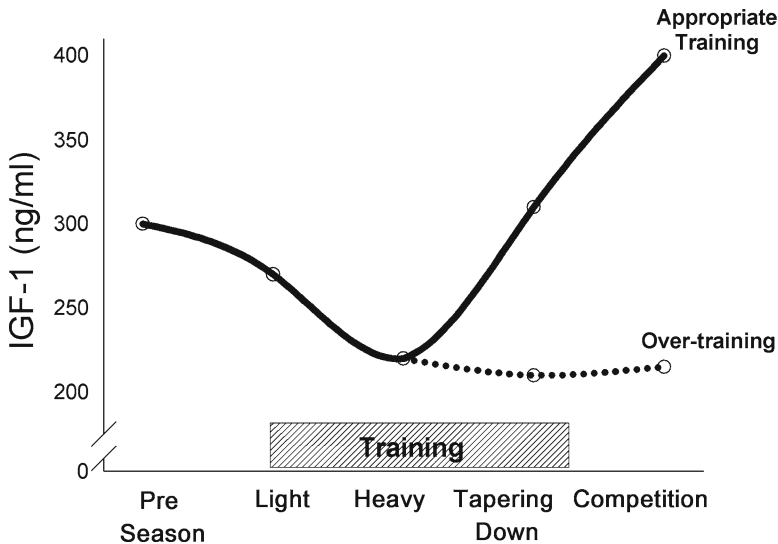


Fig. 2. Training season effects on IGF-I circulating levels. IGF-I level decreased significantly during heavy training, and with proper training increased above baseline levels before the target competition. With overtraining, IGF-I levels remain low.

elevation in free fatty acids, or alterations in parasympathetic–sympathetic tone can explain the pituitary refractoriness. Ronsen et al. showed a recovery from pituitary refractoriness to GH secretion if a second bout of high-intensity endurance exercise was performed 3 h after the first session (34). Consistent with this report, integrated 1.5 h GH concentrations were significantly greater if differences between the exercise bouts (30 min, 70% VO_2max) were 3.5 h and not 1 h apart (35). These studies suggest that a practical application for young athletes should be that in order to achieve optimal GH secretion, the rest interval between multiple daily training sessions should be long enough (probably more than 3 h) to allow pituitary recovery.

Anaerobic Exercise

A major progress was achieved in recent years in the understanding of the anaerobic exercise effects on the GH–IGF-I axis. Stokes et al. (36) studied the effect of a single supramaximal 30-s sprint on a cycle ergometer against different levels of resistance. They found that the increase in GH levels was significantly greater when resistance was 7% (faster cycling) and not 9% (slower cycling) of body mass. Consistent with that, it was shown that if heavier loads were lifted, more total work was performed, and higher IGF-I levels were found using faster compared to slower tempo resistance training (37). The possible implication for athletes is that lower levels of resistance and faster anaerobic efforts may better stimulate the GH–IGF-I axis, and thus are preferred by coaches and athletes.

Interval training is one of the most frequent training methods used in anaerobic and aerobic-type sports (38). The intensity of such training depends on the running distance (sprint vs. long distance), running speed (percent of maximal speed), the number of repetitions, and the length of the rest interval between runs. In addition, coaches and

athletes change very often the style of the interval training and use constant running distances (e.g., 6×200 m), increasing distance interval session (e.g., 100–200–300–400 m), decreasing distance interval session (e.g., 400–300–200–100 m), or a combination of increasing–decreasing distance interval session (e.g., 100–200–300–200–100 m). While these style differences may seem negligible, they may involve different physiological demands, since in the increasing distance protocol, metabolic demands (e.g., lactate levels) increase gradually and are highest toward the end of the session, while in the decreasing distance protocol the metabolic demands are higher from the beginning of the session (39).

Recently, we demonstrated a significant increase in GH and IL-6 levels following a typical constant distance (4×250 m) interval training (9). Consistent with previous findings in aerobic exercise, changes in the GH-IGF-I axis following the brief sprint interval exercise suggested exercise-related anabolic adaptations. The increase in IL-6 probably indicates its important role in muscle tissue repair following anaerobic exercise. We suggested that changes in the anabolic/catabolic/inflammatory balance can be used as an objective tool to gauge the training intensity of different types of anaerobic exercises and training periods as well.

More recently, we evaluated the effect of increasing (100–200–300–400 m) and decreasing distance (400–300–200–100 m) sprint interval training protocols, two other common types of sprint interval training, on the balance between anabolic, catabolic, and inflammatory mediators (39). Both types of sprint interval trainings led to a significant increase in lactate and the anabolic factors GH and IGF-I. Both types of sprint interval sessions led to a significant increase in the circulating pro- and anti-inflammatory mediators IL-1, IL-6, and IL1ra. Interestingly, the lactate and GH area under the curve was significantly greater in the decreasing distance session. In contrast, rate of perceived exertion (RPE) was higher in the increasing distance session. Thus, despite similar running distance, running speed, and total resting period in the two interval training sessions, the decreasing distance interval was associated with a greater metabolic (lactate) and anabolic (GH) response (see Fig. 3). Interestingly, these greater metabolic and anabolic responses were not accompanied by an increase in RPE suggesting that physiological and psychological responses to interval training do not necessarily correlate. When the athletes were asked to explain why the increasing distance training protocol was perceived as more intense, they replied that the fact that the longest and hardest run (400 m) was only at the end of the session was very difficult to tolerate. Coaches and athletes should be aware of these differences and, as a consequence, of the need for specific recovery adaptations after different types of interval training sessions. Differences in physiological and psychological responses to competitive sport training, and their influence on the training course and recovery process, should also be addressed.

Finally, in contrast to the observation that both aerobic and anaerobic exercise require a high metabolic demand in order to stimulate GH secretion, we previously demonstrated a small but significant GH response to an exercise input that was perceived as difficult by the participants (i.e., 10 min of unilateral wrist flexion; a small and relatively unused muscle group), but had no effect on heart rate or circulating lactate levels (26, 27). This suggests that factors like the individual's perceived exertion and associated psychological stress play an important role in the activation of the hypothalamic–pituitary axis and to GH release even in exercise protocols involving small muscle groups.

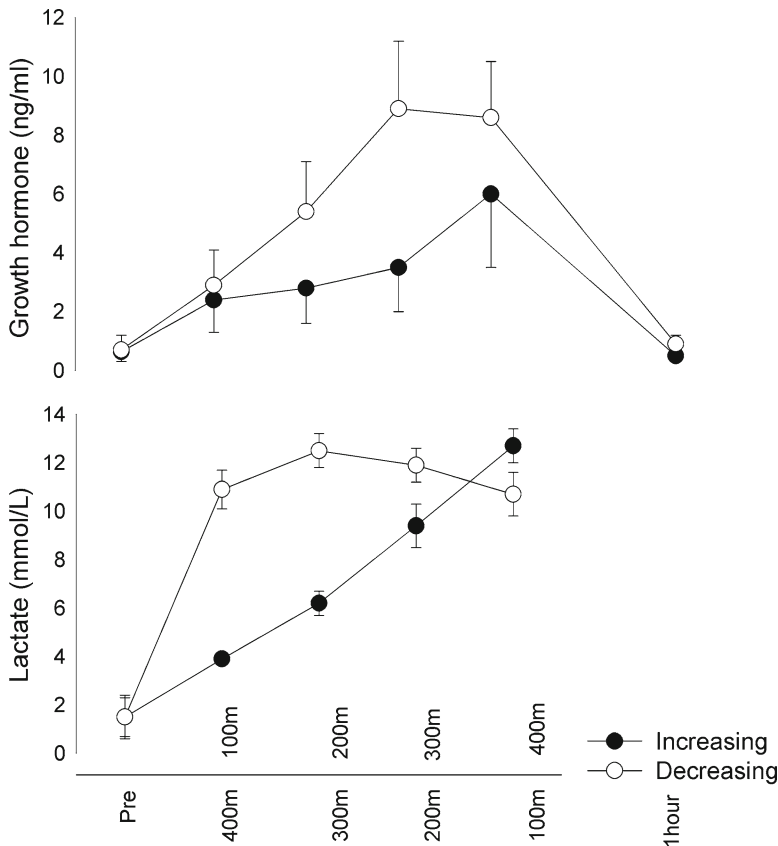


Fig. 3. The effect of increasing and decreasing distance sprint interval exercise on circulating lactate and growth hormone levels.

Recovery Modalities and the GH-IGF-I Axis

Athletic success depends primarily on the combination of genetic endowment, training, technique, equipment, and proper nutrition (40). Thus, the use of additional aids in the training process is thought to play a relatively minor role in athletic performance. However, in competitive sports, where one hundredth of a second, or a few millimeters could make a difference between “fame and shame” in the life of an athlete, the search for legal methods to improve training ability and performance becomes critical.

The development of methods to enhance the recovery of elite athletes from intense training and/or competition has been a major target of athletes and their accompanying staff for many years. Recently, it was shown that bicarbonate supplementation prior to high-intensity interval training attenuated the GH response (41). The authors suggested that acidosis increase the GH response to interval training, and that these findings might be relevant to selection of active or passive recovery. While active recovery between intervals improves lactate clearance, reduces acidosis, and allows longer training, its effect may attenuate the GH response to training. This information has to be accounted for by the athletes and coaches when planning interval training.

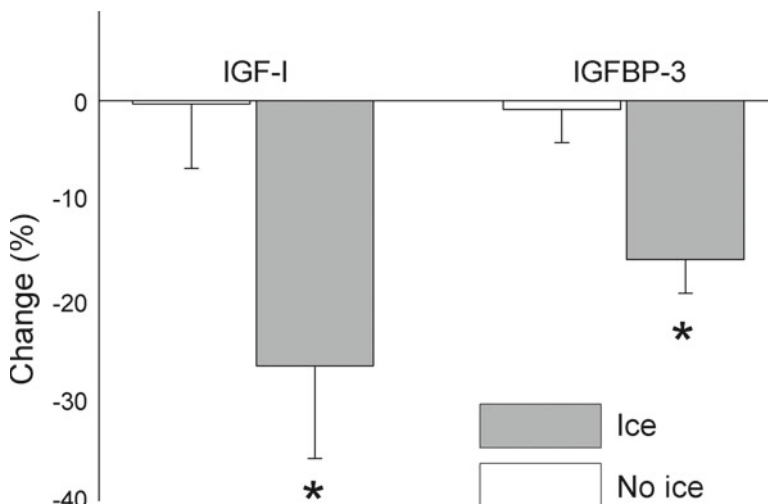


Fig. 4. The effect of local cold-pack application on recovery changes in IGF-I and IGFBP3 circulating levels from postexercise levels.

Cryotherapy is used widely to treat sports-associated traumatic injuries and as a recovery modality following training and competition that may cause some level of traumatic muscle injury (42, 43). However, evidence regarding the effectiveness and appropriate guidelines for the use of cryotherapy are limited. Recently, we evaluated the effect of cold ice pack application following a brief sprint interval training on the balance between anabolic, catabolic, and circulating pro- and anti-inflammatory cytokines in twelve male, elite junior handball players (44). The interval practice (4 × 250 m) was associated with a significant increase in GH and IL-6 levels. Local cold-pack application was associated with significant decreases in the anabolic hormones IGF-I and IGF binding protein-3 during the recovery from exercise (see Fig. 4), supporting some clinical evidence for possible negative effects on athletic performance. These results, along with the previously reported evidence for some negative effects of ice application on athletic performance, and no clear effect on muscle damage or DOMS, may suggest that the use of cold packs should probably be reserved for traumatic injuries or used in combination with active recovery and not with complete rest. However, this is an example of how exercise-induced changes in the GH–IGF-I axis and inflammatory markers may be used to fit in the complete puzzle of optimizing competitive training. Further studies are needed to explore the beneficial use of anabolic, catabolic, and inflammatory markers measurement in many other aspects of the recovery from exercise.

Nutrition, Performance, and the GH–IGF-I Axis

Nutritional factors may interfere with the GH response to exercise. For example, intravenous administration of the amino acid arginine is a strong stimulator of GH release and therefore used, for example, as one of the more common provocation tests for GH secretion in the diagnosis of short stature. Recently, it was demonstrated that oral arginine stimulates GH secretion as well (45). Therefore, the ingestion of arginine

prior to exercise may attenuate the GH response, most probably due to induction of a refractory period (46).

Ingestion of a lipid-rich meal 45–60 min prior to an intermittent 30 min cycle-ergometer exercise resulted in a significant, more than 40% reduction in the exercise-induced GH elevation in healthy children (47). The effect of prior high-fat meal ingestion appeared to be GH selective, as other counter-regulatory hormone responses to exercise, such as glucagon, cortisol, and epinephrine, were not affected. Similarly, administration of high-fat meal attenuated the magnitude of GH response to exercise also in adults, and this inhibition was correlated with circulating levels of somatostatin (48). Interestingly, high-carbohydrate meal with a similar caloric content was also associated with a small decrease in GH response to exercise; however, this decrease was not statistically significant. These studies indicate that food consumption prior to practice should be carefully selected, since a consumption of high-fat meal may limit the beneficial effect of training.

Very few studies examined the effect of nutrition on longer periods of training. Several studies suggested that the timing of nutritional supplementation may affect the training-associated response of the GH–IGF-I axis. Only a combination of postexercise essential amino acid and carbohydrate supplementation (compared to carbohydrate only or placebo) during 3 weeks of high-intensity interval training was accompanied by significant increases in free IGF-I (49). Protein supplementation 1 h before and after practice during 10 weeks of resistance training (4 times/week) was more effective than carbohydrate placebo in increasing muscle mass and muscle strength and was associated with greater increases in IGF-I and IGF-I mRNA in untrained males (50). Consistent with these findings, twice daily protein compared to carbohydrate supplementation during 6 months of strength and conditioning training (5 times/week) was associated with greater increase in IGF-I levels in untrained late pubertal and young adult males and females (51). These results suggest beneficial effect for protein supplementation during prolonged period of resistance training.

Exercise-associated GH release is attenuated in amenorrheic athletes, due to decreased GHRH response to exercise compared to eumenorrheic athletes (52–55). This is particularly relevant to the adolescent female athlete, since the prevalence of amenorrhea among these athletes is 4–20 times higher than the general population. “Athletic amenorrhea” appears mainly in younger athletes, in sports types that leanness provide a competitive advantage (e.g., esthetic-type sports, long distance running) and, in particular, when intense training is accompanied by inadequate nutrition (56). The reduced exercise-induced GH response in these athletes should be considered since it indicates probably reduced training effectiveness.

PREPARATION FOR COMPETITION

Measurements of IGF-I levels can also assist the athlete and coach in the preparation for competition. The effect of 4 weeks of training on fitness, self-assessment physical conditioning scores, and circulating IGF-I was previously determined (57) in elite professional handball players during their preparation for the junior world championships. Training consisted of 2 weeks of intense training followed by 2 weeks of relative tapering. Circulating IGF-I and physical conditioning scores decreased initially, and returned

to baseline levels at the end of training. There was a significant positive correlation between the changes in circulating IGF-I and the physical conditioning scores, suggesting that the athlete's self-assessment might serve as a reliable tool when laboratory assistance is unavailable. Consistent with these findings, a follow-up of IGF-I levels during the training season in elite adolescent wrestlers showed also a decrease in IGF-I level during periods of heavy training, and return to baseline during tapering down and prior to the competition season (58). Interestingly, changes in the pro- and anti-inflammatory mediators IL-6 and IL1ra correlated negatively with changes in IGF-I, being high when IGF-I level were low and normalized when IGF-I levels normalized, emphasizing their potential contributing role for the training-associated change in IGF-I (see Fig. 2). Similarly, levels of IGF-I and the ratio of IGF-I/BP-3 as an index of the bioactive-free IGF-I increased significantly throughout the competitive season in late pubertal triathletes (59).

Tapering down the training intensity prior to the competition is a well-known training methodology to help the athlete to achieve his best performance (60). This strategy is indeed associated with a parallel increase in circulating IGF-I levels. Therefore, these measures may assist coaches and athletes in their training preparations. Interestingly, in type of sports that do not plan their training for a specific target, and train in the relative same intensity throughout the season (e.g., soccer), changes in IGF-I level and its major binding protein IGFBP-3 were not found (61).

In optimal conditions, during the tapering of training intensity, IGF-I level will increase above baseline levels and will be associated with improved performance; however, this does not occur always. Since IGF-I can be reduced by weight loss, it is possible that a deliberate decrease in body weight in athletes who participate in weight category sports (e.g., judokas, wrestlers) prior to major championships may prevent further increase in this anabolic hormone (62), and will be associated “only” with a significant return to baseline values (58, 63). Moreover, previous studies demonstrated training-associated negative correlation between circulating IGF-I and ghrelin, a hormone that is secreted by the stomach and pancreas and known to stimulate hunger, in athletes (64). These hormonal relationships can play a role in the training-induced changes in energy balance and body composition, particularly in weight category sports.

As noted earlier, despite the decrease in *circulating* IGF-I (65–68) during periods of intense training, fitness may still improve. This suggests that while changes in *circulating* IGF-I are good markers of the general condition and energy balance of the athlete, they are not necessarily good predictors of the athlete's performance. Probably, it is the local muscle levels of these hormones, and their autocrine or paracrine secretion, that is more indicative of skeletal muscle performance (69, 70). Tapering of the training intensity, however, was found to be associated with both increased IGF-I level and with improvement of exercise performance of the athletes (60, 71).

It is still unknown what should be the permitted decrease of IGF-I levels during periods of heavy training, or what should be the optimal increase of this substance during periods of tapering down and reduced training intensity. However, we believe that an inability to increase circulating IGF-I levels before the target competition should be an alarming sign for both the athlete and his/her coach that the athlete's general condition is not optimal. Collection of baseline and training-related hormonal changes, with a comparison to the hormonal response in previous seasons, and the knowledge and experience of the past success may prove to be of a very significant relevance as well.

In summary, research in recent years has made substantial progress in the field of exercise endocrinology. In particular, it is now clear that changes in the balance of anabolic and catabolic hormones and inflammatory mediators following different types of single exercise and prolonged training and during different stages of the training season may help elite athletes and their coaches in planning and following the “optimal” training program and in preparation for competition. New methodologies, such as transdermal fluid sampling, may provide a more accurate, noninvasive means of monitoring acute exercise-induced changes in the GH-IGF-I axis when sampled in proximity to exercising muscle (72), and may be advantageous in particular for the child and adolescent athletes. Further studies are needed, however, to clarify the complex relationship of hormonal response, nutritional supplementation, different types and phases of training, and optimal athletic performance in competitive sports.

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6

Exercise and Thyroid Function

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INTRODUCTION

Thyroid hormone receptors are present in virtually every tissue in the body, thereby permitting an important physiologic role for the two thyroid hormones, thyroxine (T4) and triiodothyronine (T3). Skeletal and cardiac muscle function, pulmonary performance, metabolism, and the neurophysiologic axis are only a few of the important areas that are affected by thyroid hormone level (1). Any abnormality in thyroid function causing either an excess or deficiency in circulating thyroid hormone levels can lead to changes in body function at rest and during exercise. The presence of thyroid disease can have a major impact on exercise tolerance resulting in reduced performance of strenuous activities. On the other hand, exercise itself may have direct or indirect effects on thyroid function, either secondary to acute alterations in the integrity of the pituitary thyroid axis or to the more long-lasting changes noted in well-trained athletes to be discussed below. Alterations in thyroid function in well-trained athletes might be viewed as an adaptive mechanism associated with enhanced performance possibly serving to provide a better balance between energy consumption and expenditure. Underlying energy balance does

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appear to play an important role in the effects exercise may have on the hypothalamus-pituitary-thyroid axis. Reports in the literature indicate that athletes with excessive weight loss may exhibit a “low T3 syndrome” accompanied by amenorrhea (in women) as well as other alterations in pituitary function (2). Fortunately, thyroid diseases usually can be treated effectively, and most individuals with thyroid disorders should expect to obtain resolution of their thyroid-related symptoms, including those associated with a negative impact on their exercise tolerance. Gail Devers, who has been very public about her experience with Graves’ disease, is a well-known sprinter who went on to win Olympic fame following treatment for her Graves’ disease and may act as a case in point. After a brief overview of normal thyroid physiology, this chapter will provide a survey of the literature describing effects of abnormal thyroid hormone levels on exercise tolerance, with a special focus on alterations in cardiac, muscle, and respiratory function. The chapter will conclude with a review of existing data on the response of the pituitary–thyroid axis to varying levels and types of exercise.

Thyroid Physiology

All steps in thyroid hormone (TH) biosynthesis are driven by thyrotropin (TSH) and are intimately linked to iodine metabolism. Dietary iodine is reduced to iodide, absorbed by the small intestine, and then enters the circulation. Tissues that extract iodide from plasma include the thyroid, kidneys, salivary glands, choroid plexus, gastric glands, and lactating breast tissue. However, iodide clearance is predominately a function of either the thyroid gland or the kidneys. Iodide “trapped” by the thyroid gland subsequently undergoes oxidation by thyroid peroxidase (TPO), iodinating tyrosyl residues in the storage protein, thyroglobulin, to form the iodothyronines, monoiodotyrosine (MIT), and diiodotyrosine (DIT). MIT and DIT molecules can then couple to form either tetraiodothyronine (thyroxine or T4) and/or triiodothyronine (T3), which are the two major thyroid hormones (3). T4 and T3 are bound within peptide linkage within thyroglobulin and stored within the thyroid follicles. Under control of TSH, thyroglobulin undergoes endocytosis and proteolytic digestion, releasing T4 and T3 into the circulation. The feedback loop is completed at the hypothalamic level where declining levels of circulating T4 or T3 will prompt secretion of thyrotropin-releasing hormone (TRH), which stimulates synthesis and secretion of TSH. After binding to its specific receptor on the thyroid cell membrane, TSH leads to stimulation of T4 and T3 production and secretion mediated by cyclic AMP. Only 20% of circulating T3 is derived from thyroid secretion, whereas 80% is derived from the monodeiodination of T4 by 5-deiodinase (type I) in the periphery (see Fig. 1) (4). Since T3 is some 10–15 times more biologically potent than T4 is, this latter conversion has been termed the “activating” pathway of thyroid hormone metabolism. Alternatively and in certain physiologic and pathologic states, the deiodination of T4 proceeds via a 5-deiodinase (type II), which leads instead to reverse T3 (rT3) or 3,3,5-triiodothyronine production. Since rT3 is a biologically inactive thyroid compound (4), this route of metabolism has been termed the “inactivating” pathway. A precise metabolic role for rT3 has not been described, but diversion of T4 metabolism from the activating to the inactivating pathway serves a nitrogen-sparing and protective effect for the body during times of stress and has been viewed as a homeostatic mechanism. After binding to a cellular receptor, the thyroid hormones have both genomic (5) and nongenomic (6) effects, the former leading to increased

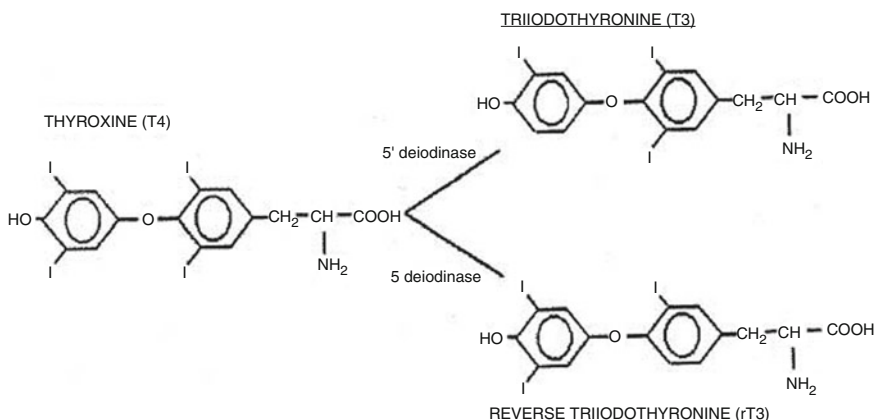


Fig. 1. Thyroxine, triiodothyronine, and reverse triiodothyronine.

Table 1
Genomic and nongenomic actions of thyroid hormones

<i>Genomic actions of thyroid hormones</i>	
<i>Positive regulation</i>	<i>Negative regulation</i>
Sarcoplasmic reticulum calcium adenosine triphosphatase	T3 nuclear receptor α 1
Myosin heavy chain α	Myosin heavy chain β
β 1-adrenergic receptors	Phospholamban
Sodium/potassium adenosine triphosphatase	Sodium/calcium exchanger
Voltage-gated potassium channels (Kv1.5, Kv4.2, Kv4.3)	Adenylyl cyclase types V,VI
Adenine nucleotide translocator 1	
<i>Nongenomic actions of thyroid hormones</i>	
Conductivity of sodium, potassium, and calcium channels	
Actin polymerization status	
Activation of PI3K/Akt/mTOR signaling pathway	
Deiodination and decarboxylation of T4 resulting in thyronamine synthesis	

expression of nuclear actions, whereas the latter appears to involve plasma membrane/mitochondrial responses (7) (Table 1).

The T3 nuclear receptor is encoded by c-erb-A genes, which have several T3 binding protein isoforms (8). Genomic effects, such as induction of gene expression, require some time for response. Nongenomic effects utilize messenger pathways, such as cyclic AMP, and hence have a more rapid response time (9).

THYROID HORMONE EFFECTS

Hyper- and hypothyroidism, associated with either excess or deficiency of TH, respectively, may have a negative impact on exercise performance. Although TH has pervasive effects on virtually all functions of the body, the following discussion emphasizes thyroid-related influences on exercise tolerance as mediated via involvement with cellular metabolism and the function of skeletal muscle and the cardiac, vascular, and pulmonary systems.

CARDIOVASCULAR EFFECTS OF THYROID HORMONES

Cardiac performance/output is dependent on the contractility of the heart as well as systemic vascular resistance. Resting tachycardia is very common in hyperthyroidism, and many patients complain of having a “racing” or “pounding” heart. The heart, being itself a muscle, is affected by thyroid hormone levels as is skeletal muscle. The heart relies mainly on serum T3 because there is no significant myocyte intracellular deiodinase activity (10, 11). TH can affect cardiac action via direct genomic and nongenomic effects on cardiac myocytes and hemodynamic alterations in the periphery that result in increased cardiac filling and modification of cardiac contraction (12). TH mediates the expression of both structural and regulatory genes in the cardiac myocyte (10, 13, 14). Thyroid hormone-responsive cardiac genes include sarcoplasmic reticulum Ca/ATPase and its inhibitor phospholamban, which are involved in regulation of calcium uptake by the sarcoplasmic reticulum during diastole (15), α - and β -myosin heavy chains, the ion channels coordinating the electrochemical responses of the myocardium: sodium/potassium ATPase (Na/K-ATPase), voltage-gated potassium channels (Kv1.5, Kv4.2, Kv4.3), and sodium/calcium exchanger (12, 16–20). TH increases the expression of β 1-adrenergic receptors and downregulates TR α 1 receptors (21, 22). In summary, the genomic action of TH on the heart involves genes which are largely responsible for enhanced contractile function and diastolic relaxation (23, 24). Thus, T3 markedly shortens diastolic relaxation, i.e., the hyperthyroid heart relaxes with a higher speed (lusitropic activity), whereas diastole is prolonged in hypothyroid states.

The nongenomic effects of TH on the cardiac myocyte and on the systemic vasculature tend to occur rapidly. Schmidt et al. documented that T3-enhanced myocardial contractility and reduced systemic vascular resistance in normal adult males occur within 3 min (25). These rapid T3-mediated effects include changes in membrane ion channels for sodium, potassium, and calcium; effects on actin polymerization; adenine nucleotide translocator 1 in the mitochondrial membrane; and a variety of intracellular signaling pathways in the heart and vascular smooth muscle cells (26–34). The actions on channels may determine set points of myocardial excitability and duration of the action potential and contribute to development of tachyarrhythmias (35, 36).

Additional mechanism of T3 actions that have been observed *in vitro* includes rapid activation of PI3K leading to Akt phosphorylation that in turn translocates to the nucleus and promotes mTOR phosphorylation (37, 38). As mTOR is important to regulate ribosomal biogenesis and protein translation, the signaling pathway described in these studies may underlie at least one of the nongenomic mechanisms by which T3 regulates cardiac growth and hypertrophy.

Moreover, it has been discovered that deiodination and decarboxylation of T4 could generate a biologically active metabolite, thyronamine, which is characterized by actions opposite to those of TH (39, 40). It has been demonstrated that thyronamine reduces cardiac output, heart rate, systolic pressure, and coronary flow in isolated heart within minutes (41). This action is initiated by a novel aminergic system coupled to the trace amine-associated receptor (TAAR) (39, 42, 43). Conceivably, a balance between T3 and thyronamine might be responsible for maintaining cardiac homeostasis. Changes in this equilibrium might contribute to the cardiovascular alterations that occur in patients with thyroid disease (44).

In Vivo Animal Studies on the Role of Abnormal Thyroid Function in the Regulation of Cardiac Response to Exercise

It has been believed that one of the main mechanisms of increased cardiac work during hyperthyroidism was the sensitization to catecholamines. However, Hoit et al. in a study on thyrotoxic baboons refuted a role of β 1- or β 2-adrenergic receptors in any cardiac response to hyperthyroidism (45). Interestingly, abnormal cardiac response to exercise has been described as being due to an inefficient use of chemical energy stored in ATP. In hyperthyroid hearts, a larger fraction of energy goes to heat production, whereas in euthyroid animals more is spent for useful contractile energy. Finally, TH modifies the secretory activity of the heart—T3 has been found to increase mRNA and protein levels of atrial natriuretic factor (46, 47).

Several studies have indicated overactivation of the renin–angiotensin–aldosterone (RAA) system in hyperthyroid animals, documenting increased plasma renin (48–50) and upregulated synthesis and secretion of angiotensinogen (51) in hyperthyroid rats. In contrast, the plasma renin activity is reduced in experimental hypothyroidism (49). There is also an evidence of tissue-specific regulation of RAA. TH activates some components of cardiac RAA, and hyperthyroidism can promote an increase in cardiac levels of renin, stimulates Ang II generation (52), and raises the levels of AT1 and AT2 receptors (49). In the heart, Ang II exhibits growth-promoting effects by inducing hypertrophy and fibrosis, mediated by the AT1 receptor (53). Although most of the effects of Ang II related to cardiac remodeling have been attributed to the AT1 receptor, the AT2 receptor is also involved in the development of some cardiac hypertrophy models (54–56). There are several literature reports showing that AT1 receptor blockade and ACE inhibition attenuate or prevent the development of cardiac hypertrophy induced by TH in vivo (50, 57–59). Some authors suggest that the mechanism of action of these compounds is associated with the alterations in calcium handling (60) while the others suggest that these drugs may inhibit AT1 receptor induced activation of PI3K/Akt/mTOR pathway (37, 61).

Clinical Findings

In thyroid disease cardiac structures and function may remain normal at rest; however, impaired LV function and cardiovascular adaptation to effort become unmasked during exercise (62).

HYPOTHYROIDISM

Hypothyroidism has been associated with a decrease in intravascular volume, stroke volume, and cardiac index, and an increase in systemic vascular resistance, resulting in diastolic hypertension (Table 2) (63). In patients with transient hypothyroidism owing to thyroidectomy, radionuclide ventriculography and right heart catheterization revealed lower cardiac output, stroke volume, and end-diastolic volume at rest, but increased systemic peripheral resistance (64). Pulmonary capillary wedge pressure, right atrial pressure, heart rate, and left ventricular ejection fraction (LVEF) were unchanged. In comparison to the euthyroid state in the same individuals, heart rate, cardiac output, end-diastolic volume, and stroke volume were lower during exercise with hypothyroidism. LVEF at rest and with exercise appears to be altered with TH deficiency. The baseline

Table 2
Cardiovascular changes observed in hyper- and hypothyroidism

	<i>Hyperthyroidism</i>	<i>Hypothyroidism</i>
Heart rate	↑ NC	↓ NC
Vascular volume	↑	↓
Stroke volume	↑	↓
Cardiac output	↑	↓
SVR	↓	↑ NC
LVEF		
Rest	↑ ↓ NC	↓ NC
Exercise	↓	↓
Diastolic blood pressure	↓	↑ NC
Systolic blood pressure	↑ NC	↓ NC
LV pre-ejection period	↓	↑
LV ejection time	↓	↑

↑ increased; ↓ decreased; NC no change

LVEF and peak LVEF were shown to be lower in hypothyroid subjects (average age 52 years) in comparison with results when the same individuals were euthyroid, although the rise of LVEF in the two states was similar (65). As assessed by radionuclide-gated pool ventriculography in a younger group (average age 24 years), there was no noticeable change in LVEF with hypothyroidism, although exercise tolerance did improve after levothyroxine (LT4) replacement (66). Even hypothyroidism of a brief duration of only 10 days was associated with an impaired LVEF response to exercise; LVEF response returned to normal with restoration of the euthyroid state (67). Of interest, the patients still achieved the same workload in either state.

Interesting observations have been found in patients with subclinical hypothyroidism. Subclinical hypothyroidism is a term that has been applied to patients with mild elevations of serum TSH (usually in the range of 5–15 mIU/L), but with normal levels of T4, fT4, T3, and fT3. It has been a matter of investigative interest whether the mild hypofunction associated with subclinical hypothyroidism affected any measurable cardiac parameters (68). Patients with TSH levels of 14.8 ± 9.4 mIU/L were studied during rest and exercise, pre- and post-LT4 therapy. Overall cardiac structure and function were not significantly altered, except for left ventricle diastolic dimension, which was slightly larger with the euthyroid state, and shortening of the pre-ejection period from 51 to 39 s. However, this study was somewhat limited by a small sample size. Resting LVEF may be lower in hypothyroid subjects than in controls (69), although in one study, treatment of individuals with subclinical hypothyroidism revealed a similar resting LVEF in comparison to untreated subjects. A small increase in LVEF was seen with submaximal exercise with LT4 therapy, but no difference was noted with moderate exercise (70).

An accurate assessment of left ventricle function at rest has been performed by means of Doppler echocardiography in 26 young and middle-aged patients with stable subclinical hypothyroidism caused by Hashimoto thyroiditis. Compared to 30 normal control subjects, patients with subclinical hypothyroidism showed no changes in left

ventricle morphology. Patients with subclinical hypothyroidism showed prolonged isovolumic relaxation time, and a reduced early-to-late ratio of Doppler derived transmitral peak flow velocities, a finding suggestive of impaired diastolic function in the sense of slowed relaxation (71). In the same study, 10 randomly selected patients were reevaluated after achieving euthyroidism by means of 6 months of LT4 administration at a mean replacement dose of 68 µg/day. The treatment caused no change in the parameters of left ventricle morphology, whereas it normalized systolic and diastolic function. Interestingly, although systemic vascular resistance was comparable in untreated patients and control subjects, it was significantly decreased after LT4 therapy. Similar findings have been documented by Kahaly (72), who assessed cardiac function on effort and physical exercise capacity by means of stress two-dimensional and Doppler echocardiography and cardiopulmonary exercise testing in 20 patients with subclinical hypothyroidism before and after restoration of euthyroidism. Compared to 20 age- and gender-matched healthy controls, patients with subclinical hypothyroidism showed no abnormalities in various cardiac parameters at rest, either before or after LT4 treatment. However, stroke volume, cardiac index, and peak aortic flow velocity were significantly lower, and the pre-ejection period was significantly prolonged during exercise in the untreated patients versus controls.

Further evidence supporting reversible left ventricle diastolic dysfunction in patients with subclinical hypothyroidism was documented employing radionuclide ventriculography (73). The authors found that the time to peak-filling rate was prolonged in ten patients with subclinical hypothyroidism compared to 10 normal control subjects. This accurate index of diastolic function normalized after achieving euthyroidism with LT4 therapy.

Abnormal diastolic function may impair coronary flow reserve. Hypothyroid individuals may have a form of reversible coronary dysfunction as found in a study of six patients undergoing stress testing before and after LT4 replacement therapy. Prior to replacement therapy, SPECT (thallium-201) scanning revealed notable regional perfusion defects in four of six patients, which resolved within 8 weeks of LT4 therapy (74). Similarly, Oflaz et al. (75) found that coronary flow reserve was lower in patients with subclinical hypothyroidism (TSH: 7.1 ± 2.1 mIU/L) than in euthyroid subjects. On the contrary, Owen et al. (76) using stress echocardiography with i.v. dobutamine found no differences in resting global, regional left ventricular function or regional myocardial velocities during maximal dobutamine stress between patients and controls, or in patients treated with replacement therapy compared with baseline values. To summarize, the vast majority of clinical studies show impaired left ventricular diastolic function during exercise in patients with overt and subclinical hypothyroidism (71, 73, 77–79).

HYPERTHYROIDISM

The effects of hyperthyroidism on cardiac function both during rest and exercise are numerous (*see* Table 2) (63). In thyrotoxicosis, the extent of the various cardiac responses to excess TH is somewhat dependent on the duration and severity of the disorder. Resting tachycardia, a slow decline in postexercise heart rate, atrial fibrillation, decreased exercise tolerance, and rarely, congestive heart failure (CHF) are seen in thyrotoxic patients. Cardiac complications from hyperthyroidism tend to occur in patients with a history of prior ischemia, hypertension, and valvular heart

disease (80). Augmented blood volume and blood flow to the skin, muscles, and kidneys are seen and may be owing to vasodilators released secondary to increased cellular respiration (81). A rise in cellular oxygen consumption leads to a higher demand for oxygen and the need to get fuel to the peripheral tissues (81). An increase in the velocity of cardiac muscle contraction is present, as well as a rise in myosin ATPase activity (82). Evaluation of systolic time intervals in thyrotoxic subjects reveals a shortening of the LV pre-ejection period along with quicker LV ejection time and isovolumetric contraction (83, 84).

Kahaly et al. analyzed alterations of cardiovascular function and work capacity using stress echocardiography as well as spiroergometry and spirometry in subjects with untreated thyroid dysfunction, then again after restoration of euthyroidism, and as compared to age- and gender-matched healthy controls. At rest, LVEF, stroke volume, and cardiac indices were significantly increased in hyperthyroidism, but exhibited a blunted response to exercise, which normalized after restoration of euthyroidism. During exercise, negative correlations were found between free T3 (fT3) and diastolic blood pressure, maximal workload, heart rate, and LVEF. This impaired cardiac response to exercise was specifically apparent in older subjects (85–87).

“Subclinical” hyperthyroidism is a term that has been applied to patients with undetectable levels of serum TSH in a highly sensitive assay, but with normal levels of T4, fT4, T3, and fT3. In one study, there was no difference in LVEF at rest and exercise between subclinical hyperthyroidism and controls, whereas overt hyperthyroid subjects had a reduction in LVEF with exercise, increased heart rate, and cardiac output at both rest and exercise (88). An exogenously administered excess of LT4 can lead to growth in left ventricle mass that usually reverts with resolution of the hyperthyroidism (89).

To summarize, LVEF may be greater at rest in hyperthyroidism, but the lack of an increase or even a drop in LVEF with exercise seems to be a reproducible finding (90).

EFFECTS ON SYSTEMIC VASCULAR RESISTANCE (SVR)

TH causes decreased resistance in peripheral arterioles through a direct effect on vascular smooth muscle and decreased mean arterial pressure, which, when sensed in the kidneys, activates the RAA system and increases renal sodium absorption. T3 also increases erythropoietin synthesis, which leads to an increase in red cell mass. The combination of both leads to an increased blood volume and preload. In hyperthyroidism, these effects increase cardiac output 50–300% while a 30–50% reduction is seen in hypothyroidism (10).

In the vascular smooth muscle cell, TH-mediated effects are the result of both genomic and nongenomic actions. Nongenomic actions target membrane ion channels and endothelial nitric oxide (NO) synthase, which serves to decrease SVR (91–96). Indeed, it was recently reported that the PI3K/Akt-signaling pathway plays a role in T3-induced NO production by vascular smooth muscle cells and by endothelial cells (27, 97, 98).

Furthermore, T3 has been shown to inhibit vascular remodeling via the inhibition of the cAMP response element binding protein, a nuclear transcription factor involved in the remodeling process (99).

Clinical Findings

HYPOTHYROIDISM

Vascular control mechanisms may be abnormal in hypothyroidism with blunted vasodilatation secondary to reduced endothelium-dependent vasodilatation (100, 101). In overt hypothyroidism, arterial compliance is reduced, which leads to increased SVR. Increased arterial stiffness with higher central augmentation pressure and lower pulse wave velocities was shown in hypothyroid patients. These abnormalities were reversible with adequate LT4 treatment (102–104). However, in subclinical hypothyroidism, the study results have been equivocal. Several studies have not found any association between subclinical hypothyroidism and blood pressure at rest (105–107). In one cross-sectional study (108) including 806 subjects with subclinical hypothyroidism and 5,669 euthyroid controls, subclinical hypothyroidism was not associated with increased resting blood pressure. Similar results were observed in the cross-sectional Busselton thyroid study (109) that included 105 subjects with subclinical hypothyroidism and 1,859 euthyroid controls from Western Australia. On the other hand, two large population-based studies with 5,872 (110) and 30,728 (111) subjects reported a modest association between high-normal serum TSH levels and resting blood pressure. This observation has been confirmed in other studies, suggesting that mild thyroid hormone deficiency also may affect vascular tone (112–115). Several studies documented an improvement of SVR after LT4 replacement (71, 79, 116). Endothelial dysfunction in patients with hypothyroidism, borderline hypothyroidism, and those with high-normal TSH values using flow-mediated arterial dilation (FMD) has been demonstrated with TSH levels correlating inversely to endothelium-dependent dilatation (115). Multiple studies have found that elevated plasma total cholesterol, LDL cholesterol, triglycerides, lipoprotein(a), C-reactive protein (CRP) levels, and impaired thyroid function status are related to the attenuation of FMD (117, 118). Impaired endothelium-dependent vasodilatation as a result of a reduction in nitric oxide availability has been demonstrated in subclinical hypothyroidism by Taddei et al. in a study of 14 patients with subclinical hypothyroidism and 28 euthyroid controls (119).

Studies have also shown that FMD is associated with plasma osteoprotegerin levels in hypothyroid patients. Osteoprotegerin, a member of the tumor necrosis factor (TNF) receptor family involved in regulation of receptor activator for nuclear factor kappa B ligand (RANKL)-mediated osteoclastic bone resorption, has been found to play an important regulatory role in the vasculature (120). High osteoprotegerin values are associated with increased cardiovascular mortality (121, 122). Elevated plasma osteoprotegerin levels in hypothyroidism had been found to be associated with FMD and exercise-induced silent myocardial ischemia (123). The underlying mechanisms of elevated plasma osteoprotegerin are unclear. In vitro studies suggest that TH and TSH are involved in regulation of osteoprotegerin expression (124, 125).

HYPERTHYROIDISM

Endothelium-dependent arterial dilatation is increased in hyperthyroid patients and is reversible after subtotal thyroidectomy (126). Ojamaa et al. (127) demonstrated vascular relaxation due to the action of excess thyroid hormone on the vascular smooth muscle cells. Conceivably, an inability to lower SVR during exercise in the hyperthyroid

state might lead to impaired exercise tolerance (128). In this regard, phenylephrine administration was associated with an increase in SVR and a decrease in cardiac output not seen in euthyroid subjects (129). On the contrary, a case-control study of 42 patients with untreated overt hyperthyroidism documented similar systolic and diastolic blood pressures during maximal exercise as in 22 healthy controls. Moreover, no changes in systolic and diastolic blood pressure responses to exercise were observed in these patients after restoration of euthyroidism during 6-month follow-up (85). Similar findings hold true for the patients with subclinical hyperthyroidism. In a recent population-based prospective cohort study, Völzke et al. (130) found that subclinical hyperthyroidism is not associated with changes in blood pressure, pulse pressure, or incident hypertension. Some smaller studies have reported similar results (85, 131).

EFFECTS IN MUSCLES

TH plays a critical role in maintaining homeostasis and influencing the rate of metabolism and energy expenditure. Skeletal muscles contribute to about 20–30% of resting metabolic rate (132). It has become increasingly apparent that TH exerts a profound effect on the plasticity of striated muscle given its strong influence on the expression of the major histocompatibility complex (MHC) gene family of motor proteins (133–135). TH controls the expression of myocyte-specific genes coding for myosin isoforms (62), the Na–K adenosine triphosphatase (ATPase) pumps, and the Ca–ATPase canals of the sarcoplasmic reticulum. This explains the increase of contractility and relaxation of skeletal muscles observed in hyperthyroidism, as opposed to hypothyroidism. Control of key enzymes of the main energetic pathways accounts for inhibition of oxidative metabolisms in hypothyroidism and excessive glycolysis recruitment in hyperthyroidism. In both cases muscle performance is reduced, with accumulation of lactic acid at exercise. This is because of defective pyruvate oxidation and proton expulsion in hypothyroidism and of acceleration of glycolysis in hyperthyroidism. Muscle glycolysis exceeds mitochondria oxidation of pyruvate enhancing the shunting of pyruvate to lactate via lactic dehydrogenate activity, thus leading to an increased lactic acid concentrations resulting in intracellular acidosis. An additional explanation of impaired exercise tolerance with dysthyroidism is the fact that TH increases fast myosin and fast-twitch fibers in skeletal muscle, which are less economic in oxygen utilization during contraction than slow-twitch muscle fibers. Reduced exercise efficiency may also be induced by excessive heat production in hyperthyroidism as well as being due to accelerated protein catabolism.

In Vivo Animal Studies on the Role of Abnormal Thyroid Function in the Regulation of Muscle Response to Exercise

Animal studies of hypothyroidism reveal that glycogen levels in muscle appear to be normal to increased at rest, whereas during exercise, muscle utilization of glycogen rises as may lactate production (136, 137). In hypothyroidism, inadequate cardiovascular support appears to be one of the principal factors involved. However, studies do not reveal a global hypoperfusion of muscle but, instead, a reduction in flow to the fast-twitch

Table 3
Muscle changes observed in hyper- and hypothyroidism

	<i>Hyperthyroidism</i>	<i>Hypothyroidism</i>
Muscle strength	↓	↓
Type II fibers	↑	↓
Lactate: exercise response	↑	↑
Sarcoplasmic reticulum Ca ²⁺ uptake	↑	↓
PCr/Pi ratio—exercise	↑	↓
PCr recovery rate	↑	↓

↑ increased; ↓ decreased

type II fibers of high-oxidative type muscles (138). Hypothyroidism has not been found to alter the metabolic cost of exercise (139). Insufficient skeletal muscle blood flow compromises exercise capacity via reduced oxygen delivery, and endurance through decreased delivery of blood-borne substrates (140, 141). The latter effect results in increased dependence on intramuscular glycogen. Additionally, decreased mobilization of free fatty acids (FFA) from adipose tissue and, consequently, lower plasma FFA levels compound the problem of reduced lipid delivery to active skeletal muscle in the hypothyroid state (142). After exercise it has been observed that the rate of glycogenolysis and muscle lactate accumulation in hypothyroid dogs exceeded those in controls, showing diminished oxidative capacity resulting in lowering the adenosine triphosphate (ATP) content. Thus, inadequate fuel utilization may be considered as a factor limiting ability for heavy exercise in hypothyroidism (136). The compensatory mechanisms observed in hypothyroid animals may involve shifts in MHC gene expression in striated muscles resulting in transformation to a slower phenotype (133, 143–146). On the contrary, hyperthyroidism induces muscle transformation to a faster phenotype (133, 147–153). Moreover, in distinction to hypothyroid individuals, muscle blood flow is enhanced in hyperthyroid subjects including fast-twitch sections of muscle (142). The effect of T3-induced thyrotoxicosis on exercise tolerance has been studied, with increases noted in resting oxygen uptake and increased lactic acid levels, protein breakdown, and loss of lean body mass (154).

Clinical Findings

HYPOTHYROIDISM

Hypothyroidism is characterized by a decrease in Ca²⁺ uptake and ATP hydrolysis by sarcoplasmic/endoplasmic reticulum calcium ATPase SERCA (Table 3) (155). Hypothyroidism may underlie up to 5% of acquired myopathies (156). At least mild elevations in creatine kinase levels are seen in about 90% of hypothyroid patients (157). Exertional rhabdomyolysis has been reported with hypothyroidism and may be secondary to an acquired, reversible defect in muscle glycogenolysis (158). One case of exercise-induced rhabdomyolysis was associated with significant hypothyroidism (TSH >100 mIU/L) after the patient had walked just a 4-km distance. Type II fiber atrophy

has been reported in muscle biopsies in hypothyroid subjects, and a subsequent muscle biopsy of the latter patient's quadriceps revealed marked type II group atrophy (159, 160). In hypothyroid subjects, the alterations in lipid, protein, and carbohydrate metabolism in muscle may have pronounced effects on muscle function. Thus, exercise may exacerbate this situation and be associated with rhabdomyolysis. In Hoffmann's syndrome, another muscle disorder related to hypothyroidism, abnormalities include increased muscle mass, muscle stiffness and weakness, creatine kinase of as much as >10 times normal levels, and repetitive positive waves on electromyography (EMG) (161). Resolution of symptoms is expected with thyroid hormone replacement. Electromyographic testing in hypothyroidism may reveal normal patterns or possibly myopathic characteristics (162). EMG patterns that can be seen with hypothyroidism include fibrillations, increased polyphasic waves, unusual high-frequency discharges, and reduced motor unit recruitment (161).

An abnormal increase in lactate during exercise has been described in "subclinical" hypothyroidism. In one study, baseline lactate and pyruvate levels were similar between euthyroid and normal subjects (163). However, during exercise, lactate was significantly higher in subclinical hypothyroidism in comparison to controls, whereas pyruvate response was similar between the groups. It was also noted that the increments in lactate were positively correlated with the duration of subclinical hypothyroidism, but not with the levels of TSH, free T4 (fT4), or free T3 (fT3). It was hypothesized that mitochondrial oxidative dysfunction was present and that this dysfunction worsens with length of disease; glycolysis may exceed pyruvate oxidation explaining the lactate buildup. T3 receptors on the mitochondrial membranes of skeletal muscle point to a possible direct effect of thyroid hormones on oxidative metabolism.

Phosphorous nuclear magnetic resonance spectroscopy (MRS) has been extensively used to investigate noninvasively the energy metabolism of human muscle. It allows tracking of real-time changes in the relative concentrations of the metabolites that are involved in high-energy phosphate metabolism such as adenosine triphosphate (ATP), phosphocreatine (PCr), inorganic phosphate (Pi), phosphodiester (PDE), changes in the muscle pH, as well as phosphocreatine recovery rate constant (kPCr) that describes PCr recovery following exercise, which is proportional to oxidative enzyme activity of the muscle (164–167). Kaminsky et al. assessed these parameters in hypothyroid women subdivided into either moderate hypothyroidism (normal fT4 with elevated TSH), subacute thyroid deficiency (status post-therapy with radioactive iodine or thyroidectomy with low fT4 and high TSH >50 mIU/L), and severe/chronic hypothyroidism (of which one had overt myopathy). In comparison to controls, the hypothyroid group had a significant increase in the Pi/ATP ratio at rest and a decrease in the PCr/Pi ratio during exercise, although the latter ratio change is not specific and can be seen in other disease states. Chronic hypothyroidism was associated with an increased PDE/ATP ratio. The pH decreased during exercise to a lower nadir in the hypothyroid patients, and more so in the subacute or moderately hypothyroid groups. Finally, PCr recovery rate was much slower in hypothyroid than control subjects (168). This study demonstrated a dysfunction of muscle bioenergetics with even mild TH deficiencies. The increased PCr/Pi ratio at rest and PCr recovery rate following exercise indicate an impairment of oxidative phosphorylation. Several postulates exist for the aforementioned metabolic muscle changes:

(1) transition of white type II to red type I fibers, which could change PCr/Pi ratio and/or an increased PDE/ATP ratio in severe hypothyroidism consistent with fiber change, and (2) PCr resynthesis and PCr/Pi have been shown to be lower in conditions with mitochondrial myopathies (169). It was postulated that PCr decline was most likely owing to altered ATP synthesis, either by impaired oxidative phosphorylation or slower rate of oxidative substrate availability. Argov et al. also reported results of ³¹P-nuclear magnetic resonance in two hypothyroid patients. PCr/Pi was lower at rest in these subjects (4.8 and 5.5) in comparison to controls (8.5). There was a depletion of PCr during exercise and a delayed recovery of PCr/Pi that was also noted in parallel studies on thyroidectomized rats. LT4 reversed the abnormalities in both groups (rats and humans) after about 1 month of therapy. The exercise and recovery response in the hypothyroid group point to an in vivo mitochondrial impairment in hypothyroid muscle (170). Recent studies on relatively larger populations of patients confirm these findings. Khushu et al. documented similar abnormalities in the bioenergetic profile in 32 hypothyroid patients (171). Similarly, Bose et al. showed shifting of equilibrium toward ATP breakdown in to ADP and Pi after the exercises confirming impaired oxidative phosphorylation in mitochondria (172).

Haluzik et al. described metabolic changes in 12 hypothyroid women with comparisons to 6 hyperthyroid and 12 euthyroid women. All patients were studied a few days after the diagnosis of thyroid dysfunction. Compared to healthy subjects, hypothyroidism was associated with significantly decreased noradrenaline and glycerol concentrations whereas the opposite applied to hyperthyroid patients. These findings suggest altered adrenergic and lipolytic activities in thyroid disorders (173).

HYPERTHYROIDISM

Hyperthyroid subjects also have impairment in cellular respiration and reduced exercise endurance (154). Excess heat generation from the elevated metabolic activity associated with thyrotoxicosis and secondary hyperthermia may adversely impact heat dissipation during exercise and exercise tolerance. However, despite a baseline temperature increase of 0.5°C in thyrotoxic subjects, exercise-induced temperature rise has been observed not to differ from that in controls (174). Reduced duration of action potentials and increased polyphasic potentials can be seen with thyrotoxicosis (175). Muscle weakness is a common complaint in patients with TH excess, and a variety of investigations have addressed muscle changes secondary to hyperthyroidism. Hyperthyroidism is associated with an increase in fast and a decrease in slow-twitch muscle fibers. Thyrotoxicosis appears to induce an oxidative muscular injury secondary to an increase in mitochondrial metabolism and a decrease in glutathione peroxidase, which may be protective against such injury (176). Glycogen is lower at baseline in thyrotoxicosis and is utilized at a faster rate with an associated increase of serum lactate (177).

Thyrotoxic periodic paralysis (TPP) is an unusual complication of hyperthyroidism more typically seen in thyrotoxic Asian subjects, although not exclusively so. Patients with TPP suffer from attacks of para- or quadriplegia incited by exercise, high-carbohydrate meals, or high-salt intake. We have seen patients present with flaccid, paralytic attacks several hours following their military physical fitness test as the presenting manifestation of hyperthyroidism. The muscular function of these patients may appear grossly

normal before and between episodes, although some patients have a prodrome of muscle stiffness and aching. Other patients may have a chronic myopathy and tremor from the hyperthyroidism. The proximal muscles of the lower extremities are usually first involved, and episodes usually resolve over a time period of several hours to a day. The pathophysiology revolves around an imbalance in Na/K pump. Nerve conduction and EMG studies reveal that the muscle has reduced excitability during TPP episodes, and low-amplitude muscle action potentials are seen following a paralytic episode (178). Decreased compound motor action potential amplitudes are found postexercise in TPP (179) and improve following treatment (180). Of note, muscle fiber conduction velocity measured in two patients with TPP was within normal limits (4.0 and 3.6 ms, respectively) before and during paralysis episodes, although muscle strength was reduced by 40% during an attack (181). The attacks usually resolve once the patient's TH levels are lowered into the euthyroid range. Arimura et al. compared the electrophysiologic response to prolonged exercise between 21 patients with TPP and 11 patients with thyrotoxicosis without paralytic attacks. The authors documented that paralytic attack in TPP patients is primarily due to a preexisting latent abnormal excitability of the muscle membrane (182). TH regulates muscle membrane excitability by increasing Na/K pump-dependent potassium influx (183). There are also a few case reports documenting rhabdomyolysis as a complication of hyperthyroidism (184–187). Some authors describe significant metabolic changes in exercising muscle exposed to the excess TH. Reduced metabolic efficiency of skeletal muscle energetic with decreased PCr in hyperthyroid patients has been documented by MRS (188). Under thyrotoxic conditions, ATP is promptly depleted, and myopathy easily develops, as the intramuscular glycogen content decreases due to the suppression of glycogenesis and glycogenolysis. During vigorous exercise, glycogen is rapidly consumed, and ATP consumption by the skeletal muscles increases more than the ATP supply. At that time, the compensatory mechanisms include involvement of purine catabolism as a source of energy (189, 190). Fukui et al. compared the levels of glycolytic metabolites (lactate and pyruvate) as well as purine metabolites (ammonia and hypoxanthine) in four groups of patients: (1) with untreated thyrotoxic Graves' disease, (2) with Graves' disease treated with methimazole, (3) Graves' in remission, (4) and healthy volunteers (191). This study revealed that glycolysis and purine catabolism were remarkably accelerated in hyperthyroidism and thyrotoxic myopathy could be closely related to the acceleration of purine catabolism, which can be normalized only after long-lasting euthyroidism. An unbalanced ATP supply or conversion of muscle fiber type (192) possibly could account for the acceleration of the purine nucleotide cycle under thyrotoxicosis. Such acceleration of the purine nucleotide cycle is thought to be in part a protective mechanism against a rapid collapse of the ATP energy balance in exercising muscles of patients with hyperthyroidism (191, 193). Another important question facing clinicians is the effect of treatment with suppressive doses of LT4 necessary in some patients with differentiated thyroid cancer. Vigario et al. (194) addressed this question in a randomized controlled trial and documented that muscle mass was lower in the patients on suppressive LT4 treatment than in euthyroid control subjects, but aerobic training, twice a week, during 3 months partially reversed this deteriorating effect of excess TH on muscle mass.

EFFECTS ON PULMONARY FUNCTION

Performance of any strenuous activity especially of endurance training requires the ability of the respiratory system to augment oxygen utilization. Exercise capacity, the maximal capacity for oxygen consumption ($\text{VO}_2 \text{ max}$), and endurance, the ability to perform prolonged exercise at 75% $\text{VO}_2 \text{ max}$, are the two main components of exercise tolerance (195).

Clinical Findings

Large goiters, especially firm, nodular substernal goiters, can cause an extrathoracic tracheal obstruction, which can limit air flow to the lungs (196).

HYPOTHYROIDISM

Altered TH levels can lead to impairment in optimal pulmonary function. Myxedema or profound hypothyroidism is associated with alveolar hypoventilation related to a reversible reduction in hypoxic ventilatory drive (197). Reductions in lung volumes are seen and include vital capacity, total lung capacity, functional residual capacity, and expiratory reserve volume, as well as a decrease in diffusing capacity for carbon monoxide (DLCO) (198). LT4 replacement therapy is associated with resolution of the aforementioned changes, but a concomitant reduction in patient weight may also be an important factor in pulmonary function improvement (199). Frank respiratory failure is unusual. Exercise capacity in hypothyroid subjects has been measured objectively by an estimation of anaerobic threshold obtained by respiratory gas analysis on a ramp-loading cycle ergometer (200). During exercise hypothyroid subjects were characterized by reduced forced vital capacity and tidal volume at the anaerobic threshold. Also, the increment of minute ventilation and oxygen uptake was significantly lower.

HYPERTHYROIDISM

Thyrotoxicosis has been implicated as a primary cause of decreased cardiorespiratory exercise tolerance (85, 201, 202). Thyroid hormones may affect regulatory mechanisms of adaptation to incremental effort. In hyperthyroidism, already at rest, cardiorespiratory capacity is maximally increased, leading to a limited functional reserve, which may explain the inadequate response of ventilation (86). Dyspnea on exertion is a common complaint in patients with hyperthyroidism, but the causes of this symptom remain unclear and may vary from one patient to another (203–206). In hyperthyroidism, oxygen demand is increased, and the respiratory systems adjust to the increase in demand by increasing respiratory rate and minute ventilation (201). Alveolar ventilation remains normal, but a rise in dead space ventilation is seen. DLCO may be reduced during periods of strenuous exercise during thyrotoxicosis (207).

Pulmonary function is dependent on not just intrinsic lung function, but also the accessory muscles for respiration. Pulmonary compliance and airway resistance tend to remain unchanged, whereas vital capacity and expiratory reserve volume are reduced, implicating respiratory muscle weakness (208). Other supporting evidence for respiratory muscle dysfunction in thyrotoxic patients is the reduction of maximal inspiratory and expiratory efforts, which are seen to resolve on restoration of euthyroidism (209). It appears that ventilation is increased beyond the oxygen uptake and is

related to dead space ventilation (210). These changes also appear to resolve with appropriate therapeutic intervention (210). Studies have shown that changes in TH levels modify diaphragmatic function as well as muscle fiber type. Currently, the most accurate and reproducible clinical assessment of diaphragmatic muscle strength and its movement is obtained by esophageal pressure measurement during the sniff maneuver (SniffPoeso) and real-time ultrasonography, respectively. Using this method, Goswami et al. documented that patients with active Graves' disease have significant functional weakness of the diaphragm. This weakness was more marked during maximal respiratory maneuvers, indicating a diminished diaphragmatic reserve which could cause the dyspnea on exertion. These changes were reversible after achieving euthyroidism with carbimazole therapy (211).

With cardiac and muscular function being adversely affected by excess TH, one would postulate that work capacity must be reduced in hyperthyroid individuals. A study of maximum power output in hyperthyroid individuals with measurements of work capacity both while thyrotoxic and then euthyroid on propylthiouracil (PTU) therapy revealed a 19% increase from a low maximum power output during the thyrotoxic phase at $1.65 \pm 0.15 \text{ W/kg}^{-1}$ to $1.84 \pm 0.15 \text{ W/kg}^{-1}$ in a euthyroid state 3 months later. A subset of patients were retested 12 months later, and maximum power output in comparison to controls was in the low normal range at $2.75 \pm 0.15 \text{ W/kg}^{-1}$, representing a +13% rise from the 3-month test (212). Oxygen uptake at maximal effort was low during thyrotoxicosis and did not increase at 3 and 12 months. Net mechanical efficiency was also low at baseline and at 3 months, but returned back up to normal at 25.2% by 12 months. Using spirometry and spiroergometry, Kahaly et al. showed reduced forced vital capacity, 1-second capacity, and increased respiratory resting oxygen uptake (VO_2) rate in 42 hyperthyroid patients compared to euthyroid controls. During exercise, decreased tidal volume at the anaerobic threshold was observed as well as a lowered increment of minute ventilation, VO_2 , and oxygen pulse (86).

The studies are equivocal in terms of the effect of treatment with suppressive doses of LT4 on exercise capacity. Some studies revealed similar blood pressure, heart rate, VO_2 , VCO_2 , and anaerobic threshold response to exercise in LT4-treated patients as in healthy control subjects (213). Other studies found that ventilation parameters between patients and controls were comparable only at rest, but the patients treated with suppressive doses of LT4 had a worse response to exercise (lower maximal workload, lower peak VO_2 , and lower anaerobic threshold) (214). In conclusion, analysis of respiratory gas exchange showed low efficiency of cardiopulmonary function, respiratory muscle weakness, and impaired work capacity in hyperthyroidism which was reversible with restoration of euthyroidism.

EXERCISE AND THYROID AXIS RESPONSE

Exercise is a stressful situation that challenges body homeostasis, so that the organism has to reestablish a new dynamic equilibrium in order to minimize cell damage. One of the systems affected is the hypothalamic–pituitary–thyroid axis (215). While data have been reported on effects of exercise on TH metabolism, the results have been inconsistent or even contradictory (see Table 4). These divergent results may be due to differences in the intensity of work, duration of exercise, frequency and design of the training program, and to differences in gender, age, and baseline individual physical

Table 4
Reported changes in hypothalamus–pituitary–thyroid axis in association with exercise

Ref	Exercise type	Caloric status	TSH	T4	FT4	T3	FT3	rT3	Comments
(223)	Pre-exercise	NA	↑	↑	NC	↑			Anticipation of exercise
(219)	Ergometry ^a	Deficient and sufficient	NC	↑	NC	↑		↑	TSH response to TRH reduced
(224)	Ergometry	NA	NC	↓		↓			Normal TRH stim
(225)	Ergometry	NA	↑	↑		↑			Untrained athletes
(234)	Ergometry	NA	NC	NC		↓			Well-trained athletes
(234)	Ergometry	NA	↓	↓	NC	↑			Glucose infusion ^b
(248)	Ergometry	NA	↑	↑		↓		↑	Submaximal exercise
(226)	Ergometry	NA	NC	↑	↑				
(260)	Ergometry	NA	↓	↑	↑	NC		NC	Suspected CAD patients
(261)	Ergometry	NA	NC	NC		NC		NC	Recreational athletes
(241)	Chronic ergometry	NA	NC	NC		NC		NC	NC TSH response to TRH
(264)	Ergometry	Anorexia		↓		↓			Amenorrheic versus eumenorrheic controls and athletes ^c
(139)	Ergometry	NA				↑		↑	Hypothyroidism
(255)	Treadmill	NA	NC	NC		NC			
(221)	Maximal treadmill exercise	Sufficient	NC	NC		NC			Transient changes in TH values reflected transcapillary movements of water
(217)	Treadmill stress test	NA	NC	NC		NC		NC	Sedentary, joggers and well-trained athletes ^d
(2)	Aerobic exercise	Deficient		↑		↓		↓	Not seen in energy-balanced group
(242)	Aerobic exercise 4 weeks	NA	NC	NC		NC		NC	Healthy women
(228)	Running	NA	↓	↓	↓			NC	Maximal exercise
(235)	Running	NA	NC	NC	↓	↑		NC	Endurance athletes ^e versus controls

(continued)

Table 4
(continued)

Ref	Exercise type	Caloric status	TSH	T4	fT4	T3	fT3	rT3	Comments
(228)	Running	NA	↑		NC	NC		NC	Submaximal exercise
(245)	Runners	Deficient	NC	↑	NC	↓			Prevented by caloric increase
(257)	Rowing, running, and weight lifting	NA	↓		NC		↓		10/17 athletes responded with decline in TSH and fT3, remaining group – unchanged values
(229)	Ultradistance	NA		↑		NC		↑	75 km ^f
(229)	Ultradistance	NA	↓	↓		↓		↑	45 km ^f
(229)	Ultradistance	NA	NC	↑		↓		NC	42.2 km ^f
(230)	Marathon	NA	NC	NC		NC		NC	Groups age 23 and age 60
(231)	Triathlon	NA	↑	↑		NC		NC	
(232)	Marathon	NA	NC	NC		NC		NC	
(233)	Marathon	NA	↑	↑		NC		↓	T4 to rT3 conversion
(249)	Cross-country skiing	Deficient	↑	↑		↑		↑	Cold exposure
(250)	Military training	Deficient	↓	↓ ^s		↑ ^s		↓	Arctic weather ^s , sleep deprivation
(219)	Military training	Deficient	↓	↓ ^h		↑ ^h		↑↑	Sleep deprivation
(219)	Military training	Sufficient	↓	↑		↑		↑	
(222)	Swimming	NA		↑		↑		↑	
(236)	Swimming	NA	↑	↑		NC			20°C ⁱ
(236)	Swimming	NA	NC	NC		NC			26°C
(236)	Swimming	NA	↓	↓		NC			32°C
(247)	Gymnastic exercise	Deficient	NC	↓		↓			Leptin levels correlated with TSH levels
(265)	Ballet	NA	↓	NC		NC			Same group of rowers underwent 3 weeks of resistance and 3 weeks of endurance training
(256)	High-intensity resistance training	NA	↑	NC		↓		NC	
(256)	High-intensity endurance training	NA	↑	NC		NC		NC	

(240)	Chronic endurance exercise	NA	↓	NC	↓	↓	Identical twins
(245)	Chronic endurance exercise	NA	NC ⁱ	↓	↓	↓	Amenorrheic ^j
(224)	High-altitude exercise	NA	NC	↑	↑	↑	TT4/FT4 increase with higher-altitude pre-exercise
(259)	High-altitude exercise	NA	NC	NC	↓	↓	Increased GH/IGF-1 axis and low T3 syndrome

T4 thyroxine; T3 triiodothyronine; FT4 free thyroxine; FT3 free triiodothyronine; rT3 reverse triiodothyronine; TSH thyrotropin; TRH TSH-releasing hormone;

NA not applicable; NC no change; ↑ increase; ↓ decrease

^aAcute exercise challenge during military training

^bA glucose infusion blunted the exercise-induced changes of rT3, T3, and T4

^cTSH response to TRH stimulation was blunted in amenorrheic versus eumenorrheic athletes

^dTSH response to TRH stimulation was unchanged by exercise in all three groups. TSH response to TRH reduced more in energy-deficient than energy-sufficient group

^eEndurance athletes had balanced increase in T3 production and disposal rates in comparison to active and sedentary men

^fTSH, T4, and T3 lower in older runners, whereas faster runners had higher T4 and TSH in relation to slower runners

^gInitial increase of T4/FT4 and T3 first 24 h, then decrease, and then resolve 48 h post-training

^hInitial increase of T4/FT4 and T3 first 24 h, then decrease. However, T3 continued to rise in the energy-sufficient group, whereas T4 initially rose, then fell, and finally rose again

ⁱHigh TSH with longer cold water exposure

^jT4/FT4, T3/FT3, and rT3 were lower in exercising amenorrheic versus sedentary group. The eumenorrheic exercise group only a slightly lower FT4 level, but T4 and T3 were slightly lower than the eumenorrheic sedentary group

status of the subjects. In addition, different duration of studies, timing of sampling after exercise, and methodological factors in hormonal assay and data analysis may also be responsible for the discrepancies (216–220).

Some studies indicated no major changes in the thyroid axis response to exercise. In one study of 26 healthy male military recruits aged 23–27, given identical diet and physical activity for a week before the test, revealed no significant changes of serum mean TH values before and after a maximal treadmill exercise. To determine the possible effect of hemodynamic changes, hematocrit (Hct)-adjusted data were analyzed in this study. The authors showed hemoconcentration, as reflected by increased Hct, immediately after exercise. Values for T3, T4, and TSH increased significantly immediately after exercise, as compared to other postexercise measurements values. However, the changes became insignificant after Hct adjustment. Therefore, this study suggested that changes in hormone values after exercise may reflect only acute transcapillary movements of water, which resolve shortly after exercise ceases, and, consequently, maximal treadmill exercise does not greatly affect concentrations of circulating TH (221). Another study in subjects swimming either 0.9 or 1.8 km or exercising with bicycle ergometry for 60–90 min showed similar results: no significant change in FT4 and a small increase (partially from hemoconcentration) in serum T3 and rT3 (222).

Interestingly, some studies indicate that TSH increases after the exercise. TSH was even noted to rise from 3.1 to 4.0 mIU/L just in anticipation of strenuous exercise, although the response dissipated with repetitive testing, which might implicate a psychological influence on the TSH rise (223). In one study, healthy men undergoing bicycle ergometry for 20 min were noted to have a drop in T4 and T3 levels at 20 and 40 min postexercise, respectively (224). TRH stimulation yielded a normal TSH response. An FT4 increase of 25% has been seen postexercise (225), although an associated rise in FFAs was seen in that study, and interference by the FFAs in the assay results is possible. TSH also rose by 41%, but could not be correlated with T4/T3 levels. Another ergometry study examining the response to 30 min of submaximal exercise revealed no change in TSH and a 35% rise in FT4 above controls (226). The response of thyroid function to short-duration, graded exercise at 47%, 77%, and 100% maximal oxygen uptake and prolonged exercise at 76% oxygen uptake has been assessed. TSH was noted to rise progressively with increasing workload to a peak TSH of 107% of baseline serum level. A rise in TSH was also seen with prolonged exercise, but with a peak 33% lower than graded exercise (227). Another study compared the effect of submaximal and maximal exercise effect on TH levels (228). Maximal exercise was associated with a decrease in TSH, FT4, and stable rT3, and rises in T3 during activity, whereas submaximal exercise was associated with an increase in TSH, but T3, rT3, and FT4 were unchanged. TH changes in ultradistance and long-distance runners have also been investigated (100). Hesse et al. studied the effect of three distances of 75 km, 45 km, and marathon (42.2 km) with the subjects performing the 45-km run being slightly older (36 years) than the other two groups. T4 levels increased in the 75 km and marathon group, but decreased in the 45-km group postrace. T3 also dropped only in the 45-km group; rT3, measured only in the marathon and 75-km groups, rose in both groups. Other correlations revealed that TSH, T4, and T3 were lower in the older-age runners, whereas faster runners with better conditioning had higher T4 and TSH levels in comparison to slower runners. The authors speculated whether the increase in rT3 might be

protective against excess glucose metabolism, especially if intracellular glucose deficiency were present (229). Dessypris et al. investigated TH responses in triathletes grouped as either young (mean age 23 years) or older (mean age 60 years) subjects (230). Both groups had a rise in TSH, which returned to normal over 18 h post-event (231). Semple et al. report on marathon runners revealed no change in TSH, T4, T3, or rT3 levels before and after the marathon (232). However, another study revealed an increase in TSH and fT4 post-marathon, with a decrease in fT3 and rise in T4 to rT3 conversion, which was still detectable 22 h following race completion (233). The level of training of athletes has been shown to affect the TH response to acute exercise. In one investigation, untrained athletes had a rise in T3, a decrease in rT3, and no change in T4, whereas the well-trained athletes were found to have a rise in rT3, no change in T3, and a decrease in T4 levels. It was hypothesized that the rT3 elevation in well-trained athletes might be adaptive in that there is a more efficient cellular oxidation process (234). Of note, Rone et al. found an increase in T3 production and turnover in well-trained male athletes in comparison to sedentary men (235). Following a treadmill stress test, TH levels and TRH stimulation revealed responses similar in nature among sedentary subjects (VO_2 max 38.5 mL/kg/min), regular joggers (VO_2 max 45.0 mL/kg/min), and marathon runners (VO_2 max 60.3 mL/kg/min) (217). Variation in ambient temperature appears to alter the body's TH response to exercise. One study looked at proficient swimmers exercising in three water temperatures, 20, 26, and 32°C, for approx. 30 min at a moderate speed; TSH and fT4 rose in the colder water, were unchanged at 26°C, and fell at the warmer temperature, but T3 levels were not affected (236). Cold receptors appear to regulate a rise in TRH and TSH level in cold water, and exposure duration may affect the peak TSH with higher levels owing to longer times in the water (237, 238).

The chronic effects on thyroid hormone parameters have also been studied in endurance athletes. The results of the studies conflict with regard to whether or not baseline TH levels are shifted in well-trained athletes. Baseline TH levels have been found to be unchanged, increased, or decreased (239). Identical twins that were studied during an observed 93 days endurance training period with stable energy intake had an average 5-kg weight loss (primarily fat), and lower baseline fT3, T3, and T4 with a nonsignificant drop in fT4 by the end of the 93 days exercise period (240). A shorter study in recreational athletes over 6 weeks revealed no change in TSH or TSH response to TRH stimulation with regular bicycle ergometry training (although the exercise endurance improved) (241). Also, a 1-month aerobic conditioning program in six healthy women revealed no change in TSH, T4, T3, or rT3 (242). Rone et al. reported no difference in baseline values for T4, T3, and TSH between endurance athletes and sedentary controls over time (235). In the latter study, oral liothyronine sodium was administered, and its metabolism assessed. The endurance group exhibited an increase in the following parameters: T3 metabolic clearance rate, T3 total volume of distribution, T3 disposal rate, and total body T3 pool even when normalized for total body weight, lean body mass, and body surface area.

There is evidence that T3 production and utilization correlate strongly with the amount of lean body mass in physically trained males (235). Radioactive iodine uptake (RAIU) may be altered secondary to chronic exercise. T3 metabolism may be altered in endurance athletes as compared with sedentary controls. A lower thyroid uptake of ^{123}I

has been found in regular exercising rats and humans (RAIU 8%) in comparison to sedentary subjects (RAIU 14%) (239). Energy balance plays a role in the body's TH response to exercise. Energy balance amounts to the difference between daily caloric intake minus the energy used by the body in the form of metabolism, muscle work, and baseline functional needs of various organs. Much data exist on the response of TH to fasting or malnutrition (243, 244). T3 tends to decrease, whereas rT3 tends to increase with fasting, which appears to be a regulatory mechanism to regulate catabolism and energy expenditure. The TH levels return to normal with refeeding. Loucks and Heath (245) found a decrease in T3 (−15%) and fT3 (−18%) along with an increase in rT3 (+24%) in healthy women undergoing aerobic exercise testing with low-caloric intake of 8 kcal/kg body weight/day. This “low T3 syndrome” was not seen in individuals receiving a diet of 30 kcal/kg body weight/day, a level more in balance with energy expenditure. Low-caloric diets high in carbohydrate appear to blunt the drop in T3 in comparison to low-carbohydrate intake (246). Even a mild energy deficiency may have an effect on TH levels. Female gymnasts with borderline energy deficit had a decrease in T3 and increase in T4 during 3 days of heavy workouts (247). Glucose infusion has been found to diminish the increase in rT3 and T4 along with decrease in T3 from a 30-min ergometry test (248). Participants in a 90-km cross-country ski race exhibited an increase in T4 and fT4, which resolved by 1 day posttrace. Skiers were exposed to cold temperatures over a period of 5.4–8.1 h. With an approximate 7,000-kcal exercise demand noted, an acute negative energy balance was believed to be evident in TH changes (249). A study of military troop exercises in the Arctic lasting 3 days revealed a TSH decline throughout the time period, which took >48 h for return to baseline. Serum T4/fT4 and T3 initially increased, then declined below baseline, and then reverted toward normal by 48 h posttests (250). Cold, sleep deprivation, and varying physical activity were all mitigating factors. A negative energy balance may have been present secondary to the cold and significant physical activity. In another military study, rangers were assessed over 4 days of grueling training in conjunction with sleep and caloric deprivation. The physical strain associated with this training was associated with an initial increase of T4, fT4, T3, rT3, and thyroxine-binding globulin (TBG) during the first 24 h. After 4 days of training, there was a gradual decrease in T4, fT4, T3 (65%), and TBG, whereas rT3 continued to rise. The group that received a higher caloric intake, and therefore less energy deficiency, had a continued increase in T3 instead. Also, T4 increased in the energy-sufficient group, and TBG remained elevated, too. TSH decreased during the first day and remained low throughout the training period in energy-deficient groups. An acute exercise challenge (bicycle ergometry) during this training period was associated with a small but significant increase in T4, rT3, and TBG, but TSH and fT4 remained unchanged. Subsequently, T4, T3, rT3, and TBG also decreased significantly following the exercise challenge. The response of TSH to TRH was reduced in all groups, but much less so in the energy-sufficient group. Energy deficiency correlates with a decrease in T3 and increase in rT3 (219).

Higher altitudes have been shown to be associated with an increase in T4 and fT4 (251). Stock et al. reported that exercise at elevated altitudes is also notable for a significant increase in T4 and fT4 with even mild activity (252). Energy balance in women runners has also been investigated. Subjects with negative energy balance had a decrease in T3 (−15%) and fT3 (−18%), but a +24% increase in rT3 was noted (245).

This “low T3 syndrome” was prevented by dietary adjustment, but not by reduction in exercise intensity. Rat studies indicate that serum T4 and T3 remain stable with exercise-induced weight loss, whereas TH changes are noted in underfed, sedentary rats (253). Other animal studies revealed a time-course-dependent changes in T3 levels with an increase in serum T3 immediately after exercise, with a gradual decrease thereafter to significantly lower values than in controls. Concomitantly, T4 levels progressively increased, resulting in the T3/T4 ratio being significantly decreased 60 and 120 min after the exercise, indicating impaired T4-to-T3 conversion (254).

A study of subjects on bicycle ergometry revealed a rise in rT3 from 29 to 40 ng/dL with a decrease in T3 from 154 to 147 ng/dL. Glucose infusion during a repeat test reduced the rT3 and T3 changes. A positive correlation during exercise between rT3 and FFA was found ($r=0.95$). The glucose infusion reduced this correlation to $r=0.81$. Of note, exercise stress was minimal in this study with heart rate of 120 beats/min and working at 40% of maximal oxygen consumption (139). As mentioned above, restriction of dietary carbohydrate has been associated with lower serum T3 level. Thyroidal responses to exercise were measured in groups with either a fat-enriched or carbohydrate-enriched diet. The T3 level tended to be lower at any point in exercise with the fat diet in comparison to the carbohydrate diet (255).

Simsch et al. assessed hypothalamic–thyroid axis and leptin concentrations in six highly trained rowers, who undertook high-intensity resistance training for three weeks followed by three weeks of endurance training. After each training cycle the subjects had one week for recovery. After resistance training a significant reduction in TSH, fT3, and leptin was found ($p<0.05$), while fT4 was unchanged. Interestingly, leptin levels correlated with basal TSH levels ($r=0.49$, $p=0.006$). In contrast, after endurance training a significant increase of TSH was observed. Body mass index (BMI) and body fat were unchanged throughout the study. The authors interpreted these data to indicate that depression of the hypothalamic–thyroid axis and leptin is associated with training intensity (256). Further supporting these observations was a study comparing TH and leptin levels in collegiate athletes ($n=17$) and sedentary controls ($n=4$), who underwent a training consisting of daily athletic activity such as rowing, running, and weight lifting. Ten of the 17 athletes responded to exercises with declines in fT3, TSH, and leptin levels. These hormonal changes were not significantly correlated with changes in body composition or hydration status during the study. Remaining subjects did not respond to training with hormonal changes (257). Hackney et al. studied 63 girls and 62 boys 12–16 years of age exposed to the physical training. They found a negative correlation between physical activity scores and fT3 in the girl adolescents, which was not the case in boys (258). Another study supporting a concept of low T3 syndrome as an adaptive mechanism to intense training included nine male well-trained elite climbers of the Italian expedition “K2 2004—50 years later.” Five of the climbers reached the summit at 8,852 m, three of them reached an altitude of 8,600 m, and one an altitude of 7,500 m. None used oxygen supplementation. This strenuous physical exercise resulted in increased activity of the GH/IGF-I axis and a low T3 syndrome but no significant change in ghrelin and leptin despite decrease in body weight (259). Similar results were documented by Hackney et al. (260) who showed in a group of 15 males participating in an expedition to climb Mount McKinley low T3, increased rT3, and high cortisol levels.

TH changes secondary to exercise have been also assessed in patients with known or suspected coronary artery disease. Test results are conflicting with at least one exercise study revealing no changes in TSH, T4, T3, or rT3, but another one yielding a TSH decline and increase in T4 and fT4, which all resolved within 1 hour posttest (261, 262). Of note, combined oral LT4/LT3 overdosage has been reported to cause ST wave depressions with treadmill stress testing that resolve with the euthyroid state (263). In general, diagnostic treadmill testing is best delayed until patients are euthyroid.

Amenorrhea is commonly seen in well-trained female athletes. Studies have compared the TH differences between women athletes with normal menses and amenorrhea. One study found that T4/fT4, T3/fT3, and rT3 levels were all lower in the amenorrheic group in comparison to sedentary women, whereas the athletes with regular menses had only a slightly lower fT4 level. TSH circadian rhythm was unaltered (264). Caloric balance was similar in the two groups. Thyroid hormone levels in eumenorrheic nonathletes and eumenorrheic endurance-trained athletes were compared with amenorrheic endurance-trained athletes. The amenorrheic subjects had lower T4 and T3 levels than the eumenorrheic groups, but the trained eumenorrheic females had slightly lower T4 and T3 levels than the eumenorrheic nonathletes as well. Of interest, the amenorrheic athletes tended to eat less fat and eat more carbohydrates with a similar caloric intake in comparison to the two other groups. Also, the amenorrheic group tended to train more hours and more strenuously than the other two groups. VO_2 was similar in the trained groups, who also weighed less and had lower body fat. As measured by ^31P -MRS, Pi/Cr was not different at rest or at exercise, and pH did not differ at any activity level. However, PCr recovery was substantially faster in the eumenorrheic endurance-trained group (26.3 ± 3.3 s) than in the eumenorrheic nonathletes (42.6 ± 4.6 s) and amenorrheic athletes (41.2 ± 6.6 s); however, the Pi/Cr recovery was only different between the eumenorrheic-trained athletes and nonathletes (265). It is of interest that T3 was lower in the trained athletes versus in the nonathletes. Also, one must wonder that even if the caloric intake in the amenorrheic athletes was equal, the T3 changes may have been secondary to an energy deficiency, since their activity level was higher. However, even the trained eumenorrheic athletes had lower levels of TH, yet many trained athletes have been found to have higher levels of T3 at rest. PCr recovery is related to oxidative metabolism, and the fast recovery in trained eumenorrheic athletes indicates a potentially more efficient metabolism, yet amenorrheic individuals did not appear to obtain this advantage. The other parameters for exercise metabolism were similar. In another study, levels of TSH, T3, and T4 were not found to be different in oligomenorrheic heavily trained adolescents versus adolescent athletes without “strenuous” exercise with regular menses (266).

SUMMARY

In summary, the thyroid function changes secondary to exercise represent a complex physiologic response, which is difficult to characterize fully. Mitigating factors in the TH response to exercise include age, baseline fitness, nutrition status, ambient temperature, altitude, as well as time, intensity, and type of exercise performed. Another important factor in interpretation of the extant literature is that not all TH blood tests were assessed in every study. Moreover, older studies employed less sensitive assay

techniques, for example, first-generation TSH immunoassay, whereas our various assays have improved over the collective time span of these many studies. The detection of increased FFA in several studies, which may interfere with some fT4 assays, also cannot be overlooked. However, despite these issues, review of the literature does reveal certain trends, and some are more evident than others (Table 4). One of the more consistent findings is that rT3 tends to increase with exercise especially with associated caloric energy deficiency or ultradistance exercise. TSH appears to be unaffected by exercise in about 50% of studies with an increase in TSH secondary to cold exposure being a noted exception. T4 was found to increase in 46%, decrease in 26%, and be unchanged in 28% of investigations, although an increase was more typically found with caloric energy deficiency, cold exposure, or ultradistance exercise; fT4 follows a similar pattern to T4. T3 was found to be decreased or be unchanged in 73% of study subjects, and usually is low with caloric energy deficiency (as in low T3 syndrome or euthyroid sick states); fT3 was infrequently assessed in the protocols but, when measured, tended to follow the similar to T3 pattern. Many of the TH changes seen especially in athletes with negative energy balance can be reversed with either a high-carbohydrate intake or even glucose infusion. Although well-trained athletes may exhibit an increased production and turnover of T4, baseline TH levels do not appear to be affected by chronic endurance exercise.

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7

The Male Reproductive System, Exercise, and Training: Endocrine Adaptations

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INTRODUCTION

Androgens exert strong anabolic effects on skeletal muscle protein synthesis (1, 2), satellite cell number (3), and skeletal muscle growth (4, 5). Because these changes are of great importance to muscle strength, androgens have been recognized as important hormones that influence sports performance (6). Exercise-induced changes in testosterone concentrations can moderate or support neuromuscular performance through various short-term mechanisms (e.g. second messengers, lipid/protein pathways, neuronal activity, behaviour, cognition, motor-system function, muscle properties, and energy metabolism) (7).

On the other hand, the gonadal axis function is strongly affected by physical exercise depending on the intensity and duration of the activity, the fitness level, and the nutritional-metabolic status of the individual (8, 9). Moreover, circulating testosterone and its bioavailable fractions are affected by weight and age. They are also changed by different kinds of stress which may appear as physical stress (i.e. endurance training,

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sleep deprivation in extreme sports, changes of air pressure in altitude training) or mental stress in relation to sport events and training (9).

In this chapter, the effects of physical exercise on testicular steroidogenesis and on spermatogenesis will be revised.

PHYSIOLOGY OF THE MALE GONADAL AXIS

The male gonadal axis consists of the testes and the hypothalamus–pituitary unit that controls their function. The testes possess a dual function, i.e. the production of androgens and of sperm.

Figure 1 depicts an outline of the male gonadal axis and of the hormonal regulation of the testicular function.

The pituitary gland is the central structure controlling gonadal function: it releases the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) and is regulated by the hypothalamic gonadotropin-releasing hormone (GnRH), which is secreted in a pulsatile fashion with peaks every 90–120 min. In man, the major hormone controlling GnRH secretion is testosterone, which inhibits gonadotropin secretion via negative feedback both at the hypothalamic and pituitary level. Dihydrotestosterone (DHT) and estradiol also modulate gonadotropin secretion acting at the hypothalamic and/or pituitary level (10, 11). In addition, several neurotransmitters and neuromodulators might influence GnRH secretion: the noradrenergic system and neuropeptide Y (NPY) show stimulatory activity, whereas interleukin-1, opioid peptides, dopamine, serotonin, and gamma-aminobutyric acid (GABA) are inhibitory. Leptin, which is produced by the fat cells, has been shown to stimulate GnRH and gonadotropin secretions (11). Ghrelin, a peptide hormone with growth hormone-releasing action, exerts multiple endocrine and non-endocrine effects including inhibition of the gonadal axis at both the central and peripheral level (12, 13). Furthermore, the adverse effect of stress on reproductive function is well known. Several factors are involved: corticotropin-releasing hormone (CRH) inhibits GnRH secretion, prolactin further reduces the GnRH pulse rate (10), and cortisol inhibits both the hypothalamus–pituitary and gonadal functions.

LH and FSH are produced and secreted by the gonadotropic cells of the anterior pituitary. LH regulates testicular androgenesis whereas FSH, together with locally produced testosterone, is responsible for spermatogenesis. LH binds to specific receptors on the surface of Leydig cells in the testis and regulates the biosynthesis of testosterone. FSH binds to receptors on the Sertoli cells and promotes spermatogenesis: in addition to a number of other proteins, the hormones inhibin B and activins are formed in the Sertoli cells under the influence of FSH. Inhibin B plays an important role in the feedback regulation of FSH secretion, whereas the physiological role of activins has not been conclusively clarified (10).

Testosterone is the most important steroid produced by the testis and is responsible for the development and maintenance of male sex characteristics as well as a number of other anabolic and metabolic effects (e.g. muscle and bone metabolism). Normal testosterone concentrations in adult males range between 12 and 30 nmol/L: testosterone concentrations in blood follow a circadian rhythm with higher levels in the morning hours and about 25% lower levels in the evening (11).

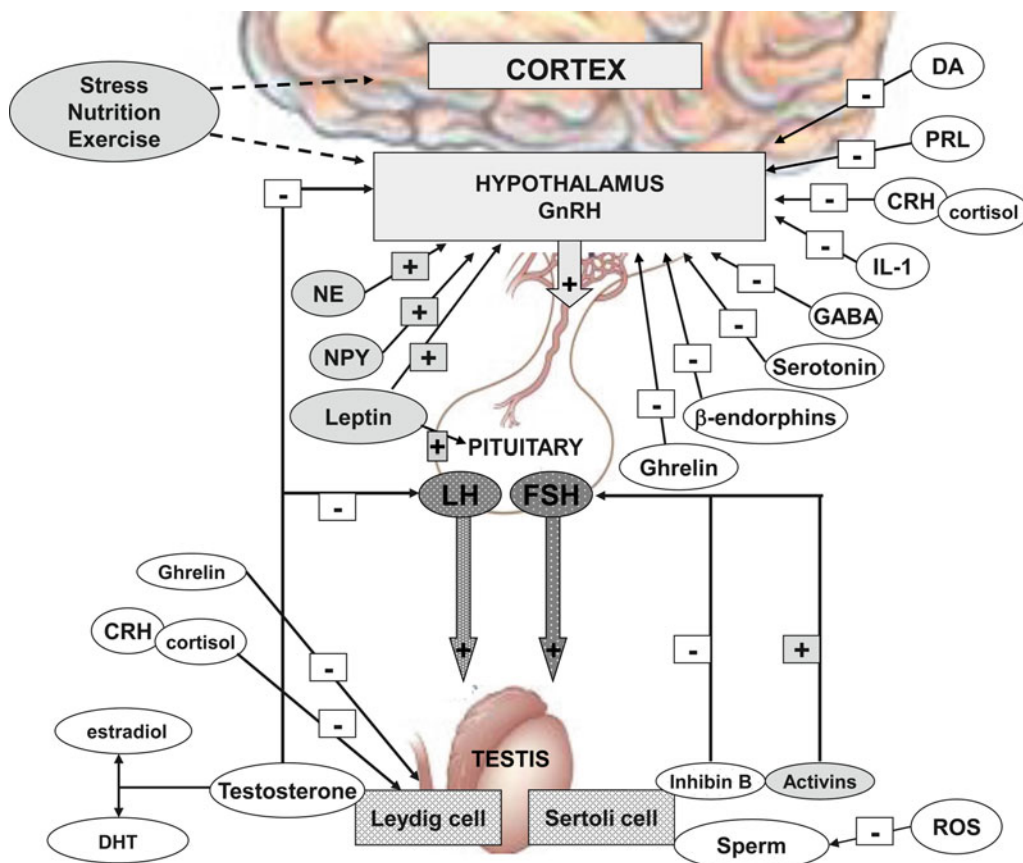


Fig. 1. Schematic diagram of the male gonadal axis. *CRH* corticotropin releasing hormone; *DA* dopamine; *DHT* dihydrotestosterone; *FSH* follicle-stimulating hormone; *GABA* gamma-aminobutyric acid; *GnRH* gonadotropin-releasing hormone; *IL-1* interleukin-1; *LH* luteinizing hormone; *NE* norepinephrine; *NPY* neuropeptide Y; *PRL* prolactin; *ROS* reactive oxygen species.

EFFECTS OF PHYSICAL EXERCISE ON TESTICULAR STEROIDOGENESIS

Short, Intense Exercise Increases Circulating Testosterone

The effects of physical activity on the male gonadal axis vary with the intensity and duration of the activity, the fitness level of the individual, and his nutritional-metabolic status. Relatively short, intense exercise usually increases while more prolonged exercise usually decreases serum testosterone levels (8, 9, 14). Increased serum testosterone levels have been reported during relatively strenuous free and treadmill running, weight training, rock climbing, and ergometer cycling (15–17). Short-term sprints can be seen as strength outburst and are comparable to strength training rather than endurance training: sprint exercise increased plasma testosterone concentrations in adolescent boys (18).

The testosterone response increases with increased exercise load (19). Similar workloads produce similar responses, regardless of whether the load is aerobic or anaerobic (20).

Immediate and 5 min post-exercise measurements showed an increase in testosterone levels both in men and women (21). Acute exercise-induced testosterone increments are also seen in older men (22). This acute hormone response was confirmed and described to be markedly stronger in young men compared to old in a study involving ten men with mean age 26.5 years and ten men with mean age 70.0 years (23).

As muscle mass increases with strength training (4) and is correlated with testosterone levels, it could be expected that the testosterone response to acute exercise is higher in persons constantly involved in strength training. Consistently, a 6-month sprint training programme increased plasma testosterone concentrations in response to sprint exercise in adolescent boys (18). Experienced weight lifters compared to beginners showed similar basal levels of testosterone but were able to evoke a stronger testosterone response during exercise (15). Contrary to these findings, a long-term training period of 12 weeks involving younger (mean 23 years) and older men (mean 63 years) showed no significant changes concerning testosterone levels before or immediately after exercise (24).

Rønnestad et al. (25) have recently investigated the effects of testosterone and growth hormone (GH) transient increase during exercise, indicating that performing leg exercises prior to arm exercises, thereby increasing the levels of testosterone and GH, induced superior strength training adaptations compared to arm training without acute elevation of hormones. It has been found that acute elevation in endogenous testosterone (by strength training) potentiates the androgen receptor (AR) response to a strength training session compared to no acute elevation of endogenous testosterone (26). It may thus be speculated that the results by Rønnestad et al. are due to an increased AR expression and, through an improved testosterone-receptor interaction, an increased protein synthesis, leading to superior strength training adaptations. This hypothesis has also been evaluated by Ahtiainen et al. (27), who have described a correlation of individual pre- to post-training changes in resting AR protein concentration with the changes in cross-sectional area of muscle fibres in a combined group of young and elderly subjects who performed heavy resistance exercise bouts before and after a training period. Overall, these findings suggested that the individual changes of AR protein concentration in skeletal muscle following resistance training may have an impact on training-induced muscular adaptations.

Mechanisms Underlying Increases in Circulating Testosterone Following Short, Intense Exercise

No conclusive evidence about gonadotropin response to an acute exercise bout is available. In fact, LH and FSH levels have been reported to be increased, decreased, or unchanged by short-term strenuous exercise (28–31).

The exercise-associated increment in circulating testosterone is considered not to be mediated by LH, due to the inconsistent LH response and to the evidence that testosterone levels increase more quickly than LH in response to exercise. Possible mechanisms such as hemoconcentration, reduced clearance and/or increased testosterone synthesis may be involved (29, 31–33). However, the timing of testosterone response differs from that of other circulating steroids (e.g. androstenedione and dehydroepiandrosterone

increase simultaneously with cortisol) thus suggesting that specific testicular mechanisms are involved (31). These mechanisms may include the activation of the sympathetic system, which stimulates testicular testosterone production during exercise via a direct neural pathway in some species (34). Catecholamine levels also increase significantly during exercise. Beta-adrenergic blockade inhibits testosterone responses to exercise, whereas l-dopa, phentolamine, and clonidine had no effect (35). An anticipatory elevation in resting testosterone levels has also been described pre-exercise and seems to be independent of hepatic perfusion or hemoconcentration (28, 31). Ultimately, the exact mechanisms involved in increasing testosterone concentrations in specific exercise protocols are yet to be defined.

Prolonged, Submaximal Exercise and Chronic Exercise Training Decrease Circulating Testosterone

In contrast to the short-term testosterone increment during and immediately after short, intense exercise, a suppression of serum testosterone levels occurs during and subsequent to prolonged exercise, in the hours following intense exercise, as well as during chronic exercise training.

During the last decades, an increasing number of investigative research studies have pointed to how chronic exposure to endurance exercise training can result in the development of a dysfunction within the reproductive components of the neuroendocrine system. The majority of these studies have concentrated upon women. However, the effects of endurance exercise training on the male reproductive neuroendocrine system have been investigated beginning in the 1980s (36). Most studies observed athletes during training and competition, giving the impression of generally lowered androgen levels, but lack the comparison with a control group (9).

A controlled study examining the effects of endurance training on the hypothalamus–pituitary–testis axis in males involved 53 men undergoing endurance training for at least 5 years and a control group of 35 age-matched, sedentary men. Baseline serum testosterone levels of the exercising men were significantly lower than in the control group. Differences in gonadotropins were not seen. Normal regulation would require LH levels to rise with falling testosterone levels, as these have a positive feedback on pituitary gonadotropin release. A suppression in the regulatory axis could explain this finding (37).

Contrary to these observations, basal testosterone levels in trained weight lifters were not altered, nor did an increase in the daily training volume change these levels (38). Similarly, basal testosterone, free testosterone, bioavailable testosterone, and sex hormone-binding globulin concentrations were not significantly different in high top-class athletes (sprinters and jumpers) vs. untrained subjects (17).

Endurance training can be seen as a factor of exposure not only to physical but also to psychological stress. It has been demonstrated in a controlled study that the reactivity patterns of mental/psychological and physical stress response of the hypothalamus–pituitary–adrenal axis are the same in a specific individual. Differential reactivity is rather seen between the so-called high and low responders. Each group has a specific endocrine reactivity pattern concerning the hypothalamus–pituitary–adrenal axis (39). It seems that the decrease of testosterone levels under the stressful situations of endurance

sport is not sufficiently answered by the pituitary. There is no adequate rise in LH levels, which seem to be unaltered or even show a tendency to decrease with the growing amount of stress impact. Nevertheless, age-dependent effects seem to exist in this regard, and the ratio of androgen to estradiol is shifted by physical activity to a more favourable pattern (higher androgen and lower estradiol levels) in older men compared to younger men performing regular mild physical activity (40).

The “Exercise-Hypogonadal Male”: Clinical Issues

It has recently been demonstrated that among subjects engaged in chronic exercise training, a selected group of men develop alterations in their reproductive hormonal profile, i.e. persistently low basal resting testosterone concentrations (41). In particular, the majority of these men exhibit clinically “normal” testosterone concentrations, but these concentrations are at the low end of normal range or even reach subclinical status.

The health consequences of such hormonal changes are increased risk of abnormal spermatogenesis, male infertility problems, and compromised bone mineralisation (41–43). The prevalence of such health problems seems low, but investigative studies examining this condition and its consequences are few in number (41, 42). The specific terminology used to refer to this condition has not been universally agreed upon. In 2005, Hackney and associates proposed the use of “the Exercise-Hypogonadal Male” as a label for this condition (44).

The “Exercise-Hypogonadal Male”: Pathophysiological Mechanisms

Exercise-hypogonadal men frequently display a lack of significant elevation in basal LH in correspondence with the reduced testosterone concentration, reflecting hypogonadotropic-hypogonadism characteristics (36, 41, 45). These LH abnormalities may involve disparities in luteinizing pulsatility (i.e. pulse frequency and amplitude), although evidence for altered LH pulsatile release is conflicting (46, 47). Moreover, gonadotropin response to GnRH has been reported both reduced and increased following prolonged, exhaustive exercise (48, 49).

Exercise-hypogonadal men have been shown to have altered basal prolactin (41). At either excessively low or high circulating levels, PRL can result in suppression of testosterone levels in men (50). It has been speculated that the absence of prolactin at the testicle alters the effectiveness of LH to stimulate testosterone production. This theory is based upon the proposed synergistic effects of prolactin upon testicular LH receptors (36). However, not all investigators reporting low resting testosterone in endurance-trained men have reported the concomitant existence of low resting prolactin levels (50). Some investigations have looked at a potential relationship between high prolactin levels and low testosterone, speculating that any “stressful” situation might provoke disproportionate prolactin responses in exercise-hypogonadal men, and this ultimately promotes a reproductive axis disruption (51).

Leptin is an adipocyte-released hormone associated in part with communicating to the hypothalamus satiety and energy reserves status (52). It is also linked to reproductive function both in women and in men. Acute and chronic exercise can impact upon resting leptin concentrations, independent of changes in body adiposity (53). However,

to date no research studies have examined whether leptin concentrations are altered in exercise-hypogonadal men.

Ghrelin is another hormone associated with appetite regulation. Newly emerging experimental evidence in animals and in humans suggests that ghrelin may function as a metabolic modulator of the gonadal axis, with predominant inhibitory effects in line with its role as signal of energy deficit (12, 13). Acute and chronic exercise has been shown to influence ghrelin concentration levels (54). However, no research has yet examined whether ghrelin levels in exercise-hypogonadal men are normal.

Other research investigations have focused on alterations in testicular ability to produce and secrete testosterone and to respond to exogenous stimuli (i.e. LH or hCG). Whereas animal studies have demonstrated that exercise training compromises testicular enzymatic activity (55), data in exercise-hypogonadal men are contradictory. In fact, some investigations suggest testicular steroidogenesis is normal, while some indicate it is marginally impaired when challenged with exogenous stimuli (41).

Another potential disruptive hormone to the gonadal axis is cortisol. Studies in a wide range of sports (e.g. cycling, marathon running, football, handball, rugby, tennis, swimming, and wrestling) have almost all shown increased cortisol concentrations during exercise (56, 57). Cortisol secretion increases in response to exercise intensity and duration, as well as to the training level of subjects (58–61), at least in part to mobilize energy stores. An inhibitory effect of the hypothalamus–pituitary–adrenal axis on the reproductive system has been demonstrated in both sexes (62, 63). In fact, glucocorticoids suppress gonadal axis function at the hypothalamic–pituitary level (62). Moreover, Inder et al. (64) have demonstrated that dexamethasone administration in humans reduces circulating testosterone and downregulates the muscular expression of the AR. Finally, CRH and its receptors have been identified in the Leydig cells of the testis, where CRH exerts inhibitory actions on testosterone biosynthesis (65).

Interestingly, a sport event and also training for such represent both a physical and a mental stress (9). The release of cortisol by activation of the hypothalamic–pituitary–adrenal axis as reaction to mental stress is well documented (39, 66). Stress responses by the hypothalamic–pituitary–gonadal axis are constantly found as well.

Along this line, anticipatory stress was measured in 50 males before a one-day experimental stress event (participation in stressful clinical research protocol). Cortisol levels rose significantly, while both testosterone and LH secretion were decreased (67). Psychological stress markers as measured by scales for anxiety, hostility, and depression were correlated with serum levels of testosterone in a group of males aged 30–55 years. Those classified as highly stressed had significantly lower testosterone levels than their counterparts (68). A cross-sectional study involving 439 males all aged 51 years showed those with low levels of testosterone (adjusted for body mass index) to exhibit a cluster of psychosocial stress indicators (69). Nevertheless, other hormonal profile studies reporting the existence of low testosterone in trained men did not show elevated resting cortisol levels (36, 70, 71). However, resting cortisol levels do not necessarily reflect a hyperactivity of the hypothalamus–pituitary–adrenal axis, which can be better defined either by serial blood or salivary sampling (72) or by assay of urinary free cortisol.

Thus, at this time the role of cortisol to the changes found in the gonadal axis of trained men is in need of further study.

EFFECTS OF PHYSICAL EXERCISE ON SPERMATOGENESIS

Clinical expression of impaired reproductive function in men engaged in chronic exercise training seems uncommon (42, 47, 73). However, chronic physical exercise may induce a state of oligospermia, a reduction of the total number of motile sperm and an increase in abnormal or immature spermatozoa.

Vaamonde et al. (74) have analysed the semen profiles of three male populations with different types and levels of physical activity (physically active non-professional subjects, water polo players, and triathletes) and found that sperm concentration, velocity, and morphology were significantly different among the practitioners of the three different training modalities. The differences were more marked as intensity and volume of exercise increased, especially for morphology which was the parameter showing the greatest difference (74).

Safarinejad et al. (49) performed a longitudinal study on the effects of intensive, long-term treadmill running on reproductive hormones and semen quality. A total of 286 subjects were randomly assigned to moderate-intensity exercise ($\sim 60\% \text{VO}_{2\text{max}}$) and high-intensity exercise ($\sim 80\% \text{VO}_{2\text{max}}$) groups. The two groups exercised for 60 weeks in five sessions per week. This was followed by a 36-week low-intensity exercise recovery period. After 24 weeks of exercise, the subjects exercising with high intensity demonstrated significantly declined semen parameters (sperm density, motility, and morphology) compared with those exercising with moderate intensity. At 36 and 48 weeks, these differences were more significant. A significant correlation was found between high-intensity exercise, its duration, and sperm count, as well as mean sperm motility and sperm morphology. Serum testosterone and free testosterone began to decrease, and serum SHBG began to increase at the end of 12 weeks with both moderate- and high-intensity exercises. Both semen and hormone parameters improved to their pre-exercise level during the recovery period (49).

In a recent study, Wise et al. (75) have examined the association between regular physical activity and semen quality in a large cohort of 2,261 men attending an infertility clinic. They found that none of the semen parameters (semen volume, sperm concentration, sperm motility, sperm morphology, and total motile sperm) were materially associated with regular exercise. However, in the subgroup of men who reported bicycling as their primary form of exercise, bicycling at levels of >5 h/week was associated with low sperm concentration and total motile sperm. These findings generally agree with earlier studies that have shown deleterious effects of bicycling on semen parameters among competitive cyclists (73, 76). It remains unclear as to whether the changes associated with bicycling are due to mechanical trauma (i.e. caused by compression of scrotum on the bicycle saddle), to a prolonged increase in core scrotal temperature (i.e. related to exercise itself or wearing of constrictive clothing), or some other factors (77).

Oxidative Stress as a Putative Mechanism Underlying Impaired Spermato-genesis in Exercise-Hypogonadal Men

Several mechanisms have been reported to affect the male reproductive function in exercising subjects. Alterations in the hormonal milieu, as discussed in the previous paragraph, may well play a role, since qualitatively and quantitatively normal spermatogenesis

is critically dependent on an intact hypothalamus–pituitary–testis axis. On the other hand, it has been reported that endurance exercise is associated with oxidative stress (78). During endurance exercise, there is a 10- to 20-fold increase in whole-body oxygen (O_2) consumption, and O_2 uptake in the active skeletal muscle increases 100- to 200-fold (79). This increase in O_2 utilization may result in the production of reactive oxygen species (ROS) at the rates that exceed the body's capacity to detoxify them (80). An increase in the formation of ROS decreases fertility, as the ROS will attack the membranes of the spermatozoa, decreasing their viability (81). However, some studies have suggested that exercise training enhances antioxidant capacity (82, 83). Indeed, the machinery eliminating ROS adapts after regular exercise and actually lowers the amount of ROS that is produced, especially in the major organs (muscles) of oxygen consumption and ROS production. Exercise training tends to decrease ROS also in body fluids, although no data concerning seminal fluid seem to be available.

Regardless of the exercise protocol studied, increases in DNA damage in peripheral human white cells have been reported, generating the consensus that exercise does indeed induce DNA damage (84). After an exercise bout, DNA damage persists for up to 7 days (85). The presence of high ROS levels has been reported in the semen of between 25 and 40% of infertile men (86). This is because ROS, at high levels, are potentially toxic to sperm quality and function (87). Therefore, persistent ROS formation during continuous strenuous exercise might be harmful for normal spermatogenesis. However, the participation of other maybe unknown factors affecting sperm quality seems plausible (49).

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8

Exercise and the Hypothalamus. Ovulatory Adaptations

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INTRODUCTION

As early as 1939, Hans Selye, who later received the Nobel prize for work on the endocrinology of the adaptation response, reported that muscular exercise was often a cause for “menstrual irregularities” in women (1). Selye performed controlled animal experiments showing that whether or not exercise suppresses reproduction depends on the abruptness of exercise onset (1). Forty years later, Shangold et al. (2) published the first prospective observational study documenting gradual shortening of the luteal-phase length with increased running activity in one woman with regular menstrual cycles. Despite these early observations indicating that subtle alterations of ovulatory function occur within cycles of normal length, the exercise science literature has since focused on the absence (amenorrhea) or presence (eumenorrhea) of menstrual flow in women athletes. The purpose of this chapter is to review the subtle (and clinically important) ovulatory changes in response to exercise.

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Hundreds of cross-sectional studies report “athletic amenorrhea,” and inappropriately imply causal relationships between loss of flow and exercise. However, better-designed prospective studies observing normally ovulatory women and closely examining ovulatory function during progressively increasing exercise (subsequently termed “exercise training”) show only subclinical changes and no amenorrhea when exercise training is the only stressor (3–5). Prevalent but subtle changes in ovulatory function are the first and most subtle hypothalamic adaptation to exercise training (2, 6). Failure of hypothalamic adaptation in response to intense stressors such as starvation, psychological distress, illness, or rapidly increasing exercise, results in significant disability. Overwhelming stress associated with excessive exercise training (Chap. 27) and extreme nutritional imbalance (Chap. 10) are discussed elsewhere in this volume.

In this chapter, we describe the subtle alterations in ovulatory function that occur as a result of hypothalamic adaptation (rather than failure to adapt) to exercise training and other “stressors.” We will also discuss the consequences of ovulatory disturbances, including infertility and a negative bone balance. Before beginning that discussion, however, it is necessary to define both the language and the physiological processes of ovulation.

THE OVULATORY CYCLE

The words used to describe the release of an egg and the hormonal characteristics of a cycle in which that occurs need to be defined and described because both are usually obscured by the persuasive, yet unfounded expectation that regular cycles are normally ovulatory. We will start by defining the language of reproduction.

Terminology

In the exercise science literature, women are commonly classified as eumenorrheic if their menstrual flow occurs monthly, or oligo/amenorrheic if flow is sporadic or has been absent for 3 or more months (7). However, cycles of normal length can also be described by their luteal phase or ovulatory characteristics. Ovulatory and cycle interval characteristics form a complex continuum (Fig. 1). This starts with the most normal cycle type, which is ovulatory with a normal luteal-phase length of 10–16 d and a normal cycle length of 21–36 d (8). This spectrum ends with the most disturbed ovarian function, which is amenorrhea, the absence of flow for 6 or more months. Between these extremes, cycles that are normal in length may have a short (<10 d) or insufficient (normal length, but low progesterone) luteal phase, or be anovulatory. Anovulatory cycles are ones in which cycle intervals may be short, normal, or long, but no egg is released and progesterone levels never exceed 16 nmol/L (5 ng/mL).

DEFINITIONS

Given the importance of clarity in science, it is useful to define the terms meant to describe cycle types when ovulatory status is not known. Eumenorrhea implies menstrual cycles that are normal in length, with flow occurring each 21–36 d (9). When a woman’s flow occurs between 36 and 180 d, the term oligomenorrhea is appropriate. For cycle lengths longer than 180 d, women are classified as experiencing amenorrhea. Cycles of varying and abnormal lengths are called “cycle disturbances.”

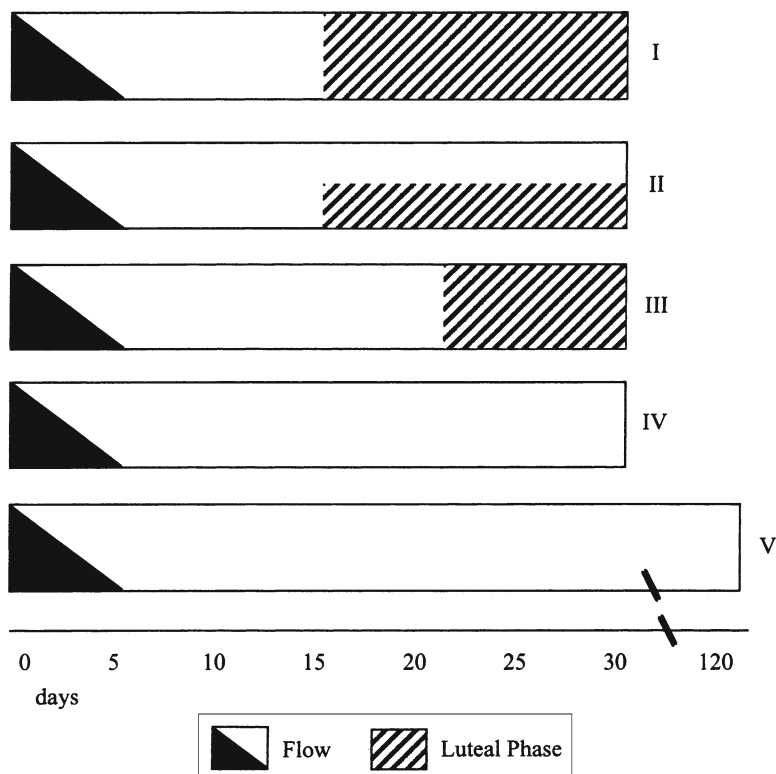


Fig. 1. A spectrum of cycle types starting at the top with the most normal, which is of normal length and ovulatory with a normal luteal-phase length. The next cycle is also of normal length and ovulatory, but has an insufficient but normal-length luteal phase. The third cycle, also of normal length, illustrates a short luteal-phase cycle. The fourth cycle is an anovulatory cycle of normal length, and the final cycle is a anovulatory cycle that is longer than normal in cycle length (oligomenorrhea).

The words used to describe the ovulatory characteristics of a cycle are nearly independent of cycle intervals. Cycles are defined as having short luteal-phase lengths if ovulation occurs but the time from ovulation to the day before the start of flow (luteal phase) is <10 d by quantitative basal temperature (QBT) analysis (8, 10), or <12 d using the midcycle luteinizing hormone (LH) peak as the indicator of luteal-phase onset. An inadequate or insufficient luteal phase means ovulation occurs and luteal-phase length is normal, but progesterone levels during the luteal phase are lower than the usual midluteal peak progesterone level of 45 nmol/L. If ovulation and subsequent corpus luteal formation do not occur, the cycle is anovulatory. Therefore, anovulation refers to cycles in which no eggs are formed (and released). “Subtle disturbances of ovulation” refers to cycles that are normal in length, but have either a short or inadequate luteal phase or are anovulatory. “Ovulatory disturbance” is a general term that includes the range of abnormal ovulatory characteristics, including short and inadequate luteal phase and anovulatory cycles, and generally implies that the cycle length is normal. Cycles that are irregular or abnormal in length and about which ovulatory characteristics are unknown should be termed “cycle disturbances,” and include polymenorrhea (cycles shorter in interval than 21 d) as well as oligomenorrhea and amenorrhea.

IMPRECISE LANGUAGE ABOUT REPRODUCTION

There are several problems with classifying women as eumenorrheic. The term has recently been applied to women who “experienced at least 10 menstrual periods per year” (11), even though this would give average cycle lengths of 36.5 d (which are abnormally long) and should be described as “oligomenorrhea.” Another difficulty with the term eumenorrheic is that it presumes that all cycles of normal length display the same ovulatory and hormonal characteristics. Data from our 1-year prospective study in ovulatory women of varying exercise habits (4) show that normal-length cycles can as easily be anovulatory as ovulatory. In that study, all of the anovulatory cycles were normal in length. Therefore, a further erroneous assumption often made in the literature is that only long or short cycles are hormonally abnormal.

The term “ovulatory” menstrual cycle is also often misused. Researchers often assume a woman is ovulatory if she reports that her cycles are normal in length and regular. Likewise, the term “anovulation” is commonly used as a synonym for amenorrhea, because women whose periods have stopped (unless they have become pregnant or have had a hysterectomy) are not ovulating.

Classifying women only by their cycle intervals implies that the reproductive system works in an on–off manner, rather than displaying the broad spectrum of potential responses described above. Classification of women’s cycles needs to include the entire range of cycle types, because a distinctly different hormonal profile is present in each case. The variability and hormonal physiology of cycles, even those of normal length, are important to understand.

Physiology

Just as cycles vary in interval and ovulatory characteristics, so does the cascade of signals from the hypothalamic gonadotrophin-releasing hormone (GnRH) nucleus to the pituitary gonadotrophin-producing cells. Pituitary messages to the ovarian follicle also change, as do hormones from the ovary that give feedback to the pituitary and the hypothalamus. What follows is an effort to clarify the cycle manifestations of the hypothalamic changes described in earlier chapters.

OVARIAN HORMONE LEVELS DURING THE NORMAL CYCLE

An ovulatory menstrual cycle is characterized by systematic and major changes in the levels of estrogen prior to ovulation (follicular phase) and variations of both estrogen and progesterone postovulation (luteal phase). Follicular-phase estradiol levels during and just after flow average 60–200 pmol/L (levels that are similar to those in children and men). Estradiol levels subsequently rise over the next 7–18 d to a peak just prior to ovulation that is, on average, 220% above the follicular phase baseline (12). There is then a decrease to about 100% above baseline for most of the luteal phase before estradiol levels again return to baseline just prior to menstruation (12). In contrast, progesterone levels, which remain low during the follicular phase (approx 0.5 nmol/L, similar to levels in children and men) increase after ovulation to over 1,400% of follicular-phase baseline values. Progesterone levels remain elevated over 1,000% above baseline during the 10–16 d of active hormonal production by one corpus luteum (12).

The production of estradiol and progesterone is coordinated by, and ultimately dependent on, the timing and magnitude of GnRH pulsatility in the hypothalamus. GnRH stimulates the gonadotrophins, LH and follicle-stimulating hormone (FSH), to be released from the pituitary. LH peaks at midcycle, and directly triggers follicle rupture and egg release. FSH plays an important role in recruiting intermediate-sized follicles and stimulating the dominant follicle that eventually ovulates. In addition, FSH increases LH receptors on ovarian granulosa cells. GnRH, LH, and FSH are all in feedback regulation by ovarian estradiol and progesterone levels. Also, FSH production is actively suppressed by inhibin, a polypeptide hormone whose probably important role in reproductive physiology is still poorly understood (13).

HORMONAL PROFILE CHANGES DURING DISTURBED CYCLES

Hormonal characteristics of cycles that are abnormal in length will be briefly discussed followed by the hormonal characteristics of cycles that are abnormal in ovulatory characteristics. Although few studies have systematically measured estradiol levels in cycles that are short or long, the generalization that shorter cycles have higher estradiol levels is supported by a study in which hormone levels were measured daily during 68 cycles (14). That study documents that shorter follicular-phase lengths are associated with statistically higher estradiol levels. The logic of this observation is that the more estradiol stimulation of the endometrium, the more likely it is to shed causing bleeding. The opposite is true of long cycles—less estradiol stimulation of the endometrium leads to delayed shedding and flow.

The hormonal characteristics of cycles with disturbed ovulation are less clear. The common feature of all disturbed cycles is the lower amount and/or duration of progesterone production. Estrogen and androgen productions are highly variable in individuals with ovulatory disturbances. Evidence for high estrogen levels with anovulatory cycles is most clearly found in studies of women shortly after puberty (15) and in perimenopausal women before menopause (16). In both instances, estrogen levels exceed the midcycle peak equivalent levels for prolonged periods of time. Androgen excess, which is associated with anovulation, is also associated with high estradiol levels (17), obesity, insulin resistance, and varying hirsutism.

Evidence that estradiol levels may be normal in anovulatory cycles comes from our observational prospective study (4). In that group of initially ovulatory women (in whom perimenopause and androgen excess were excluded), the cycles without ovulation were normal in length, and the women who had entirely normal ovulation did not differ in mean estradiol level (measured twice in two cycles a year apart) from the women who experienced anovulation. This flies in the face of the expectation that cycles with disturbed ovulation will have low estradiol levels as had been observed in four women studied by Sherman and Korenman (18). Sowers et al. also have reported low midfollicular estradiol levels in premenopausal women with disturbed ovulation (19). However, several other authors in addition to ourselves have not observed consistently low estradiol levels associated with anovulation (20, 21).

In summary, disturbances of cycle interval are often associated with abnormally low or high estradiol levels (inversely related to cycle length), but ovulatory disturbances may have high, normal, or low estradiol levels and rates of production.

Documentation of Ovulatory Function

This section describes the currently available methods for documenting ovulatory function and the advantages and disadvantages of each. Our primary focus will be to describe the use of QBT, which we have found to be the best available method for continuous, longitudinal monitoring of ovulatory function.

CURRENTLY USED INDIRECT METHODS

All of the currently available methods for assessing ovulation are indirect, except actual visualization of extrusion of a secondary oocyte from the ovary. Because ovulation requires an LH surge and progesterone levels do not rise if ovulation does not occur, serum or urinary measures of the midcycle LH peak and/or progesterone levels are often used as indicators of ovulation. One method is to perform serial samples of serum or urine daily during the midcycle to detect the LH peak. Alternatively, in the week prior to menses, serum (or plasma) samples showing levels of progesterone above 16 nmol/L (5 ng/mL) are indicative of ovulation. The postovulatory increase in progesterone can also be measured in spots of whole blood (22), urine (23), or saliva (24), or by its effect to increase the basal temperature or to inhibit the elasticity of cervical mucus (although this latter effect has not been scientifically evaluated to date).

An estradiol peak is necessary to trigger the midcycle LH peak. Therefore, another indirect assessment of ovulation involves collecting estradiol levels daily with serum samples. Samples must be taken until an estradiol level double the preceding level and over 750 pmol/L is documented. However, midcycle peak estradiol levels may occur and not be followed by an LH peak or by ovulation in premenopausal (as in perimenopausal) women (21, 25, 26). Therefore, an estradiol peak level is not a specific test of ovulation. To a lesser degree, the same lack of specificity is also true of an LH peak (26).

LIMITATIONS OF AVAILABLE METHODS FOR DIAGNOSIS OF OVULATION DISTURBANCES

Serial sampling of blood, saliva, or urine is required to document adequately all of the important ovulatory characteristics (including whether ovulation occurred, as well as luteal-phase adequacy and length) of a single cycle. Using these methods to document several consecutive cycles is very labor-intensive, invasive, expensive, and imposes a high degree of burden on the participating woman. Continuous longitudinal documentation of hormone levels in large numbers of women is, therefore, virtually impossible to obtain using these sampling techniques (3, 5, 26). Similarly, although formerly endometrial biopsy analysis to show the histological changes related to progesterone was considered to be a gold standard for luteal-phase adequacy and length, it has a ± 2 -d SD and is not useful (27). In addition, although gynecologists commonly describe endometrial biopsies as a minor procedure, they are invasive and uncomfortable (and women who have had one often attempt to avoid a second!). Finally, vaginal or abdominal serial ultrasound assessments (to show a growing follicular cyst that enlarges to over 18 mm and then disappears) are considered reasonably accurate indicators of the occurrence of ovulation (28), but they are impractical because they are uncomfortable for women, require expensive equipment and complex scheduling, and are expensive for long-term prospective research.

The logical question is: why not measure ovulatory characteristics during one cycle and then just monitor cycle intervals over the necessary period of time? Could you not infer that the subsequent cycles, if they are regular and normal in length, are similar in ovulatory status? That would be an accurate strategy if women's cycles were as stable in ovulatory characteristics as they are in cycle interval. However, ovulatory characteristics are highly variable over time within women (4, 8, 29, 30). For example, Hinney et al. (29) documented "corpus luteum insufficiency" by a late-luteal-phase progesterone level below 25 nmol/L in 109 women of whom only 55, when tested in the following cycle, continued to show corpus luteum insufficiency. Likewise, 5 year after our intensive monitoring of continuous cycles for 1 year in 66 women, cycle lengths (in three cycles) correlated well with previous ones ($r=0.68$, $p<0.05$), but luteal-phase lengths correlated considerably less well ($r=0.39$, $p=<0.05$) (30).

Furthermore, as this chapter will subsequently document, ovulatory disturbances caused by hypothalamic adaptation occur rapidly and as quickly revert to normal ovulation. Thus, studies that measure ovulatory characteristics in only a few cycles or monitor cycles discontinuously (such as every other or every fourth cycle) are not likely to detect ovulatory disturbances (in general) and particularly not likely to document those related to hypothalamic adaptation to exercise. That is especially true if cycle characteristics are documented only in the cycle before exercise intensity is again increased, as has been done in two important prospective studies (3, 5).

At least 6 month of continuous sampling, in which both ovulation and luteal-phase length are assessed, are necessary to characterize adequately a sedentary, weight-stable woman's menstrual and ovulatory characteristics (31). In exercising women, it is even more critical to provide a robust baseline from which to examine potential changes associated with exercise training. For all of these reasons, a noninvasive, inexpensive, and habit-forming method for documenting ovulatory characteristics is necessary.

QUANTITATIVE BASAL TEMPERATURE

Daily basal (meaning first thing after waking in the morning, fasting, and when metabolism is stable) oral temperatures (often referred to as BBT) potentially allow continuous, longitudinal research into ovulatory characteristics to be conducted in large populations. High levels of progesterone during the luteal phase increase the basal temperature. This increase begins to be significant approx 24–48 h after the LH peak (10). A monophasic set of basal temperatures during one cycle in which our least-squares program (Maximina[®]) detects no significant shift characterizes an anovulatory cycle with progesterone levels that do not rise sufficiently to alter temperatures. A biphasic cycle is indicative of ovulation, and the day of the significant temperature shift can be used to define the onset of the luteal phase (10). In ovulatory cycles, the increased progesterone levels raise the basal temperature during the luteal phase by approx 0.3°C.

However, BBT was a clinical tool before it was a research method. Therefore, the early studies utilizing BBT as a method of detecting ovulation had a number of problems, including that women might take their temperature at different times of day, women had difficulties reading or shaking down the older mercury thermometers, women were expected to plot their own temperatures as a graph (which caused common inaccuracies of graphing), and the temperature patterns were evaluated for the presence

or absence of ovulation using nonquantitative methods and often “eyeball” or equally nonreproducible methods (32). Finally, even when more systematic methods of assessing changes in temperatures were described (33), insufficient data relating the temperature shift to hormonal data were available.

In our laboratory, these problems have been solved by better instruction of women about what, in addition to fever, alters the basal temperature (such as wakening earlier or later than usual, or being up in the night) and providing a form on which to record these factors. In addition, we asked them to take their temperature with a digital thermometer reading to two decimal places and to record temperatures in a list, rather than plotting them on a graph. We then devised and applied a computer program (Maximina®) of least-square analysis to each cycle of temperature data and showed it to be valid against the independently assessed serum LH peak ($r=0.88$) (10). This more scientific method we term QBT to differentiate it from the crude and unscientific BBT methods used in the past.

Thus, we believe we have transformed the previously inaccurate and unreliable BBT method into a scientific tool for documentation of ovulatory characteristics. Furthermore, it is a method that can be easily taught, requires only a relatively inexpensive and durable digital thermometer, and is one that interested women can and will consistently use (4, 34) for lengths of time exceeding a year. Taking of basal oral temperature quickly becomes a habit. However, for this to happen, it does require the interest and commitment of women and of those teaching them.

The major difficulty with widespread use of the QBT method is its lack of accuracy in those whose time of waking and sleeping is variable (e.g., those on shift work, with small children, or students). Ideally, a simpler method, not dependent on a stable life pattern, and requiring less commitment from women would be developed for documentation of ovulatory characteristics in longitudinal studies and for epidemiology.

HYPOTHALAMIC ADAPTATION AND OVULATORY FUNCTION

The neuroendocrine physiology of adaptation to exercise and other stressors is complex and not yet well understood. However, it was reviewed over 10 years ago and much remains the same (35). It has also been covered by earlier chapters, and therefore, it will be reviewed only briefly here. The hypothalamus functions to maintain internal homeostasis in response to internal and external factors. Numerous influences, such as ambient and core temperature changes, energy balance changes, illness (which alters eating and sleeping patterns and may cause elevated temperature levels), and psychological stress, can directly or indirectly alter the pulsatile secretion of GnRH and thus change subsequent reproductive function (36, 37).

The thesis of this chapter is that the first and most subtle adaptive response to exercise training is a shortened luteal phase length in which estradiol levels are commonly normal, but total exposure to progesterone is decreased. As discussed, studies that examine hypothalamic control and the subtle changes that lead to shortening of luteal-phase length with exercise training require long-term, continuous monitoring of ovulatory function.

Ovulatory disturbances in response to exercise training can be viewed as a functional adaptation to the increased physiological stress of the exercise, rather than as part of a disease process (Table 1). The adaptation model suggests:

Table 1
Model choices^a

	<i>Disease</i>	<i>Adaptation</i>
Cause	A single agent or presumed etiology	An integrated interaction of personal and environmental factors
Time-course	Continuous or worsening	Labile and reversible
Disability	A present detriment in function/pain/discomfort	A present positive effect, rare discomfort/concern
Prognosis	Risk for chronic disease, harm/discomfort	Excellent, no permanent impairment (if reversed)
Therapy	Specific (external agent)	Modulation of attitude/environment/lifestyle
	Effective	Do nothing to interfere with the adaptation
	Without major side effects	Cause no harm
Therapeutic relationship (patient:physician)	Passive: authoritarian	Active: consultative, supportive

^aPrior. Reprinted with permission of Human Kinetics Publisher (37)

1. Ovulatory disturbances are caused by a hypothalamic process that is conservative, e.g., protects or saves energy for the individual.
2. They are induced by a variety of physical and psychological “stressors,” which act through a common mechanism and manifest similar changes.
3. There are gradients of change in response to the severity or intensity of the threat.
4. The adaptive changes reverse to the normal baseline steady state when the threat is lessened or eliminated, or the individual has had sufficient time and is able to adapt.

Evidence for these points will be described in the following sections. The specific ovulatory adaptations to exercise training, including the gradients of change and reversibility will be described in the “[Adaptations to Exercise Training](#)” section.

Hypothalamic Adaptive Processes

Evidence that the subtle alterations that lead to shortening of luteal-phase length are controlled by the hypothalamus is largely circumstantial, because altering hypothalamic function biochemically or with direct nerve cell stimulation is impossible in humans. The strongest evidence that the hypothalamus controls changes in ovulatory function comes from the similar pattern of responses during exposures to a whole range of psychological and physiological stressors.

Corticotropin-releasing hormone (CRH) release increases when any internal or external environmental signal is perceived as stressful (as shown schematically in Fig. 2). The increased CRH may either directly or indirectly (via the β -endorphin system) slow the hypothalamic pulsatile release of GnRH (38, 39) and, therefore, decrease pulsatile LH release. Because the pulses of LH stimulate progesterone and estradiol secretion, they provide an important modulator of ovulatory function, although the exact effector mechanisms are as yet unknown.

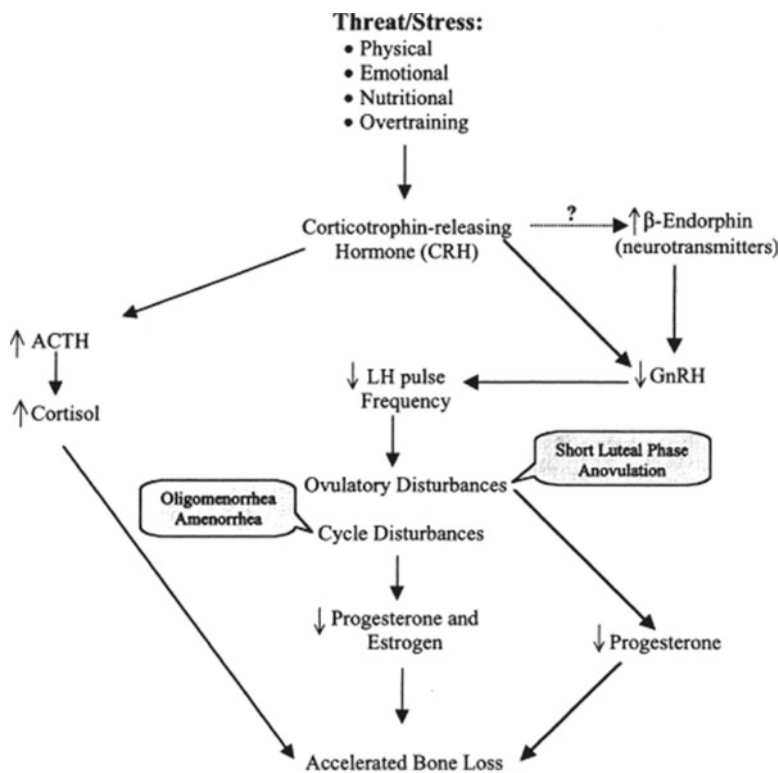


Fig. 2. Process through which physical, emotional, or nutritional challenges cause increased release of CRH from the hypothalamus. These factors suppress the reproductive system and stimulate the adrenal axis. *ACTH* corticotrophin; *LH* luteinizing hormone. Adapted from Prior (36).

Reproductive and primarily ovulatory changes are “conservative” for the individual, because through multiple pathways, they effectively prevent pregnancy when the individual woman is under duress. They are also conservative of energy because less progesterone production, which decreases the otherwise increased core temperature by approx 0.3°C, would mean about 300 fewer dietary calories were needed to ensure energy balance (40).

STRESS MECHANISM

Selye (1) observed nearly 50 years ago that the adrenal glands were hypertrophied when various kind of stressors interrupted estrus in rats. He also observed similar patterns of response of the ovaries and the adrenals to excessive exercise, to interference with normal diet, and to emotional stressors. More recently a strong relationship was also documented between social stress and nonovulation in nonhuman primates. Subordinate primates experienced 16.5% of their cycles as nonovulatory, whereas dominant female primates had only 3.5% anovulatory cycles (41) Cortisol excess that was similar to levels seen in women under stress significantly increased the metabolic clearance of progesterone as well as increasing LH pulse amplitude in experimental studies by Kowalski et al. This research showed that monkeys who were exposed to

induced hypercortisolism had lower luteal-phase serum progesterone levels and ovulatory disturbances (42, 43).

In humans, downward modulation of reproduction during illness was documented by lower than normal LH levels in very ill postmenopausal women (44). Evidence for an adaptive ovarian response to emotional or psychological stress is best illustrated by a prospective study in Japanese nursing students whose regular and apparently ovulatory cycles more commonly showed ovulatory disturbances during the stressful school year than in the summer break (45). Weight loss is known to be one of the most powerful physiological hypothalamic stressors (46, 47). An experimental protocol involving fasting for 3 d in the late follicular phase appears to be more disruptive of follicle development and more likely to alter LH pulsatility in women who are initially very lean than in those who have normal body weights and fat (48). There continues to be debate about what proportion of exercise-related effects on the reproductive system are causally related to relative energy imbalance or are caused by a separate exercise stressor per se (49). Long-term prospective monitoring of ovulatory characteristics and cycle hormone levels, rather than cross-sectional data, are needed to answer this question.

Active women with amenorrhea, like overtrained male athletes (50), have increased basal levels of cortisol (51) and blunted cortisol responses to exercise (52, 53). Berga et al. (52) reported high 24-h cortisol levels in those with hypothalamic forms of “anovulation” compared with normally menstruating women. This hypercortisolemia was not observed in women with other reasons for disturbed cycles, such as hyperandrogenism or hyperprolactemia. A few women initially deemed to have hypothalamic amenorrhea subsequently ovulated during the study and were shown to have concomitantly reduced levels of cortisol (52). Ding et al. (51) could similarly predict women whose cycle intervals would subsequently become normal because their cortisol excretion was decreased. Active women with normal cycle intervals may also have decreased LH and increased cortisol compared to sedentary controls (54). Because cycle lengths are normal, but LH and cortisol levels were altered, it is likely that these women were experiencing subtle ovulatory disturbances associated with exercise training (although ovulatory function was not assessed) (54).

High cortisol secretion or urinary excretion has become a useful marker of hypothalamic adaptive responses to stressors including exercise, because all stressors apparently act through the hypothalamic CRH pathway. Therefore, studies in both humans and non-human primates demonstrate increased cortisol levels simultaneously with decreased LH pulsatility and/or disturbed ovulatory function during reproductive disturbances coinciding with a variety of stressful situations.

It should be noted that although hypothalamic disturbances of ovulation characterized by lower pulsatile release of LH are probably the most common cause for the menstrual cycle disturbances reported in athletes, short luteal-phase cycles or anovulation associated with androgen excess (and with high, rather than low, LH levels) (55, 56) can also be documented. High androgen and LH levels were recently described as a cause of amenorrhea in swimmers (55). In addition, defects of the large corpus luteum cells have been postulated to cause lower luteal-phase progesterone levels, although LH pulsatility and estradiol levels are both normal (29).

ENERGY CONSERVATION

Cycle disturbances are termed “functional,” because they do not represent disease processes. When discussing ovulatory disturbances as protective against excess energy expenditure, the severity of the disturbance is proportional to the amount of energy conserved. Basal metabolic rate (BMR) is 32% lower in severe cases of an energy deficit, such as with anorexia nervosa and amenorrhea, as well as anovulation (57). Amenorrhea in women without an extreme eating disorder may be relatively less threatening, because compared with menstruating women, it appears to lower BMR only 17% (58). Anovulatory cycles, which are normal in length, are also less metabolically costly to maintain than ovulatory cycles and prevent the risk for the high energy demands of pregnancy. The basal temperature rise during the luteal phase increases metabolic rate as evidenced by reported increases in energy intake after ovulation. Barr et al. (40) documented that women’s dietary intake is increased approx 300 kcal/d during the luteal phase of cycles which were confirmed to be ovulatory compared with anovulatory cycles in the same women who had no exercise or weight changes during the six-cycle study.

A shortened luteal-phase length (in contrast to anovulation) occurs in response to the least threatening intensity or kind of stressor. Energy is relatively less conserved when the luteal-phase length is shortened than in anovulatory cycles, because there may be as many as 9 d of progesterone elevation. We believe that shortening of luteal-phase length is the most common adaptive response to stressors, such as weight loss, emotional stress, illness, or exercise training. It is of importance that despite the minimal alteration of ovarian physiology, fertilization and implantation of the egg are still prevented by corpus luteum insufficiency and short luteal-phase cycles.

Synergism or Interactions Among Factors Influencing Ovulatory Function

The concept of adaptation with a common hypothalamic change caused by many different stressors implies that the response to one, such as exercise, would depend on the current state of other factors, such as energy balance or emotional stress. Therefore, it is important to consider those factors that are known to influence ovulatory function and to acknowledge that individuals may respond differently to any given stress depending on the presence of many personal variables (59). Such factors as the individual’s current energy balance, underlying characteristics of the individual (i.e., the levels of reproductive maturation, weight, and emotional well-being), intensity of the threat, and the rapidity with which it is introduced all influence the adaptive response. Multiple emotional and psychological stressors, weight loss or restrictive eating, and the need to feel “in control” all are often perceived as stressful by the hypothalamus and influence reproductive function (Fig. 3). These stressors all appear to act through the common hypothalamic CRH pathway.

ENERGY BALANCE

It is likely that exercise and other stressors affect LH pulsatility through their influence on energy balance (49). This is discussed in detail elsewhere in this volume (Chap. 10, 11). It was postulated in 1982 that hypothalamic insulin receptors might provide a common signal (6). Those who are ill or overexercising would have decreases

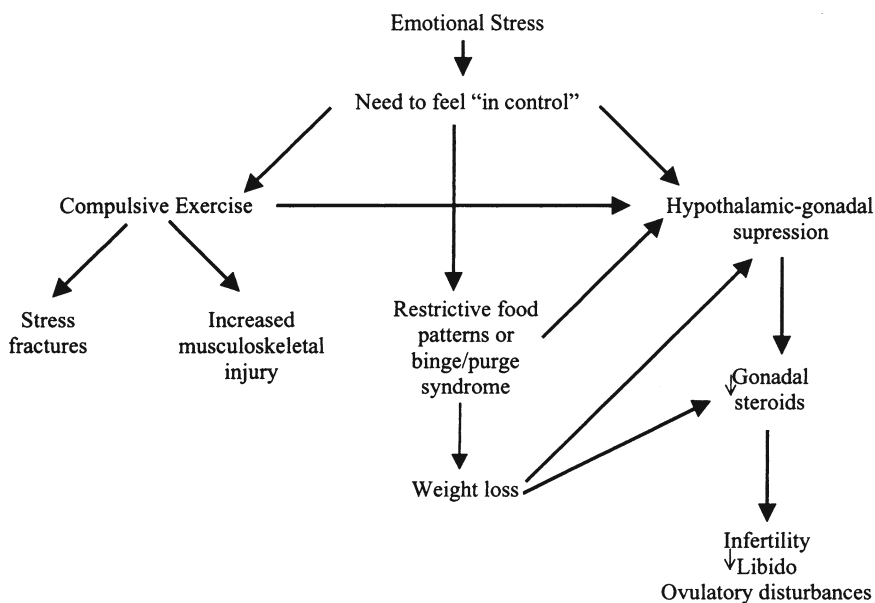


Fig. 3. Interrelationships among multiple factors (stress, compulsive exercise, and restrictive eating) that are causally related to the development of ovulatory disturbances.

in their insulin levels as a consequence of negative caloric balance. It is well accepted that severe weight loss or an extreme energy deficit, such as with anorexia nervosa, suppresses reproductive function. In such extreme cases, CRH levels are high (60), and amenorrhea will likely result. More subtle reproductive disturbances often occur when the relative threat is less severe, but conditions that facilitate pregnancy are not optimal. For example, ovulatory disturbances may occur with healthy weight loss or dieting (61), as well as when recreational exercise or emotional stress increase. In each case, the greater the need for energy conservation, the more severe the cycle disturbance (47, 62).

EATING RESTRAINT

Subtle ovulatory disturbances also occur with “eating restraint,” a psychological attitude in which women feel they must limit food intake to avoid obesity (63). Women who are classified as highly restrained (based on the Three Factor Eating Questionnaire (63)) are very conscious of their food intake, but do not necessarily consume fewer calories than same weight and age-matched controls who are not restrained (64, 65). Because maintaining or achieving their desired weight is so important to their emotional well-being, eating carries with it psychological stress for women with eating restraint. Very early, it was shown that women with higher scores on the Eating Restraint Scale of the Three Factor Eating Questionnaire were more likely to have short luteal-phase cycles (66). Two recent studies from our laboratory also examined ovulatory function and eating restraint in normal-weight, regularly cycling ovulatory women who varied in their usual activity levels (67) and in regularly cycling vegetarian and nonvegetarian women (68). The restraint scale of the Three Factor Eating Questionnaire (63) was administered initially, and menstrual cycle characteristics were documented prospectively

for at least three (67) or six cycles (68), respectively, in each study. In both studies, women in the highest tertile of restraint were significantly more likely than women in the lowest restraint tertile to experience a short luteal phase or anovulatory cycle. These findings could not be attributed to differences in energy intakes, exercise levels, or body mass index (BMI is weight in kg divided by height in m²) levels. Restrained women did not differ in BMI, weight, energy intake, or activity from the less restrained women in each respective population (67, 68). All of the women had consistently normal cycle lengths. Thus, none of these women would have known their cycles were disturbed. They would all have been classified as “eumenorrheic” if ovulation had not been assessed.

It is probable that the effect of eating restraint on ovulatory function is mediated through hypothalamic adaptation pathways. At present, because data examining the potential relationships among cortisol excretion, ovulatory disturbances, and dietary restraint have yet to be published, it is hypothetical that these are hypothalamic adaptive changes. Nonetheless, the evidence that subtle ovulatory disturbances are more common among “highly restrained” eaters, despite similar energy intakes and expenditures, emphasizes that hypothalamic ovulatory disturbances may result from relatively minor psychological as well as physiological stressors.

HYPOTHALAMIC REPRODUCTIVE “MATURATION”

Another variable influencing the ability of the hypothalamic system to respond to stressors is its relative maturity. For example, the majority of menstrual cycles are anovulatory in the first year after menarche (8). However, on average, women do not develop the highest rate of ovulatory cycles (94%) until they are approx 12 year after menarche (8) (or gynecologic age 12). This implies that some are still gynecologically immature. It fits with the adaptation hypothesis that those whose hypothalamic–reproductive axis has not yet become sturdily and regularly ovulatory are more likely than those with mature reproductive patterns to respond to stress with altered reproductive function (45).

One of the first studies documenting the reproductive hormonal characteristics of young athletes showed that both swimmers and controls had short luteal-phase cycles, but in swimmers, the luteal phase was even shorter than in sedentary controls (69, 70). Although subject numbers were small, these data confirm the more extensive data of Vollman (8) that teenagers are susceptible to subtle disturbances of ovulation. Young runners (gynecologic age <10 year, mean chronological age 20 year) are also more likely to have disturbed folliculogenesis and decreased estradiol, progesterone, gonadotrophins, and testosterone levels than are gynecologically mature women (gynecologic age >15 year, mean chronological age 31 year) (71). Therefore, data suggest that the combination of more intense training and an immature hypothalamus is potentially additive in suppressing reproduction in young women.

It has already been shown that women of mature gynecologic age who begin exercise or intensify training only experience ovulatory changes. However, evidence suggests, although appropriate experiments to document it conclusively have not been performed, that a woman in her 20s who is only intermittently ovulatory and begins to exercise or intensifies exercise training may well develop cycle as well as ovulatory disturbances. This young woman would likely develop oligomenorrhea or amenorrhea.

Age at menarche has been shown to be influenced by intense exercise training (72–74). Although genetic factors also have a strong influence on menarcheal age (75, 76), dancers and gymnasts have delayed menarche compared with their sedentary sisters even though they are genetically very similar. Puberty involves maturation of axillary and pubic hair as well as breast enlargement and maturation. Interestingly, when young athletes are forced (often because of injury) to interrupt their gymnastics or dance training, rapid development through one or more Tanner breast stages commonly occurs (74, 77).

Women of young gynecologic age have anorexia most frequently. Weight loss and young age may make them more vulnerable to anorexia. In a similar manner they will likely be more prone to exercise effects on ovulatory function, especially if exercise is combined with restricted energy intake or psychological performance pressure from coaches and parents. It is also possible that women experiencing reproductive and ovulatory disturbances in response to stress when younger will be more susceptible to exaggerated stress responses throughout life (78, 79).

The pubertal maturation of the breast is primarily dependent on ovarian hormones, with little or no influence of adrenal steroids. By contrast, pubic hair maturation can proceed with the normal adolescent increases in adrenal androgen secretion, without significant increases in ovarian hormones. Discrepancy in the degree of breast maturation compared with pubic hair maturation through the Tanner stages is probably a clue to hypothalamic adaptive changes related to exercise training and/or other stressors. Warren et al. (74) reported that pubic hair development occurred at a normal age in young women dancers, yet breast development and age at menarche tended to be delayed. Clinical data from ovarian hormone treatment of male to female transsexuals and observations during a prospective study of puberty suggest that normal breast development to the fully mature Tanner V breast stage will not be reached without adequate exposure to progesterone levels (Prior, unpublished observation).

STRESS INTENSITY

Whether ovulation becomes disturbed partially depends on the intensity of the stress and partly on the rate at which the stress is introduced. For example, all rats responded to “inescapable” shock by suppressed gonadotrophin secretion (80), whereas only some rats are susceptible to the relatively less threatening stress of endurance exercise (81).

Hans Selye coined the term “General Adaptation Syndrome” and published early controlled trials of exercise and energy restriction stress on rats (1). Selye’s experiments showed a dramatically different response to gradually increasing exercise compared with rapid imposition of exercise training (or caloric restriction). Animals who started running at 3.5 km/d developed anestrus (the rat equivalent of amenorrhea) with interstitial atrophy, few mature follicles, and increased weight of their adrenal glands. A second group of rats gradually increased exercise intensity to reach 3.5 km/d over 4 week (Fig. 4). Even though the rats in the second group maintained the same level of exercise intensity as the first group for 2 of the 3 months, reproductive function remained normal and ovarian follicle development was appropriate. Similar differences in response were observed in rats treated with rapid “semi-starvation” compared with gradual decreases in caloric intake (1). Selye subsequently showed a similar pattern of reproductive response in rats that were restrained or separated from their cage-mates or

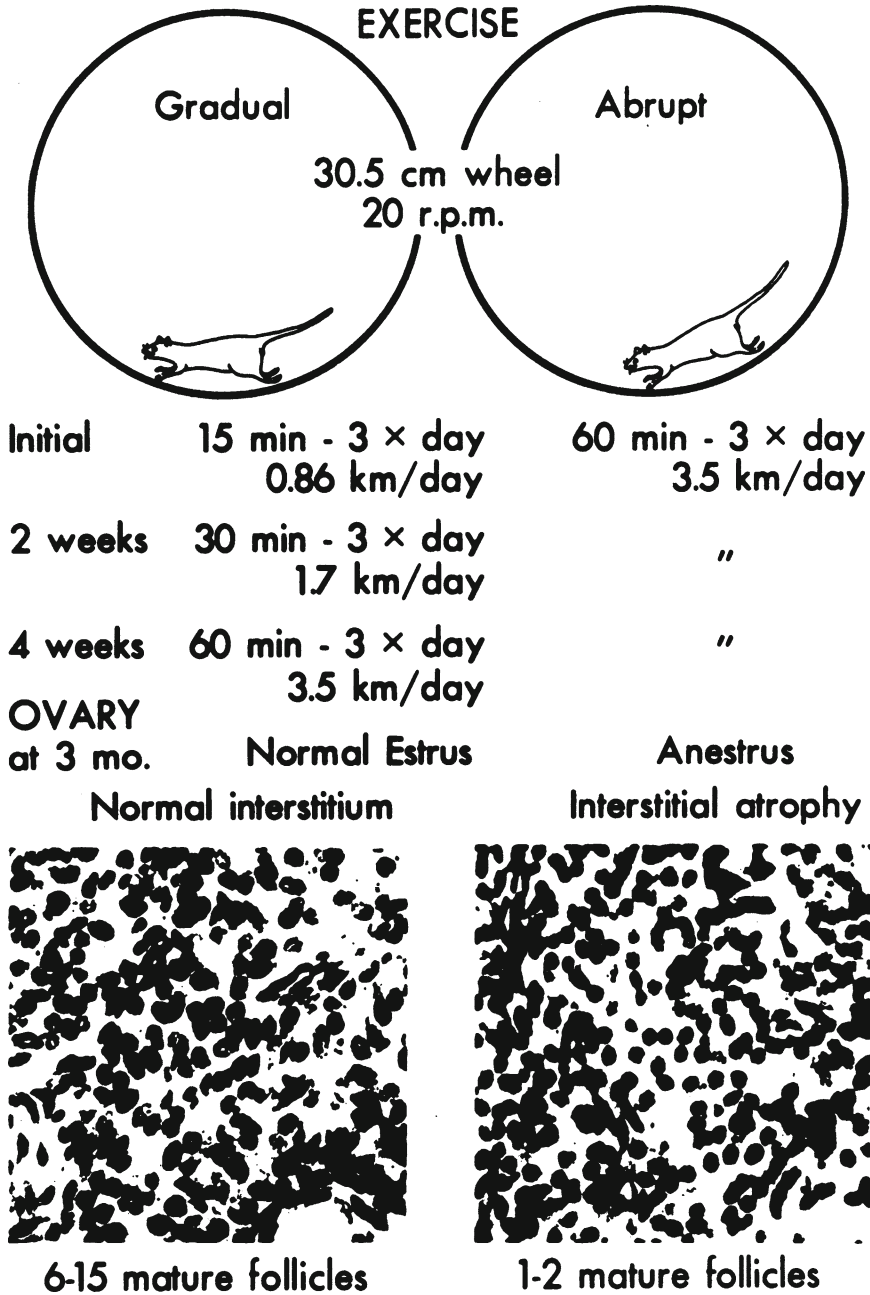


Fig. 4. Illustration of the concept of the "general adaptation syndrome" developed by Hans Selye. Exercise was introduced abruptly or gradually in rats randomized to one or the other group. The photomicrographs show ovulatory adaptation by normal interstitium and follicular development in rats with gradual increase in exercise. Abrupt introduction of exercise led to anestrus (the rat equivalent of amenorrhea), interstitial atrophy, and development of only a few mature follicles. Data redrawn from ref. (1).

siblings. These data suggest that similar mechanisms of hypothalamic adaptation on the reproductive system occur in response to exercise training, weight loss, and psychological stress as well as to illness (44).

In Selye's day, before immunoassays for hormones were available, adrenal gland weights were shown to increase in experimental animals exposed to stressors. Because reproductive disturbances occurred in parallel they were also considered to be "adaptive" and related to a generalized stress response. These observations are consistent with current data showing elevated cortisol levels in women with hypothalamic disturbances of ovulation, oligomenorrhea, and amenorrhea.

The classic animal stress experiments have not been adequately reproduced in humans. However, as will be discussed in more detail below, the data available in humans suggest that a high training intensity and volume can be well tolerated if adequate time for adaptation to that exercise is allowed.

ADAPTATIONS TO EXERCISE TRAINING

Exercise Training Studies in Mature Women

Only a few studies have prospectively documented cycle characteristics with exercise training in mature women. The first prospective documentation, in only one woman, used the elasticity of cervical mucus as a marker of the midcycle estradiol peak to show shortening of the luteal phase associated with an increase in weekly running distance (2). Other early studies showed an increased prevalence of short luteal phase or anovulatory cycles associated with increasing intensity or volume of exercise training (82, 83). In a group of 14 gynecologically mature women (gynecologic age >15 year, mean chronologic age 35 year) who had been training for a marathon, only one-third of a total of 48 cycles prior to a marathon (3 cycles/woman) were biphasic (and thus ovulatory) with normal luteal-phase lengths (83). The only difference between nonovulatory and ovulatory cycles appeared to be the length of the usual training run (from approx 2–5 miles) (83).

A study of longer duration (14–15 month) in women not proven to be initially ovulatory showed a decrease in the volume of menstrual blood and lower estradiol levels with marathon training (84). Running activity increased from 24 to 100 km/week over the study period. Ovulatory characteristics were not examined, however, and the inclusion of participants from 24 to 57 year (84) confounds these outcomes. Nevertheless, in that study and in none of the others to be subsequently described did the women develop amenorrhea, despite rapid increases in running activity/intensity mandated by some protocols.

Three important prospective studies of exercise and reproduction are compared in Table 2. These studies have all sought to establish an influence of exercise training on the reproductive hormonal characteristics of both the follicular and the luteal phases of the cycle and cycle and luteal-phase changes during exercise training: Bullen (26, 85), Bonen (3), and Rogol et al. (5). Because of their importance to this discussion, each study is described in detail below.

Bullen and colleagues (26, 85) monitored 28 college-aged women residing at a summer camp by measuring hormonal characteristics for two cycles using analysis of daily overnight urines and evening temperatures. These women (whose mean age was 20

Table 2
Published prospective studies of exercise training on menstrual cycle and luteal length

Author	<i>Bullen et al. (26)</i>	<i>Bonen (3)</i>	<i>Rogol et al. (5)</i>
Total (n)	28	57	23
Chronologic age	22 (0.6)	30.0 (1.3)	31.4 (1.3)
Gynecologic age mean (SE)	10 (0.6)	17.1 (1.4)	17.8 (0.9)
Study groups	Exercise+ weight maintenance (A) Exercise+ weight loss (B) (max of -0.45 kg/week)	<10 miles/week for 2 month (A) <10 miles/week for 4 month (B) 10-20 miles/week for 2 month (C) 10-20 miles/week for 4 month (D) 20-30 miles/week for 2 month (E) 20-30 miles/week for 4 month (F) 2-4 month	Train at lactate threshold (n=9) Train above lactate threshold (n=8)
Duration of exercise training	2 month	As described above	1 year
Exercise schedule	Running 4 miles/d progressing to 10 miles/d by week 5, plus 3.5 h of cycling, tennis, or volleyball		Start: 6.25 miles/week Week 1-20: add 1.25 miles every second week Week 20-39: hold at 24 miles/week Week 40-end: add 1.25 miles every second week (max of 40 or 65 miles/week)

Exercise intensity	70–80% of max aerobic capacity (adjusted each month)	Not reported	6 d/week ran at lactate threshold 3 d/week ran at lactate threshold and 3 d/week ran above lactate threshold Daily blood samples day 9 through end of cycle	Mean LL 13.9 (0.6)
Sampling method	Daily BBT and daily urinary sampling (overnight)	Daily blood samples	Every fourth cycle	
Sampling intervals	Continuous	Every second cycle		
Luteal length (LL)	Mean LL not available	Control cycle	Cycle 1	13.4 (0.7) 13.8 (0.7)
mean (SE)	Cycle types during training	Run cycle 1 Run cycle 3 (only includes groups B, D, and F)	Cycle 4 Cycle 8	
	Study group			
	%Ovulatory	Detrain, cycle 3 or 5 NA	Cycle 12	12.8 (0.7)
	%Short Luteal Phase			
	%Anovulatory			
Additional stressors	Young gynecologic age Weight loss Away from home Intense exercise training		NA	

year) were confirmed to be ovulatory prior to entry into the study and were also randomly assigned to either weight-loss or weight-maintenance groups. Running activity increased from 4.5 to 10 miles/d by week 5 of the 8-week camp. In addition to running 10 miles/d, women also participated in 3 h/d of varied recreational activities. Bullen and colleagues documented that none of the women in the study developed amenorrhea despite their young age and that they were exposed to several stressors, including change of residence, intense and rapidly increasing exercise training, and caloric restriction (in the weight-loss group). Ovulatory disturbances and shortened luteal-phase cycles were common, however, and only 8 of the 28 women ovulated normally in both cycles. The addition of weight loss to the exercise training caused a further significant increase in ovulation disturbances as well as oligomenorrhea in a few women (26, 85).

Bonen and colleagues set out to determine whether a dose–response between running mileage/week and reproductive function was operative. In particular, by observing sedentary, mature women who ran at varying exercise loads, they tried to determine whether or not a threshold of exercise intensity was present above which luteal-phase disturbances would begin. Bonen (3) monitored mature women over 2–4 month who were variously training at <16, 16–32, or 32–48 km/week. These investigators showed that although there were trends toward shortening of the luteal phase in the first cycle measured after training began, no consistent luteal-phase length changes were documented, nor were there any differences in ovulatory characteristics between women in different intensity groups (3).

As shown in Table 2, the design of the study by Rogol et al. (5) was similar to Bonen's, but used VO_2 max testing to document the anaerobic threshold as when increased lactate was produced. This assessment was used to increase gradually the exercise intensity to maintain it just below or above the "lactate threshold." This allowed them to document more accurately the exercise load, which was gradually increasing over 1 year for all participants whose hormone levels were intensively sampled every 4 month before the next increase in exercise intensity. Rogol et al. (5) also report that neither running intensity nor duration affected ovulatory function in women training for 1 year at increasing intensities that were maintained either above or below their own adjusted lactate threshold.

Several differences exist between the studies of Bullen and those by Bonen and Rogol, which at least partially explain their discrepant outcomes. The rapid introduction of a high volume of training, and the addition of weight loss (26) in Bullen's protocol provides a greater stress load, and would thus be more likely to lead to ovulatory disturbances than an exercise program alone in older women who remained in their own homes and communities (3, 5). In addition, the women in Bullen et al.'s (26) study were significantly younger both in chronological and gynecological age. Another important difference is in design—Bullen and colleagues increased exercise intensity rapidly, whereas the other two studies were more gradual in exercise intensification. Finally, these studies differ in the methods and time-course of monitoring. Bullen et al. (26) monitored cycles consecutively and inclusively. In contrast, Bonen (3) and Rogol et al. (5) assessed ovulatory characteristics intermittently every two or every four cycles, respectively. Shortened luteal phase or anovulatory cycles may have been missed because monitoring occurred after one or three cycles of probable adaptation to a new

exercise load. Any ovulatory disturbances would have likely occurred in the first cycle following the increase in training volume. By the second or fourth cycle after the increase in intensity/duration of training, adaptation would have occurred, homeostatic balance would be achieved, and normal ovulatory function would have returned.

We, like Bullen et al. (26), have monitored luteal length and ovulation continuously, but over 1 year in 66 women of varying self-chosen activity levels (4). As described earlier, all women were confirmed to be normally ovulatory on two consecutive cycles prior to study entry. Despite that, over 80% of the women experienced at least one short luteal phase or anovulatory cycle during the year of study. When the average cycle, luteal phase, and two cycles of hormone levels are used as previously reported (4), no differences were found by exercise habit in the number or severity of ovulatory disturbances, or in estradiol and progesterone levels. That was true regardless of whether the women were completing <1 h of aerobic exercise/week (normally active controls), running more than 1 h/week, but not training for a specific event (consistent runners), or runners increasing training in preparation for a marathon during the study year (4). The reason for ovulation disturbances that did occur was not initially understood. However, we have subsequently found them to be more prevalent in women scoring high on the Restraint Scale, suggesting they are related to dietary restraint (67, 68).

The same study was recently used to compare the characteristics of the premarathon cycle in the marathon-training women with a season-matched cycle in the consistent runners. Exercise training without weight loss can be shown to cause shortening of the luteal phase. The luteal-phase characteristics of the cycle before the marathon were compared in marathon-training women with their own initial and final cycles and the premarathon cycle with a season-matched middle cycle from the consistent runners. Compared to both their own cycles during less-intense training and all of the cycles in the consistent runners, significant shortening of the luteal-phase length before the marathon occurred in the marathon-training women (86).

Hypothalamic adaptation to the runners' baseline exercise probably had occurred before they passed the screening for two consecutive ovulatory cycles and became qualified to enter the study. However, the intensified training before the marathon appears to cause shortening of the luteal phase in the cycle prior to the marathon when their training mileage was the greatest. The detailed dietary, weight, body fat, and hormonal characteristics also monitored before the marathon are being studied for explanations other than exercise training to explain the luteal-phase shortening that was documented. These data all suggest that adaptation to increased exercise, even as intense as training for a marathon, normally occurs with only shortening of the luteal phase in well-nourished, reproductively mature women who have no major emotional distress. In addition, as discussed below, adaptation allows a woman's reproductive system to show a luteal-phase change quite rapidly and then to become normal again.

Observable Changes Prior to Ovulation Disturbances: Molimina

Prior to shortening of the luteal-phase length, which is the first objective change in reproductive function, other observable, but even more subtle changes are commonly reported by mature women who are beginning exercise training. The earliest change with moderate, recreational levels of exercise is a decrease in molimina (87). "Molimina"

whose Greek etymology means “to try hard,” includes the set of physical and emotional, but not troublesome indicators of the coming menstrual flow. Although the so-called premenstrual syndrome (PMS) occurs in both ovulatory and nonovulatory cycles, in the truest sense, molimina indicates that ovulation has occurred. Its most accurate differentiating characteristic is the development, during the week before flow, of breast tenderness only on the high sides of the breasts (Prior, 1987, unpublished observation). An additional indicator of an ovulatory cycle is the disappearance of elastic cervical mucus after the midcycle estrogen surge. Because progesterone inhibits cervical production of elastic mucus, this time pattern of presence and then disappearance of mucus is also a potential indicator that ovulation has occurred.

However, molimina is often confused with PMS, which may include only emotional symptoms and is more apt to occur in cycles with high estradiol levels and decreased progesterone productions (88). This early exercise-training-related decrease in molimina could be associated with the minimally decreased follicular-phase estradiol levels that occur early after onset of training (84). Older evidence says that increased emotional sensitivity, fluid symptoms, and appetite in the week before menstruation are all indicative of ovulatory cycles (89).

We asked the question concerning whether exercise would decrease molimina by studying a group of running women who were increasing their exercise training over 6 months. Exercise training was associated with decreased molimina, especially fluid symptoms and perception of personal stress as well as decreased anxiety despite no change in weight or cycle characteristics (87). Age and weight matched non-exercising women studied in parallel experienced no significant changes in the molimina over the same study period (87) (Fig. 5).

Time-Course of Ovulatory Adaptation

With the addition of more strenuous training, endocrine changes progress from decreased molimina to shortened luteal phase. The next and more disturbed cycle is non-ovulatory. This sometimes occurs as training workload increases (6). The sedentary woman whose training and cycle characteristics are shown in Fig. 6 developed severe back pain during the 12th cycle and did not ovulate. It is likely that she developed anovulation because she not only had the stress of the pain to deal with but also what for her was an important worry, that she would be unable to compete in and finish the marathon for which she had trained so hard.

In a woman with well-established normally ovulatory cycles (probably after gynecological age 12), exercise-training adaptive changes do not normally progress to anovulation. However, if an additional stressor is added, such as illness, weight loss, and/or emotional stress (*see* Exercise Training Studies in Mature Women and Reversibility/Adaptation), anovulation may develop. Amenorrhea will usually not develop unless the woman is of young gynecologic age, is not yet sturdily ovulatory, and has stresses in addition to exercise-training, such as eating restraint or psychological stress, energy imbalance, rapid induction of exercise, or rapid weight loss.

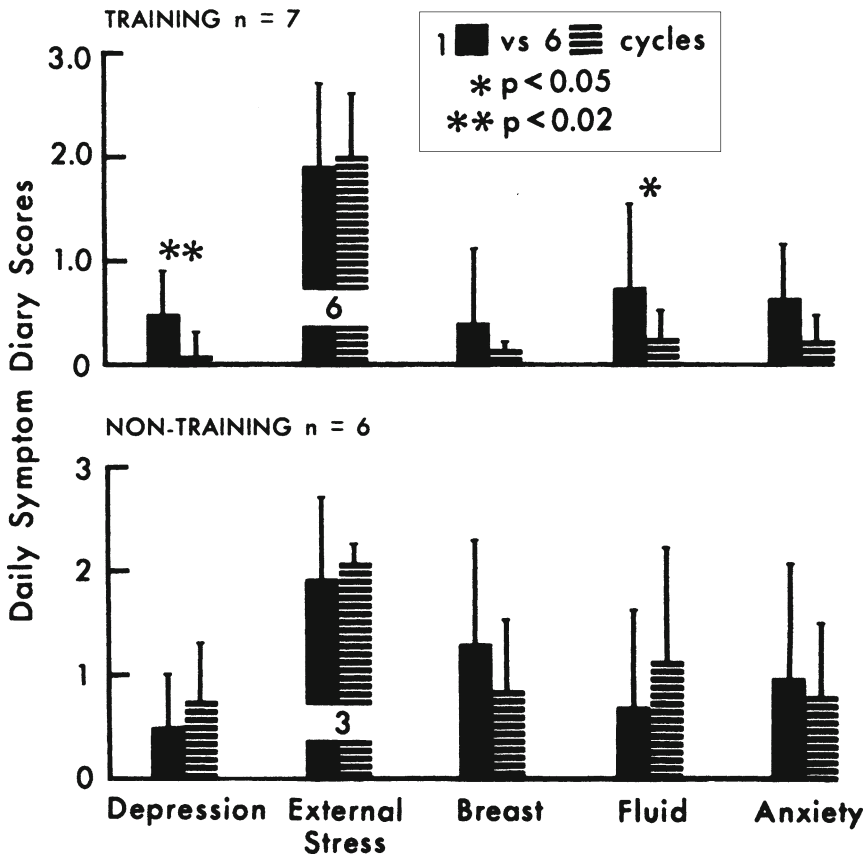


Fig. 5. The progressive changes in minimal experiences are shown as mean \pm SD of daily Menstrual Cycle Diary scores during the 14 d preceding menstrual flow with the first and sixth consecutive menstrual cycle in seven women runners who intensified exercise before a marathon race and in six nontraining women. Nonparametric statistics showed that training women had significantly lower breast symptoms compared with sedentary women during cycle 1 ($p=0.019$). At cycle 6, the two groups differed in depression ($p=0.045$), fluid ($p=0.019$), and breast symptoms ($p=0.006$). For external stress, the data were available on only six training and three nontraining women. Reprinted with permission from ref. (87).

Reversibility/Adaptation

A few case studies are useful to illustrate further the progression and reversibility of ovulatory adaptation. Figures 6 and 7 show luteal-phase lengths as documented by semiquantitative BBT (8) for 1 year of consecutive cycles in two mature, normal-weight women. One of these women, as discussed above, was a sedentary woman who trained for and ran a marathon during the year of observation (Fig. 6). The other was a rather lean and compulsive runner who wanted to become pregnant (Fig. 7) (83). The first woman's prospective record indicated alternating cycles showing short luteal-phases (<10 d) and normal luteal-phase lengths with anovulation during the cycle before and

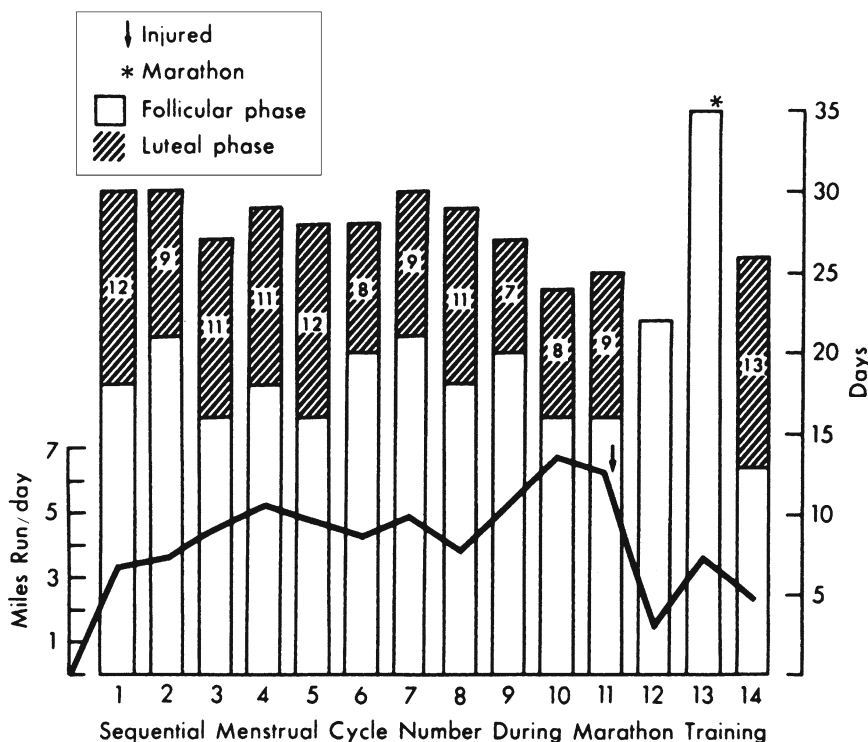


Fig. 6. This *bar graph*, illustrating cycle lengths as *open bars* and luteal-phase lengths as cross-hatched areas within those bars, shows sequential menstrual cycles during 1 year of marathon training in a previously sedentary woman. Note the alternating short and normal luteal phases and progression to anovulatory cycles (in cycles #12 and #13) just after the most intense and highest mileage of training just before and in the marathon cycle. When she decreased her training following the marathon, ovulation and her luteal-phase lengths were restored to normal. Reprinted with permission from ref. (83).

of the marathon race. As mentioned above, the pain and worry as well as exercise training likely accounted for anovulatory cycles. A normal luteal-phase length cycle returned when both her emotional stress and her training workload decreased immediately after her successful marathon.

Figure 7 shows prospective documentation of ovulatory characteristics over 1 year in another woman who was running regularly, but was quite lean and stressed. She showed consistently short luteal-phase cycles early in the year (Fig. 7). In an effort to reverse her secondary infertility, she decreased running for one cycle, but this was emotionally stressful. Inadequate or insufficient luteal-phase characteristics were documented by endometrial biopsy accepted as a cause for her secondary infertility. When she stopped running for approx 6 week, she became pregnant (before a normal luteal-phase length and appropriate endometrial histology could be documented).

These detailed case histories of two women who monitored their individual exercise and ovulatory characteristics over an extended period of time indicate the rapid hypothalamic adaptation and reversibility of ovulatory disturbances related to exercise training (83). These data have been supported in larger samples of women runners (4, 86, 90)

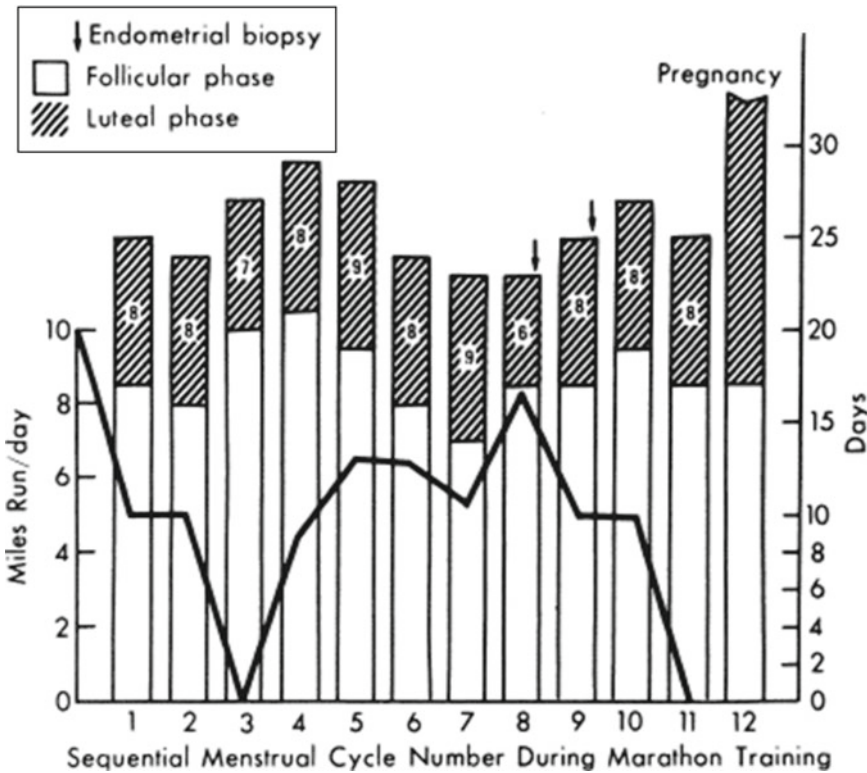


Fig. 7. This bar graph is similar to Fig. 6 and shows the sequential menstrual cycle and ovulatory characteristics in a woman who was training intensely and compulsively. Even with decreased running during the first few cycles, she continued having short luteal-phase cycles. Endometrial biopsies (arrows) were consistent with luteal-phase deficiency. In the middle of cycle 11, she stopped running and became pregnant before a normal luteal phase could be documented. She carried the child to term and delivered a healthy baby. Reprinted with permission from ref. (83).

showing shortened luteal-phase lengths with increased running mileage/intensity and a return to normal luteal length with decreased training volume or at the same exercise load when the stress of the competition is over or adaptation has had time to occur. Animal data also support that endurance exercise training is more likely to influence the first cycle after exercise is initiated rather than later cycles when adaptation has occurred (81). In contrast, perhaps because it is less psychologically stressful, voluntary exercise in rats appears to have no influence on ovulation (91).

Very few data document long-term adaptation to exercise well. As an example, it is useful to observe the second marathon-training year in the woman whose cycles were documented in Fig. 6. The characteristics of the cycle before her second marathon a year after her first are shown in Fig. 8. During the first marathon, she had shown short luteal-phase cycles progressing to anovulatory cycles the month prior to (M-1) and of (M) the marathon. In her second marathon, 1 year later, luteal length remained normal throughout training, even through her training was similar in volume and intensity to her earlier marathon. It appears possible that by the second year she had adapted to the

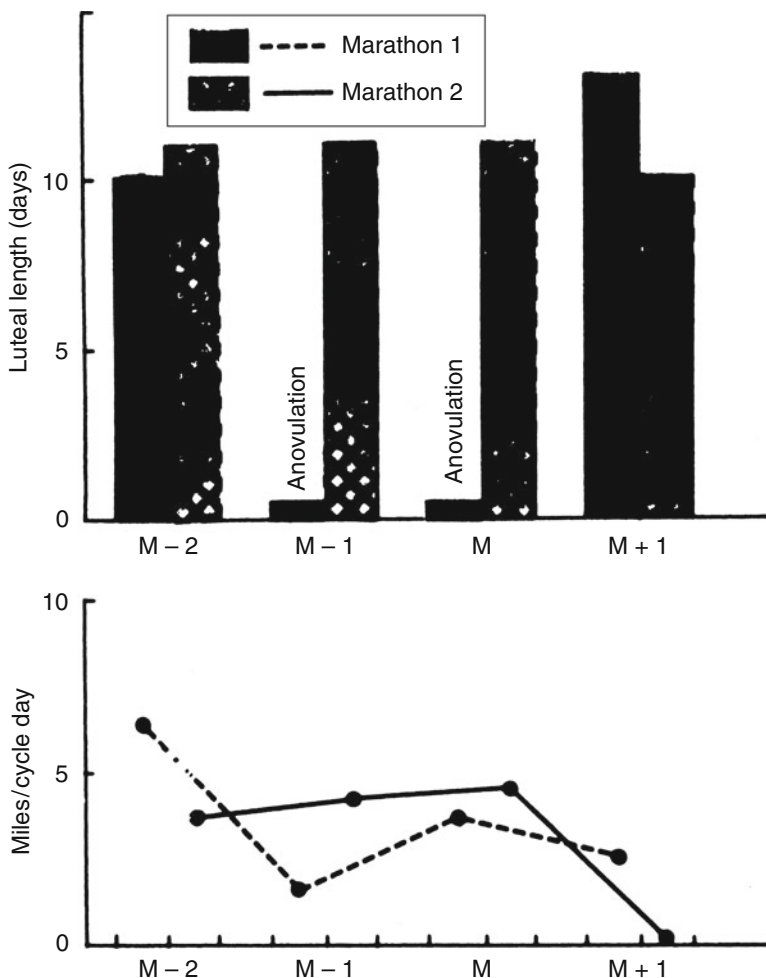


Fig. 8. Luteal-phase lengths during the cycles before and just after a marathon in the first marathon training and race (as shown in Fig. 6) and in subsequent marathon a year later. During the second marathon, despite similar or increased mileage, there were no luteal-phase disturbances documented. This illustrates reproductive adaptation to the levels of exercise this woman was now performing. Reprinted with permission from Prior et al. (92).

marathon training, which allowed her cycles to maintain normal ovulation. Key in each of these stories is the fact that the woman was basically emotionally healthy and maintained normal body weight without significant weight loss.

In mature women, adaptation to exercise training and reversal to normal commonly occur within one cycle. These adaptive changes of luteal-phase length with increasing exercise training are modeled in Fig. 9. Note that, as in the woman described above who trained for her first marathon, by the end of the year, the model suggests that a level of exercise intensity that had provoked ovulatory disturbances now no longer causes a change from a normal ovulatory cycle.

Bullen's study (26) also demonstrates rapid reversibility when training ceased. Although a few women developed oligomenorrhea as well as disturbances of ovulation,

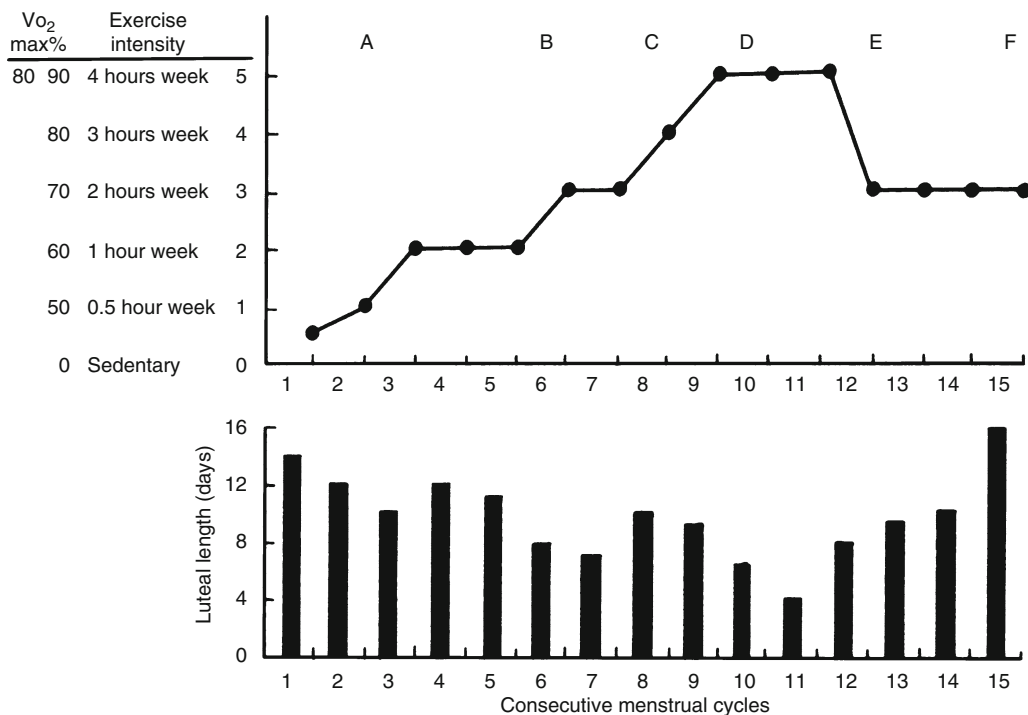


Fig. 9. Theoretical model of the luteal-phase changes that occur over time with increasing exercise in an ovulatory woman who is undergoing exercise training. Note that at the end of the year's sequence of cycles, despite a considerable exercise load, luteal-phase length and ovulation are normal. Reprinted with permission from Prior et al. (92).

all of the women experiencing ovulatory and cycle disturbances (short luteal phase or anovulatory cycles as well as oligomenorrhea) regained both normal cycle intervals and normal ovulatory function within a few months after the end of the summer training camp (26). Furthermore, it is common for athletic women to become pregnant within months of decreasing their training (and competitive stress), even though they may have had several years of anovulatory cycles or amenorrhea (83, 93, 94). However, in exercising younger women, in whom the hypothalamus has not fully matured, the return to or achievement of normal ovulatory cycles will often take longer.

Although the majority of the data just presented were collected using QBT analyses nearly 15 year ago, no studies since have closely examined ovulatory characteristics continuously during several months of exercise training. The development of new methods of monitoring ovulation and luteal-phase length (using blood spot progesterone levels (22)) should soon allow the nuances of cycle adaptation to be more specifically characterized, and mechanisms and modulating factors more carefully delineated.

CLINICAL APPLICATIONS/TREATMENT

The practical and clinical implications of ovulatory adaptation to exercise training are not the purpose of this chapter. However, it is important that the clinician and coach be alert to document persistent changes in luteal-phase length or any anovulatory cycles. These changes are useful indicators that the exercise training load is excessive for that woman's level of hypothalamic reproductive maturation and/or when combined with her other stressors (competitive anxiety, moving, weight loss or eating restraint, or even illness).

If ovulatory disturbances are documented, it is very easy to provide physiological treatment. Persistent ovulatory disturbances should be treated by prescribing either cyclic oral micronized progesterone (300 mg at bedtime) or medroxyprogesterone (10 mg) on d 14–27 of the woman's own cycle (56, 95). Although this "treatment" does not directly correct the hypothalamic stressor that led to the disturbances in the first place, feedback to the hypothalamus by progesterone may aid in the maturation process. The most useful function of cyclic progesterone is to provide physiological levels of progesterone, which will cause regular menstrual flow if estradiol levels are normal.

Cyclic medroxyprogesterone, in a randomized, placebo-controlled 1-year trial, caused a significant 2% increase in spinal bone density in athletic women with abnormal cycles (Fig. 10) (34). In contrast, no prospective controlled study of oral contraceptive use has clearly shown increased bone density, and several large, well-designed studies indicate oral contraceptives may cause skeletal harm (96), especially in young women or primates whose reproductive maturation is incomplete (97, 98).

The most important reason for the clinician to know about ovulatory adaptive responses is to teach each woman to observe and understand the menstrual cycle changes she may experience. In this era of "self-help medicine" molimina, QBT recording, and the concepts of adaptation rather than disease are all beneficial to the health-conscious woman.

CONCLUSIONS

This chapter has reviewed the subtle adaptation of the reproductive system to increasing exercise training gradually. Evidence suggests that changes in luteal-phase progesterone (and perhaps estradiol) production and duration are the first and the major adaptive response of the hypothalamic–pituitary–ovarian system to increasing exercise intensity. These ovulatory disturbances are commonly perceived by decreases in molimina—this is often seen as both a physical and emotional benefit and will motivate women to continue to exercise. If no additional stressor other than the exercise is present, the luteal-phase changes will reverse to normal in the next cycle, even though the exercise level is maintained.

Although more data are needed, it is likely that these physiological and psychological changes during exercise training are conservative for the individual, are reversible, and cause no long-term harm. However, if luteal-phase defects persist, bone loss occurs (4). In addition, fertility is impaired by luteal-phase defects. Persistence of ovulatory disturbances may be commonly related to the psychological stress of dietary restraint (67, 68).

The benefits of exercise for cardiovascular (99), skeletal (100, 101), and emotional health (102) are well supported, yet the concept persists that exercise causes women

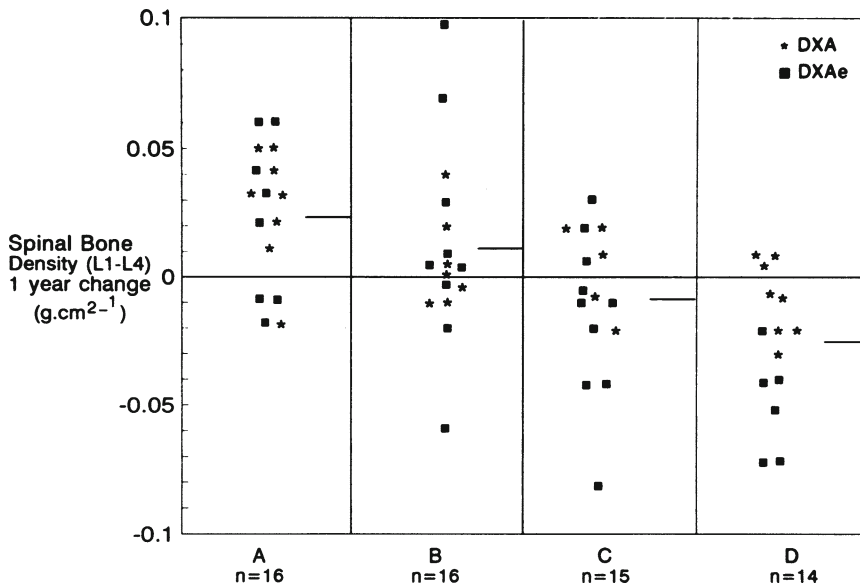


Fig. 10. This data table shows as individual marks the rates of 1-year spinal bone mineral density change by dual-energy X-ray absorptiometry (DXA) in 61 active women with abnormal menstrual cycles randomized to receive medroxyprogesterone acetate cyclically for 10 d/cycle (MPA) with or without active/calcium therapy or placebo. It shows a significant 2.5% loss of bone in D, the double-placebo group, despite the fact that these women were of normal weight, had good exercise habits, and took an average of 1,000 mg/d in their diets. By contrast, women in A who were similar and randomized to receive double-masked therapy with cyclic MPA and an extra 1,000 mg of supplemental calcium experienced an average 2% increase in bone density. Reprinted with permission from ref (34).

major reproductive harm in the form of amenorrhea. In this chapter, we have attempted to erase that perception by viewing the body's responses to exercise training as adaptive. When increasing levels of exercise are introduced gradually, adaptation can occur and the result is a minimal change. Ovulatory disturbances occur normally when initiating a more intense training program or increasing the exercise load, but will reverse rapidly to normal once adaptation has occurred (103). When taken to an extreme or combined with other psychological or physiological stressors, exercise can, as is always emphasized, become negative. In that circumstance, persistent ovulatory disturbances occur, which depending on the age, nutritional state, and emotional support of the woman, may progress to oligomenorrhea or amenorrhea.

Amenorrhea, although it is uncommonly associated with exercise in mature, ovulatory women, may occur in the face of exercise combined with a negative energy balance or when several stressors coexist, especially in women who have never established regularly ovulatory cycles. Gynecological immaturity is a significant factor in the ability of women to adapt to exercise stress. This implies that caution should be taken in the intensity and rate of exercise training with young athletes.

In summary, although the concept of adaptation to exercise training has been known for 50 years (1) and has been applied to women's reproduction for over 16 years (83) few well-controlled studies have documented the most subtle evidence of this

adaptation: ovulatory disturbances. Much work remains to be done to document the variables that influence the hypothalamic reproductive centers to change their signals and the ovarian as well as adrenal responses to these alterations.

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9

Adrenergic Regulation of Energy Metabolism

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INTRODUCTION

During exercise, energy turnover increases and adrenergic mechanisms play an important role in this regulation. In addition, increased adrenergic activity during exercise also results in an increased heart rate and in an enhanced force of myocardial contraction as well as in vasoconstriction in the splanchnic circulation, in the kidneys, and in noncontracting muscles. These circulatory changes favors a redistribution of blood flow to exercising muscle as well as an increased cardiac output (*1*). Furthermore, the adrenergic activity stimulates sweat glands and thereby influences thermoregulation, and it causes an increased contractility of skeletal muscle as well as influences exercise-induced suppression of components of the human immune system. In the present chapter, it is demonstrated how adrenergic activity can influence substrate mobilization and utilization both directly and indirectly via secretion of hormones.

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ADRENERGIC RESPONSES TO ACUTE EXERCISE

Adrenergic activity can be assessed both by direct measurements of electrical activity in superficial sympathetic nerves and by measurement of circulating norepinephrine and epinephrine in the blood. The direct recording of sympathetic activity can be performed to resting muscle only, but during exercise of, e.g., the arms, sympathetic activity to the resting leg muscle has been shown to increase with progressively increasing intensity of arm exercise (2). In addition to these measurements, a correlation has been found between sympathetic nerve activity and plasma levels of norepinephrine (3). Although a correlation between circulating norepinephrine and direct recordings of sympathetic nerve activity from the peroneal nerve has been demonstrated during exercise, the increase in sympathetic outflow to the various regions of the body differs somewhat during exercise. During exercise, using methods to measure norepinephrine spillover, it has been demonstrated that the increase in sympathetic activity during exercise is dominated by an increased sympathetic activity directed toward active muscle. During two-legged exercise, approx. 50% of all circulating norepinephrine is released from sympathetic nerve endings in active muscle. Furthermore, when arm exercise is added to leg exercise, the norepinephrine spillover from active leg muscle also increases despite unchanged work output and unchanged blood flow to the leg muscles (4).

In addition to norepinephrine released from sympathetic nerve endings, epinephrine is released from the adrenal medulla in response to sympathetic neural activity during exercise. The circulating epinephrine is responsible for the major adrenergic effect on energy metabolism during exercise compared with norepinephrine. In the present chapter, the adrenergic effect on carbohydrate and fat metabolism will be discussed, but epinephrine per se has been shown also to increase protein metabolism in isolated electrically stimulated rat muscle (5).

The levels of circulating free norepinephrine and epinephrine increase with exercise intensity expressed by the percentage of maximal individual performance ($\%VO_2$ max). This holds true both during prolonged exercise as well as in response to short-term intermittent exercise and to intense weight training. The increase in plasma norepinephrine and epinephrine occurs rapidly in arterial blood, and it has been calculated that the half-life of epinephrine is around 2–3 min during exercise. Circulating levels of catecholamines can only be considered as overall markers of sympathoadrenergic activity and are influenced not only by secretion, but also by clearance of the hormone. Whereas clearance of norepinephrine is difficult to determine on a whole-body level owing to the fact that it is extracted at two levels in series, namely both the lung and the systemic organs (6), the turnover of epinephrine can be studied in humans, using a radiolabeled tracer. It has been shown that whole-body clearance of epinephrine increases by 15% at low exercise intensities and decreases around 20% below basal levels after more intense exercise (7). However, since the increase in plasma epinephrine seen during dynamic exercise in humans is five- to tenfold, these changes are caused by increases in secretion from the adrenal medulla rather than by changes in clearance. Among the major contributors to epinephrine clearance are the hepatosplanchnic area and the kidneys.

MOTOR CONTROL AND REFLEX INFLUENCE ON ADRENERGIC RESPONSE

In experiments using partial neuromuscular blockade to weaken the muscle force and thereby increase the motor center activity needed to produce a certain force output, it was found that exercise-induced increases in levels of circulating catecholamines were augmented compared to control experiments with saline infusion (8). These findings are supported by experiments in paralyzed cats where direct stimulation of the subthalamic locomotor areas in the brain resulted in adrenergic hormonal responses similar to the ones seen during voluntary exercise (9). Together, these experiments support the view that motor center activity can directly stimulate sympathoadrenergic activity during exercise directly and independently of feedback from contracting muscle. That central factors linked to exercise intensity are not sufficient to elicit a maximal adrenergic response can be demonstrated in different ways. When exercising a small muscle group (e.g., one knee extensor) even at maximal intensity, only a small catecholamine response can be observed (4). Furthermore, when maximal work output was reduced by more than 60% with a neuromuscular blockade (tubocurarine), despite subjects working at the highest possible effort, adrenergic responses were far from maximal (10). In addition to central factors, peripheral neural feedback can be demonstrated using lumbar epidural anesthesia in doses sufficiently high to block impulses in thin afferent nerves, but preserving motor nerves and the ability to perform exercise to the highest possible degree. During static exercise, but not during dynamic exercise, catecholamine responses were inhibited when afferent responses were absent (11, 12). Interestingly, both ACTH and β -endorphin responses during submaximal exercise were abolished during epidural anesthesia (11, 13). In support of a role of afferent nerves in adrenergic responses, plasma catecholamines increased in response to direct stimulation of these nerve fibers in cats (14). An alternative model to study feedback mechanisms during exercise is to use patients with metabolic deficiencies. Both in myophosphorylase (McArdle's disease) and phosphofructokinase deficiency and in mitochondrial myopathy, an excessive neuroendocrine response and exaggerated mobilization of extramuscular substrate (glucose and free fatty acid (FFA)) was found, most likely a coupling toward the oxidative demands of the muscle cell rather than to the oxidative capacity of the working muscle (15–17).

ADRENERGIC ACTIVITY AFTER PHYSICAL TRAINING

Vigorous endurance training will reduce the catecholamine response to a given absolute workload (18), whereas neither sympathetic nerve activity nor norepinephrine levels at maximal workloads differ between individuals with different training status (19). This supports the view that physical training does not alter the capacity of the sympathetic nervous system, but that responses to submaximal exercise are linked closely to the relative rather than to the absolute workload (20). Surprisingly, however, it has in a 24-h study been found that highly trained individuals had a higher catecholamine release over the day compared with sedentary individuals (21). Epinephrine response in trained individuals vs. sedentary has been shown to be enlarged when

stimulated by a variety of stimuli, such as hypoglycemia, caffeine, glucagon, hypoxia, and hypercapnia (20, 22–25). This indicates that the capacity to secrete epinephrine from the adrenal medulla improves with training. In rats who underwent 10 weeks of intense swim training, the adrenal medullary volume and the adrenal content of epinephrine were larger in trained rats compared with controls who were either weight matched, sham-trained, or cold-stressed (26). Although these findings indicate that the improved secretion capacity of epinephrine is a result of training, this will most likely require several years of training. In well-trained athletes who underwent hypoglycemia before and 4–5 weeks after an injury that resulted in inactivity, epinephrine responses did not change with this short-lasting alteration in activity level (27). However, still it is interesting that endocrine glands apparently are able to adapt to physical training and alter their secretion capacity, similar to other tissues like muscle and heart.

HEPATO-SPLANCHNIC GLUCOSE PRODUCTION AND ADRENERGIC ACTIVITY

During intense exercise the rise in hepatic glucose production was parallel with a rise in plasma catecholamine levels (28–30). In addition, in models where electrically induced cycling was used in spinal cord-injured individuals with impaired sympathoadrenergic activity, hepatic glucose production was abolished (31).

In swimming rats, the removal of the adrenal medulla reduced the hepatic glycogenolysis (32), as well as the exercise-induced increase in hepatic glucose production in running rats (33). However, most studies have been unable to demonstrate any effect of epinephrine on liver glycogen breakdown during exercise (34–37). In running dogs, evidence has been provided that epinephrine may play a minor role in liver glucose output late during exercise (38) probably owing to an increased gluconeogenic precursor level. Furthermore, adrenalectomized individuals maintain a normal rise in hepatic glucose production during exercise (39), and only when epinephrine is infused in these patients, hepatic glucose production was augmented during the early stages of exercise (unpublished observation).

Direct stimulation of liver nerves caused an increase in hepatic glycogenolysis, and the hypothesis has been put forward that liver nerves are important for the exercise-induced rise in liver glucose output. In contrast to this, surgical or chemical denervation of the liver in various species did not reduce the exercise-induced increment in hepatic glucose production (32, 33, 40, 41), which indicates that sympathetic liver nerves are not essential during exercise. In humans, the role of liver nerves and epinephrine has been studied with application of local anesthesia around the sympathetic celiac ganglion innervating liver, pancreas, and adrenal medulla (42). Pancreatic hormones were standardized by infusion of somatostatin, glucagon, and insulin. During blockade, the exercise induced epinephrine response was inhibited by up to 90%, and presumably liver nerves were also blocked, but this did not diminish the glucose production response to exercise. This indicates that sympathoadrenergic activity is not responsible for an exercise-induced rise in splanchnic glucose output. In further support of this hypothesis, the exercise induced increase in liver glucose production was identical in liver-transplanted patients compared to healthy control subjects as well as in kidney-transplanted patients who received a similar hormonal and immunosuppressive drug treatment as

liver-transplanted patients (43). Liver-transplanted patients were investigated approx. 8 months after surgery, and no sign of reinnervation occurred in any of the patients as judged by the content of norepinephrine in liver biopsies (44). Finally, in recent experiments in exercising dogs who underwent a selective blockade of hepatic α - and β -receptors, it was demonstrated that circulating norepinephrine and epinephrine do not participate in the stimulation of glucose production during intense exercise (45, 46). Taken together, sympathetic liver nerves or circulating norepinephrine play no role in glucose mobilization from the liver during exercise, and circulating epinephrine only plays a minor role during intense exercise and late during prolonged exercise.

ADRENERGIC EFFECT ON SKELETAL MUSCLE CARBOHYDRATE METABOLISM

Muscle contractions per se increase glucose uptake, and humoral factors can modify this (47). Insulin and contractions have a synergistic effect on glucose uptake with contractions (48), whereas epinephrine has been demonstrated to decrease glucose clearance in running dogs (49). In addition to this, femoral arterial infusion of epinephrine into an exercising leg in humans caused a reduction in the normal exercise-induced glucose uptake (50). More recently, it has been shown that in adrenalectomized individuals performing leg cycling for 45 min at 50% VO_2 max followed by 15 min at 85% VO_2 max, the rise in glucose uptake during exercise was reduced when epinephrine was infused to substitute plasma epinephrine levels normally observed during exercise (unpublished observation). The mechanism behind this is at present unknown, but could be related to an enhanced glycogenolysis, increased intramuscular glucose concentration, or altered uptake of FFA, all changes that can influence glucose uptake.

It has been shown that adrenergic activity can enhance the glycogen breakdown in muscle during contraction both in exercising animals (51) and in humans (50, 52). However, those studies often used supraphysiological doses of epinephrine, and later studies in humans using lower doses have only been able to demonstrate a higher activation of phosphorylase, but could not demonstrate any marked increase in glycogen breakdown (53). Noradrenergic activity probably does not play any role in muscle glycogenolysis, since unilateral hindlimb sympathectomy did not diminish glycogen breakdown in swimming rats (54).

SYMPATHOADRENERGIC ACTIVITY AND FAT METABOLISM

Lipolysis in fat tissue is enhanced by β -adrenergic activity, and catecholamine responsiveness of β -adrenergic receptors in adipose tissue is increased after acute exercise (55). By the use of microdialysis of subcutaneous abdominal tissue, it was demonstrated that nonselective β -adrenoceptor blockade inhibited the exercise-induced increase in dialysate levels of glycerol (56). Although this indicates a role for adrenergic activity in fat metabolism during exercise, the relative role between sympathetic nerve activity and circulating norepinephrine/epinephrine is currently not known. Intravenous infusion of epinephrine in resting humans caused an increase in lipolytic activity as determined by microdialysis of subcutaneous adipose tissue, an effect that was desensitized by repeated epinephrine infusions (57). The direct role of sympathetic nerve

activity on adipose tissue has recently been addressed using microdialysis, and it was found that during hand-grip exercise, the increase in umbilical glycerol release was attenuated in spinal cord-injured individuals with impaired sympathetic nerve activity when compared with healthy control individuals (58). It should be noted that this very moderate type of stress was not able to document any increase in lipolysis in the clavicular region. Furthermore, in a recent study, glycerol output in subcutaneous abdominal adipose tissue was found to be lower during prolonged arm-cranking in spinal cord-injured individuals compared with controls performing a similar relative workload (unpublished observation). Taken together, indices are provided that sympathetic nerves to adipose tissue stimulate lipolysis directly during exercise. If regional differences (visceral vs. subcutaneous fat) exist in responsiveness of the adipose tissue toward increased sympathetic activity, this could play an important role in the treatment of adipositas.

Not only adipose tissue, but also intramuscular fat can be stimulated by catecholamines, and both lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) play important roles in this regulation (59). HSL might be under control by both contractions and epinephrine, and it has recently been shown that activation of HSL and glycogen phosphorylase occurs in parallel in adrenalectomized individuals who receive infusion with epinephrine during exercise (unpublished observation). This could indicate that mobilization of intramuscular triglyceride and glycogen occurs simultaneously stimulated by adrenergic activity, and that choice of substrate for energy production takes place at another level.

SUMMARY

Physical exercise causes an increase in adrenergic activity that can be determined both by changes in plasma catecholamines and in intraneural sympathetic activity. Release of norepinephrine from contracting muscles and release of epinephrine from the adrenal medulla are major contributors to high levels of plasma catecholamines. Both feed-forward stimulation from motor centers in the brain and afferent impulses from working muscles stimulate sympathoadrenergic activity, and a coupling to oxidative demands of the working muscle is likely. Long-term physical training increases the size and secretory capacity of the adrenal medulla, which may improve exercise capacity. Sympathoadrenergic activity only plays a minor role in regulation of hepatic glucose release, but via depressing insulin secretion and influencing target tissue adrenergic activity improves glycogen and fatty acid mobilization.

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10

Energy Balance and Weight Control (Male and Female): Considerations

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INTRODUCTION TO ENERGY BALANCE

Energy balance is a reflection of an individual's energy intake vs. expenditure, and is manifested in their body mass. When intake is similar to expenditure, neutral energy balance occurs and body mass remains stable. However, when intake exceeds expenditure, positive energy balance occurs (also termed energy excess); the body stores the excess energy and body mass increases. The opposite results when expenditure exceeds intake, termed negative energy balance or energy deficit (30). It is important to keep in mind that intake and expenditure should be compared over the long term rather than for shorter time periods as it is nearly impossible to match them on a daily basis. When even small deficits or excesses in daily energy balance occur day after day, substantial changes in body mass can result. For example, the addition of daily sugar-sweetened beverages to one's diet resulted in substantial increases in body mass and increased risk for type II diabetes in a 4-year cohort analysis of women in the Nurse's Health Study II (33). These researchers found that by increasing the consumption of sugar-sweetened beverages from less than one per week to one or more per day, these women consumed

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an extra 358 kcal/day and gained ~4.5 kg on average (33). Thus, seemingly small additions to one's daily diet can quickly add up to substantial changes in body mass and energy balance.

An individual's total daily energy expenditure is comprised of three main components: resting metabolic rate (60–75% of the total), diet-induced thermogenesis (10–15%), and activity thermogenesis, which can be further subdivided into exercise and non-exercise components (14). Resting metabolic rate (RMR) is closely related to the amount of fat-free mass (i.e., lean mass) and tends to be greater in men compared to women (29). These sex differences are attributable to the greater amount of fat-free mass in males vs. females, on average. Basal metabolic rate decreases with age, and research has shown that it is 4.6% lower in older participants, compared to younger (20). Lean mass is also related to diet-induced thermogenesis, such that greater amounts of lean mass are associated with more calories being burned following a meal. Finally, several other factors are related to diet-induced thermogenesis including age, sex, fitness level, and menstrual cycle phase. Some research has shown that body composition and physical activity levels were more closely related to energy expenditure (EE) than were age or sex (20). In fact, fat-free mass had the strongest relationship with 24-h EE, and the results were similar between women and men ($r^2=0.79$ for females and 0.76 for males) (20). The number of kilocalories an individual burns each day via activity thermogenesis varies widely. In general, daily physical activity levels and related caloric expenditure from activity thermogenesis decline with age in both men and women (14). Research on sex differences in physical activity levels is mixed; some researchers have reported lower levels of physical activity energy expenditure in women compared to men (29), while others have reported no difference (14). Similarly, sex differences in measures of EE disappear after taking body composition into account (EE per kg fat-free mass). Taken together, these sex differences in resting metabolic rate (RMR), diet-induced thermogenesis, and activity thermogenesis begin to explain how men and women differ in overall energy balance and weight control.

After reviewing the components of daily energy expenditure and related energy balance, one can see that there are many factors involved in energy balance and the propensity to change or maintain body mass. These include sex, age, menstrual cycle phase in women, body composition, level of physical fitness, genetic predisposition, as well as numerous hormones. This chapter will examine the relationship between these variables with particular emphasis on the roles of hormones in weight control for males and females.

HORMONES INVOLVED IN ENERGY BALANCE, FAT DISTRIBUTION, AND WEIGHT CONTROL

Numerous hormones influence energy expenditure and body mass in humans. Some of the same hormones also influence body fat distribution, and the patterns of storage differ greatly between men and women. For simplicity, the hormones are grouped according to their primary functions: metabolic-, sex-, and stress-related hormones. Their roles and impact on body mass and weight control are explained in each respective subsection.

Metabolic Hormones. Several hormones with metabolic functions impact energy balance in humans, namely leptin, insulin, and ghrelin. *Leptin* was discovered most recently and is a catabolic hormone that provides satiety signals to the brain (13, 15, 34, 42). It is released by adipose tissue and is closely related to the amount of fat mass in adults. Specifically, leptin is released in greater quantities from subcutaneous fat stores than visceral locations (11). Some researchers suggest that leptin is more closely related to total body fat levels in females compared to males (41). It follows that these authors reported that females are more sensitive to leptin's actions than are males. Leptin plays an important role in regulating long-term energy balance rather than acute fluctuations following each meal (16). Once released, leptin, along with insulin, targets the hypothalamus where it induces feelings of fullness, signaling for the individual to stop eating. However, leptin's role in energy balance is complex as it is influenced by numerous other hormones including thyroid hormones, cortisol, insulin, and growth hormone (27).

Insulin is another metabolic hormone involved in energy balance. It is secreted from the β cells of the pancreas in response to increases in blood glucose. Insulin levels are indicative of visceral fat levels in humans (6, 10). The correlation between body fat and insulin is particularly strong in males, and some authors suggest that men are more sensitive to insulin than females (41). Thus, these sex-related differences in insulin and leptin sensitivity provide a possible mechanism explaining the metabolic differences in weight control among men and women.

Similar to leptin, insulin reduces appetite over the long term (13, 15, 16). As a result, these hormones are often classified as anorexigenic. Ironically, individuals with excess body mass (i.e., overweight or obese) often display resistance to leptin and/or insulin (13, 15, 25). This suggests that being in a state of chronic positive energy balance alters the body's ability to respond to satiety cues and regulate blood glucose levels. These changes also explain why overweight and obese men and women are at an increased risk for developing impaired glucose tolerance and subsequently type II diabetes.

Finally, *ghrelin* is another metabolic hormone impacting energy balance. It stimulates hunger in the short term and, not surprisingly, is released in great amounts by the stomach (16, 21, 22, 44). Ghrelin levels fall after meals in a manner proportional to the energy load of the meal consumed, suggesting that this hormone plays a role in inducing satiety and regulating energy balance (16, 22). Interestingly, ghrelin's actions are opposite of insulin, although ghrelin plays a role in its release (16). Insulin and ghrelin are negatively correlated; individuals with high insulin levels tend to have low ghrelin levels. It is not surprising that this hormonal profile of elevated insulin and low ghrelin is common in overweight and obese individuals in particular, as ghrelin levels and BMI are inversely correlated (16, 26).

Ghrelin's regulation of acute energy balance in the short term is due to its effect on the hypothalamus as it stimulates the release of numerous signals that increase hunger. These orexigens (appetite stimulants) include neuropeptide Y (NPY), agouti-related protein (AgRP), and melanocyte-stimulating hormone (α -MSH) (16, 22). In one of the first studies of ghrelin during exercise, plasma acylated ghrelin levels and related ratings of hunger declined during and following an acute running bout in young men (7).

This highlights ghrelin's role in stimulating appetite, and is intuitive that ghrelin levels are suppressed during exercise. Finally, in a well-designed study of male and female twin pairs, resting plasma ghrelin levels were significantly higher in women compared to men (26). Taken together, these sex differences in anorexigens (i.e., appetite suppressants) and orexigens suggest that the signals controlling hunger and satiety differ among men and women. These differences are another important consideration for understanding sex-related differences in energy balance and weight control.

Sex Hormones. Estrogens and androgens play a large role in body weight regulation, fat distribution, and energy balance in humans and rodent models. Of these sex hormones, women tend to have higher levels of circulating estrogen while men display greater levels of androgens. Estrogen is related to decreased levels of visceral fat in men and women; alternatively, androgens are related to lower levels of visceral fat in males but higher levels of visceral fat in females (2, 4, 6).

In addition to estrogen's role guiding the development of secondary sex characteristics and bone mass, estrogen also has important metabolic roles including the reduction of appetite and body mass (1). Additionally, estrogen interacts with the metabolic hormones leptin and insulin to influence body fat distribution and overall energy balance. The release of estrogen impacts appetite by also decreasing the action and/or effectiveness of several orexigens (i.e., appetite stimulants) including ghrelin, neuropeptide Y (NPY), and melanin-concentrating hormone (MCH) (28, 38). This data supports estrogen's role in decreasing food intake through its influence on orexigens. Estrogen also leads to a reduction in food intake through its effects on anorexigenic hormones including insulin, leptin, serotonin, and cholecystokinin (CCK) (9, 12). It is important to recognize the importance of other appetite suppressants such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and peptide YY (PYY) (36). Along with leptin, insulin, and ghrelin, these factors have been shown to decrease hunger signals at hypothalamus (36).

Researchers have developed a potential model to explain the relationship between estrogen, fat distribution, and leptin and insulin (35). They theorize that in premenopausal women, estrogen reduces visceral fat through enhanced lipolysis and decreased lipogenesis. Estrogen also retains subcutaneous fat, and is related to increases in resting leptin and reductions in resting insulin levels. However, in men and postmenopausal women, these researchers propose that the lower levels of estrogen and lowered activity of estrogen receptor alpha are related to increases in visceral fat and reductions in subcutaneous fat and leptin while concomitantly insulin levels are increased (23, 35). Some of this may be explained by the direct relationship between leptin and subcutaneous fat as the latter secretes leptin. These sex-related differences in hormone levels and fat distribution have been supported by other researchers as well (8).

Androgens such as testosterone and dehydroepiandrosterone (DHEA) also play an important role in energy balance, specifically in influencing where males and females store their body fat. Women tend to deposit and retain more adipose tissue around their hips, buttocks, and thighs, known as a "gynoid" or "pear" body shape. On the other hand, men tend to store more fat around their waist and midsection, known as an "android" or "apple" body shape (4). The underlying cause of the gynoid shape in women and android shape in men may be due to differences in adipogenesis and the environment within developing adipose cells. Research has shown that women have greater levels of

early-differentiated adipocytes compared to men (measured in abdominal and femoral fat depots) (37). These authors also speculated that sex differences in regional fat distribution may be due to differences in the microenvironment of the cells and related apoptosis, innervation, blood supply, and responsiveness to hormones (37).

In addition to the aforementioned sex differences in body fat distribution, men tend to have higher levels of visceral fat, while women tend to store more fat subcutaneously. Androgen levels may play a role in these relationships as greater amounts of visceral fat has been associated with lower androgen levels in men and excess androgen levels in women (4). Unfortunately for men, visceral fat carries an increased cardiovascular disease risk compared to subcutaneous fat. This often puts men at an increased risk for cardiovascular disease (2–4). Not surprisingly, inverse correlations have been reported between body fat and energy expenditure from physical activity in men ($r=-0.34$, $p<0.03$) (29). Therefore, sex differences in estrogen and androgens are related to body fat and its distribution; these differences in turn influence energy expenditure and balance in men and women.

Stress Hormones. Stress hormones such as catecholamines and cortisol are another category of hormones that influence energy balance in men and women. To further complicate the matter, stress hormones also interact with sex hormones, thus altering their actions (24). Catecholamines such as epinephrine and norepinephrine are released in response to sympathetic nervous system stimulation when a stressor occurs, whether real or perceived; a common example is exercise. In response to catecholamine release, appetite centers in the hypothalamus are suppressed and related food intake declines.

The primary function of catecholamines and the stress hormone cortisol is to provide energy for the body to face the stressor. Rather than stimulating appetite, these hormones cause the body to break down stored energy and one example is by stimulating lipolysis. This process is also enhanced by thyroid hormones, cortisol, growth hormone, and estrogen (27). Thus, there are numerous hormonal signals triggering fat breakdown throughout the body. These hormones and their related lipolytic actions are extremely important during exercise, especially at low to moderate intensities. Catecholamines also increase available energy by increasing glycogenolysis in both the liver and the muscle (45). The data concerning sex differences in catecholamines at rest and during exercise is conflicting; some studies have shown no difference in men and women while others have reported slightly higher levels of epinephrine and norepinephrine in men (45). Likewise, research has shown that men and women have similar levels of both blood and salivary cortisol measures at rest (19). However, these authors identified sex differences in salivary cortisol in response to stress, such that women in the luteal phase of their menstrual cycle had similar responses to men and both were greater than women in the follicular phase of their menstrual cycle or women on oral contraceptives (19). This suggests that both sex and menstrual cycle phase in females should be considered when evaluating cortisol levels and their impact on energy balance.

The functions of numerous metabolic, sex, and stress hormones related to energy balance were discussed above. The following sections will describe how these hormones are affected by physical activity. The related changes in appetite, energy intake, and energy balance will also be discussed, and sex-related differences in these relationships will be discussed when possible.

INFLUENCE OF PHYSICAL ACTIVITY ON APPETITE AND ENERGY BALANCE

It is a commonly held belief that increases in physical activity lead to stimulation of appetite. However, researchers summarizing the influence of physical activity on energy balance have reported mixed results. For example, researchers have shown that in general, physical activity does *not* have a large influence on the balance between intake and expenditure (5). That is, increases in physical activity do not necessarily stimulate appetite, just as reductions in physical activity do not lead to substantial decreases in appetite. These authors noted sex differences in the relationship between physical activity and appetite. They suggested that compared to men, women exhibit a greater tendency to either increase energy intake following physical activity or have a more difficult time achieving negative caloric balance and weight loss via physical activity (5). This conclusion has been supported by other researchers as well (17, 39) and by the greater prevalence of obesity in women worldwide compared to men (~300 million vs. ~200 million, respectively) (43). Similar results were found in a review of 290 participants from 22 studies as physical activity was inversely related to percent body fat in males (partial $r=0.35$, $p<0.001$) but not in females (partial $r=0.16$, $p>0.05$) (39). While the mechanisms behind these differences were beyond the scopes of the reviews, authors have hypothesized that sex differences in fat may be attributable to women's need for sufficient fat stores for successful reproduction (5, 17).

In another study of exercise and appetite, a group of 12 normal-weight men and women exercised for 14 days, and the resulting changes in energy balance were examined (40). Participants took part in periods of no additional exercise as well as moderate- and high-intensity exercise, with the order counterbalanced, and were fed ad libitum. The authors reported that the additional energy expenditure from the exercise did not elicit equal increases in energy intake; rather the average caloric compensation was only ~30%. This yielded average negative energy balances ranging from -0.9 to -3.8 MJ/day in women and -1.6 to -4.7 MJ/day in men (40). The authors acknowledge that while tightly controlled, this study only represents the initial compensation to exercise-induced energy deficits and longer studies are needed to elucidate the chronic relationships between these variables. These studies provide additional data that contributes to sex-related differences in weight control and long-term energy balance.

EFFECTS OF EXERCISE ON HORMONES: SEX DIFFERENCES

Energy expenditure from physical activity is influenced by the metabolic-, stress-, and sex-related hormones described earlier in this chapter. In turn, hormone release changes in response to physical activity and exercise. Acute bouts of exercise are related to increases in catecholamines, growth hormone, cortisol, thyroid hormones, estrogens, and androgens while insulin and leptin tend to decrease (27). These patterns of hormonal release differ in response to chronic exercise training, however, as most are unchanged or decrease.

To further complicate these relationships, some authors have found that hormonal changes in response to exercise may differ between men and women. For example, growth hormone levels have been shown to increase to a greater degree in women,

compared to men, during exercise (31). Additionally, in a study examining the hormonal changes following exercise performed in several energy states, researchers noted reductions in resting leptin and insulin and increases in acylated ghrelin in response to exercise performed in a state of negative energy balance, and women had higher acylated ghrelin and lower insulin following the bout, compared to men (17). This supports the notion that following physical activity, women's appetite is stimulated to a greater degree than men's (17). These researchers proposed a model to help explain some trends they were observing. They proposed that in men, physical activity reduces appetite but does not change metabolic hormones such as ghrelin, insulin, and leptin. Therefore there would be no compensatory changes in energy intake for men, and the energy deficit caused by the increased expenditure would result in reduced body fat. Conversely, in women, physical activity may have no effect on appetite yet cause large hormonal changes. These changes, along with women's maintained appetite, may result in a state of positive energy balance which may preserve or even increase their levels of body fat (17). This model may explain why women tend to maintain or even gain body mass or fat in response to physical activity, whereas men typically do not. As previously described, the common theory explaining these sex differences is that the hormonal differences exist to protect women's fat mass to a greater extent than men's in order to ensure successful reproduction.

Researchers have also examined sex differences in substrate utilization during exercise. In a study of seven men and seven women endurance-trained cyclists matched by peak oxygen uptake (VO_2peak) per kg lean body mass, there were no sex differences in respiratory exchange ratio (RER) during moderate-intensity exercise, indicating similar contributions from fat and carbohydrates (32). Likewise, there were no sex differences in insulin, epinephrine, or norepinephrine concentrations during exercise. However, the sources of fat differed between the sexes as men derived less energy from myocellular triacylglycerols compared to females and males also had a larger greater proportion of energy that was unaccounted for in fat and carbohydrates sources. Other researchers have reported conflicting results regarding sex differences in fuel metabolism during cycling. Horton et al. (18) found that women rely more heavily on fats during exercise (51% vs. 44% for women and men, respectively) while men obtain more energy from carbohydrates (53% and 46%, respectively) when cycling for 2 h at 40% of their maximal oxygen uptake (VO_2max). These differences are likely attributable to the higher concentrations of epinephrine and norepinephrine seen in men compared to women. It is important to recognize that while conflicting results are often reported, readers must consider the intensity and duration of the exercise as they have a large influence on substrate use and the related hormone response. In summary, these studies provide additional data supporting sex-related differences in energy usage, energy balance, and ultimately weight control.

SUMMARY

This chapter highlighted the influence of numerous hormones on energy balance and weight control in men and women. One of the primary sex differences is that females tend to store more fat subcutaneously, while men store more in visceral locations. Similarly, females are more sensitive to leptin while men are more sensitive to insulin,

and each respective hormone is closely related to their level of adiposity. These differences often place men at an increased risk for the development of cardiovascular disease due to its association with visceral fat. In addition, women respond differently to physical activity than men as their hormonal profile favors conservation of fat mass, most likely for reproduction. These differences suggest that different strategies are necessary for maintaining or altering energy balance and body mass in men and women.

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11

Exercise Training in the Normal Female: Effects of Low Energy Availability on Reproductive Function

Anne B. Loucks, PHD

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THE FEMALE ATHLETE TRIAD

This chapter summarizes the studies in our lab and others that identified low energy availability as the key factor causing the female athlete triad, and identifies four distinct origins of low energy availability among female athletes. In 2007, the American College of Sports Medicine (ACSM) published a revised position stand on the female athlete triad (1), which replaced its earlier position stand on the same subject (2). The revised position stand corrected the former misunderstanding of the triad as a narrow syndrome consisting of disordered eating, amenorrhea, and osteoporosis by describing the triad more broadly as the harmful effects of low energy availability on menstrual function and bone mineral density. The revised position stand emphasized that energy availability can be severely reduced by exercise energy expenditure alone without clinical eating

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disorders, disordered eating, or even dietary restriction. It also explained that low energy availability induces more menstrual disorders than amenorrhea, and that these functional hypothalamic menstrual disorders must be carefully distinguished by differential diagnosis from other kinds of menstrual disorders not caused by low energy availability that are, therefore, unrelated to the triad. The revised position stand also explained that bone mineral density in young athletes must be quantified in terms of z -scores instead of t -scores, and that during adolescence low energy availability can cause z -scores to decline as t -scores increase.

HYPOTHETICAL MECHANISMS OF FUNCTIONAL HYPOTHALAMIC MENSTRUAL DISORDERS IN EXERCISING WOMEN

As in other fields of research, competing schools of thought developed to explain the high prevalence of menstrual disorders observed in exercising women. Of the several early mechanisms proposed, three were most widely held.

Body Composition

In 1974, body composition was offered as an explanation for the amenorrhea observed in anorexia nervosa patients (3). This idea was a refinement of an earlier hypothesis about body weight accounting for the timing of menarche (4). The body composition hypothesis held that menarche occurs in girls when the amount of energy stored in their bodies as fat rises to a critical 17% of their body weight, and that menstrual function is lost later when their body fat declines to less than a critical 22% of body weight (3).

The body composition hypothesis was the most widely publicized explanation for menstrual disorders in athletes in the lay community and the most widely embraced by the clinical community, even though it was the least widely accepted within the scientific community. The hypothesis was based entirely on correlations without any supporting experimental evidence (5). Actually, observations of athletes did not consistently verify an association of menstrual status with body composition (e.g., (6)), and did not display the correct temporal relationship between changes in body composition and menstrual function (for reviews, see (7–10)). Rather, eumenorrheic and amenorrheic athletes were found to span a common range of body composition (11) leaner than that of eumenorrheic sedentary women. In addition, after the growth and sexual development of prepubertal animals had been blocked by dietary restriction, normal luteinizing hormone (LH) pulsatility resumed only a few hours after ad libitum feeding was permitted, before any change in body weight or composition could occur (12). Moreover, when surgical reduction of the stomachs of severely obese women (body weight ~130 kg; BMI ~47) reduced the amount of food that they could eat, rapid weight loss and amenorrhea occurred while the patients were still obese (body weight ~97 kg; BMI ~35) (13).

Despite such criticisms, scientific interest in the body composition hypothesis was renewed with the discovery in 1994 of the adipocyte hormone leptin (14), with the observation of statistically significant correlations between leptin levels and body fatness in rodents and humans (e.g., (15)), and with the discovery of leptin receptors on hypothalamic neurons. Since then, an abundance of experimental evidence from rodents

and humans has demonstrated that a minimal level of leptin is permissive (i.e., necessary but not sufficient) for sexual development and function (16). This permissive effect occurs indirectly via receptors on hypothalamic kisspeptin neurons that communicate with the hypothalamic gonadotropin-releasing hormone (GnRH) neurons that regulate LH pulsatility (17).

Recently, a 9-month double-blind, randomized, clinical trial administered pharmacological doses of leptin to women with functional hypothalamic amenorrhea whose BMI was in the range 18–25 kg/m² (18). Prior to treatment, their leptin levels (mean \pm SD = 4.6 \pm 2.0 ng/mL) were within the lower portion of the range (7.4 \pm 3.7 ng/mL) cited by the leptin assay kit manufacturer (Millipore Corp.) for women in this range of BMI (19). Leptin levels comparable to those reported by the manufacturer have been found in other women with similar ranges of BMI (20–26). The leptin dosages administered to the women with functional hypothalamic amenorrhea in this experiment raised their leptin levels more than tenfold (mean \pm SD = 59 \pm 37 ng/mL). Yet menstrual cycles occurred only intermittently, with the number of menstruating women fluctuating from month to month between 3 of 10 (30%) and 4 of 7 (57%).

By contrast, nutritional counseling has restored spontaneous menstrual cycles in 75% of women with functional hypothalamic amenorrhea within 5 months (27). Although leptin was originally thought to communicate information about fat stores, it was later found to vary profoundly in response to fasting, dietary restriction, refeeding after dietary restriction, and overfeeding before any changes in adiposity occurred (28–31). This led to the hypothesis that leptin also signals information about dietary intake, and specifically carbohydrate intake after leptin synthesis was found to be regulated by the tiny flux of glucose through the hexosamine biosynthesis pathway in both muscle and adipose tissue (32). In eumenorrheic and amenorrheic athletes, leptin was found to differ not in its average concentration, but rather in the presence and absence, respectively, of a diurnal rhythm (20), and the diurnal rhythm was found to depend not on energy intake but rather on energy availability or more specifically on carbohydrate availability (24). Thus, if leptin does participate in the functional regulation of the GnRH pulse generator in exercising women, it seems more likely to do so as a signal of low energy or carbohydrate availability than as a signal of low energy stores.

Energy Availability

In 1980, Warren was the first to suggest that menstrual function in dancers might be disrupted by an “energy drain” (33), but an empirically testable energy availability hypothesis was first clearly stated in terms of brain energy availability by Winterer, Cutler, and Loriaux in 1984 (34). They hypothesized that failure to provide sufficient metabolic fuels to meet the energy requirements of the brain causes an alteration in brain function that disrupts the GnRH pulse generator, although the mechanism of this alteration was unknown.

At the organismal level, the energy availability hypothesis recognizes that mammals partition energy among several major metabolic activities, including cellular maintenance, immunity, thermoregulation, locomotion, growth, and reproduction (35), and that the expenditure of energy in one of these functions, such as locomotion, makes it unavailable for others, such as reproduction. Considerable observational data from biological

field trials supports this idea, and indicated that the dependence of reproductive function on energy availability operates principally in females (for reviews, see (35–39)). Experiments had induced anestrus in Syrian hamsters by food restriction, by the administration of pharmacological blockers of carbohydrate and fat metabolism, by insulin administration (which shunts metabolic fuels into storage), and by cold exposure (which consumes metabolic fuels in thermogenesis) (35). Disruptions of reproductive function were independent of body size and composition.

The energy availability hypothesis was also supported by endocrine observations of athletes. Amenorrheic athletes displayed low blood glucose levels during the feeding phase of the day (40), low insulin and high IGFBP-1 during the fasting phase (40), loss of the leptin diurnal rhythm (20), and low triiodothyronine (T_3) levels in the morning (41, 42). All of these abnormalities in metabolic substrates and hormones are signs of energy deficiency. T_3 regulates basal metabolic rate, and low T_3 occurs in numerous conditions, from fasting to cancer, in which dietary energy intake is insufficient to meet metabolic demands. In addition, eumenorrheic and amenorrheic athletes both displayed low insulin and high IGFBP-1 levels during the feeding phase of the day, as well as low leptin (20) and elevated growth hormone (GH) levels over 24 h (40). Indeed, eumenorrheic and amenorrheic athletes were found to be distinguished not by different 24-h mean concentrations of leptin, but rather by different amplitudes in the diurnal rhythm of leptin (20).

Amenorrheic and eumenorrheic athletes reported similar stable body weights, despite dietary energy intakes similar to those of sedentary women (41, 43–47). That is, they reported their dietary energy intakes to be much less than would be expected for an athlete's level of physical activity. This apparent discrepancy between stable body weight and unexpectedly low dietary energy intake was controversial. Since energy intake and expenditure are very difficult to measure accurately, the apparent discrepancy might have been attributable to methodological errors. Some investigators attributed the apparent discrepancy between energy intake and expenditure in athletic women to underreporting of dietary intake (48, 49), because such underreporting is common in all populations (50), but underreporting did not account for the abnormalities in metabolic substrates and hormones observed in athletes. Furthermore, behavior modification and endocrine-mediated alterations of resting metabolic rate operate to stabilize body weight despite dietary energy excess and deficiency (51).

Exercise Stress

The exercise stress hypothesis held that exercise disrupts the GnRH pulse generator by activating the hypothalamic-pituitary-adrenal axis. In order for the stress hypothesis to be meaningfully independent of the energy availability hypothesis, however, the adrenal axis must be activated independently of the energy cost of the exercise.

Certainly, there are central and peripheral mechanisms by which the adrenal axis can disrupt the ovarian axis (52), and prolonged aerobic exercise without glucose supplementation does activate the adrenal axis. Selye first induced anestrus and ovarian atrophy in rats by abruptly forcing them to run strenuously for prolonged periods (53). Later, others also induced anestrus by forced swimming (54, 55), by forced running (56), and by requiring animals to run farther and farther for smaller and smaller food

rewards (57, 58). The elevated cortisol levels induced in such experiments were interpreted as signs of stress, and the resulting disruptions of the HPG axis were widely interpreted as evidence that “exercise stress” has a counterregulatory influence on the female reproductive system.

Amenorrheic athletes also display mildly elevated cortisol levels (40, 43, 59–61). This observation was the basis for attributing their amenorrhea to stress. Mild hypercortisolism is also associated with amenorrhea in patients with functional hypothalamic amenorrhea (62) and anorexia nervosa (63). This interpretation overlooked the glucoregulatory functions of cortisol, which inhibit skeletal muscle glucose uptake and promote skeletal muscle proteolysis for hepatic gluconeogenesis in response to low blood glucose levels. Thus, it was possible that the mild hypercortisolism observed in amenorrheic athletes might have reflected a chronic energy deficiency rather than exercise stress.

At the time, it was not known whether the adrenal axis mechanisms that disrupt the HPG axis in forced exercise experiments on animals also operate in voluntarily exercising women. Indeed, up to that time, all animal experiments investigating the influence of the “activity stress paradigm” on reproductive function had confounded the stress of exercise with the stress of the method used to force animals to exercise. These experiments had also been confounded by the energy cost of the exercise performed, and glucose supplementation during exercise was found to blunt the usual rise in cortisol in both rats (64) and men (65). As a result, in 1990 the literature on stress contained only ambiguous evidence that the stress of exercise disrupts the HPG axis in either animals or humans.

PROSPECTIVE CLINICAL EXPERIMENTS

Experiments Confounding Exercise Stress and Energy Availability

Several investigators attempted to induce menstrual disorders through chronic exercise training, but most (66–69) applied only a moderate volume of exercise, or the volume of exercise was increased gradually over several months, and diet was uncontrolled or unquantified. In one case (69) selected physically trained subjects appeared to have been luteally suppressed before the study began (70).

Only one experiment had successfully induced menstrual disorders in regularly menstruating women (71). Modeled on Selye’s early animal experiments (53), this single successful experiment imposed a high volume of aerobic exercise abruptly, thereby suppressing follicular development, the LH surge, and luteal function in a large proportion of the subjects in the first month and in an even larger proportion in the second. Both proportions were greater in a subgroup fed a controlled weight-loss diet than in another subgroup fed for weight maintenance, but even the weight maintenance subgroup may have been underfed, since behavior modification and endocrine-mediated alterations of resting metabolic rate operate to stabilize body weight despite dietary energy excess and deficiency (51).

Such experiments, in which outcome variables are properties of the menstrual cycle, require sustained observations over a period of several weeks. Such prolonged experimental protocols suffer from practical problems with subject retention and compliance with experimental treatments. To avoid these difficulties, short-term experimental

protocols were developed in which LH pulsatility was chosen as the outcome variable, because ovarian function is critically dependent on LH pulsatility. Of course, short-term effects on LH pulsatility are not proof of chronic effects on ovarian function, but hypotheses about mechanisms regulating LH pulsatility could be tested in highly controlled short-term experiments, and then chronic effects could be confirmed in prolonged experiments later.

One such short-term experimental protocol found that a combination of increased exercise and dietary restriction disrupts LH pulsatility during the early follicular phase (72). LH pulse frequency during 12 waking hours was lower in four habitually physically active women when their exercise training regimen was increased during a few days of dietary restriction than during dietary supplementation. However, this experiment did not determine whether LH pulse frequency could be suppressed by exercise without dietary restriction, nor whether the stress of exercise had a suppressive effect on LH pulsatility beyond the impact of the energy cost of exercise on energy availability.

Experiments Distinguishing the Independent Effects of Exercise Stress and Energy Availability

For several years, we focused our efforts on a series of so-called “Excalibur” experiments that were designed to determine the independent effects of exercise stress and energy availability on the HPG axis (25, 26, 73–77). For these experiments, we defined energy availability operationally as dietary energy intake minus exercise energy expenditure. Conceptually, this corresponds to the amount of dietary energy remaining after exercise training for all other physiological functions. Although not the actual physiological quantity hypothetically affecting the HPG axis at the cellular level, our operational definition in behavioral terms had the advantage of being readily measurable and controllable. We controlled the dietary energy intake of our subjects by feeding them diets of known amount and composition as their only food during the experiments. We also required them to exercise under supervision in our laboratory on a treadmill while we measured and controlled their energy expenditure until they had expended a predetermined amount of energy. In the absence of any empirically operational definition of stress (78), we defined exercise stress independently as everything associated with exercise except its energy cost.

Through careful subject selection, we took pains to minimize the influence of potentially confounding factors. Healthy, regularly menstruating, habitually sedentary, non-obese, nonsmoking women 18–34 years of age at least 5 years past menarche, with no recent history of dieting, weight loss, or aerobic training were recruited. Before being admitted to the study, these volunteers underwent an extensive screening procedure, including written medical, menstrual, dietary, and athletic histories; a physical examination; a 12-lead resting electrocardiogram; a 7-day prospective dietary record; determination of body composition by hydrostatic weighing or whole body air-displacement plethysmography; and a treadmill test to determine their aerobic capacity. Volunteers were admitted into experiments only if they presented no current use of medications including oral contraceptives and no history of heart, liver, or renal disease, diabetes, and menstrual or thyroid disorders. They must also have had documented prospective records of menstrual cycles 26–32 days in length for at least the previous

3 months. They were required to be 18–30% body fat, with habitual energy intakes between 35 and 55 kcal/kg lean body mass (LBM)/day based on their 7-day diet records, with maximal aerobic capacities less than 42 mL O_2 /kg body weight (BW)/min, and they must have been performing less than 60 min of habitual aerobic activity per week for the previous 3 months.

The narrow range of our subjects' menstrual cycle lengths implied that we restricted our subject pool to the central 60% of menstrual cycle lengths in the population, and that from this pool we chose women whose menstrual cycle lengths were in the least variable 20% of the population (79). Thus, if anything, our subjects' reproductive systems were robust against disturbance by commonly occurring environmental and behavioral influences. We could be confident, therefore, that if our treatments disrupted the reproductive systems of these women, they would disrupt the reproductive systems of other women, too. We could also be confident that our subjects' metabolism had not been disturbed by any confounding medical conditions or dietary or exercise habits before our treatments were applied.

EXCALIBUR I

Excalibur I (73) was designed to investigate whether exercise stress had any suppressive effect on T_3 levels independent of the impact of the energy cost of exercise on energy availability. We were interested in T_3 because it regulates the rate of energy expenditure at rest, and because it was known to be suppressed in amenorrheic athletes. We reasoned that if the energy cost of exercise necessitates such major metabolic adjustments as the suppression of reproductive function, then these metabolic adjustments might be mediated in part by suppressing T_3 . We found that severely low energy availability (8 kcal per kg of body weight per day, kcal/kgBW/day) suppressed T_3 levels by 15% while exercise stress had no effect on T_3 . T_3 levels were suppressed similarly regardless of whether energy availability was reduced by dietary energy restriction or by exercise energy expenditure. Furthermore, the suppression of T_3 in exercising women was prevented by supplementing their diet in compensation for the energy cost of their exercise. These findings were consistent with the energy availability hypothesis and inconsistent with the exercise stress hypothesis.

EXCALIBUR II

Excalibur II (74) was designed to reveal whether T_3 levels in exercising women vary in linear proportion to energy availability or are suppressed abruptly at a particular threshold of energy availability. We administered various levels of energy availability to exercising women and found that the suppression of T_3 by low energy availability occurred abruptly at a threshold of energy availability near 25 kcal/kgLBM/day. For our women of average body size (59 kg) and composition (24.5% body fat) that threshold was about 1,000 kcal/day.

EXCALIBUR III

Normal ovarian function depends not on some stable concentration of LH but rather on the occurrence of pulsatile surges of LH concentrations in the blood at regular intervals. These pulses correspond to regular secretory bursts of LH from the pituitary gland in response to similar secretory bursts of GnRH from the hypothalamus. The frequency (at intervals of 70–180 min) and amplitude of these pulses vary around the

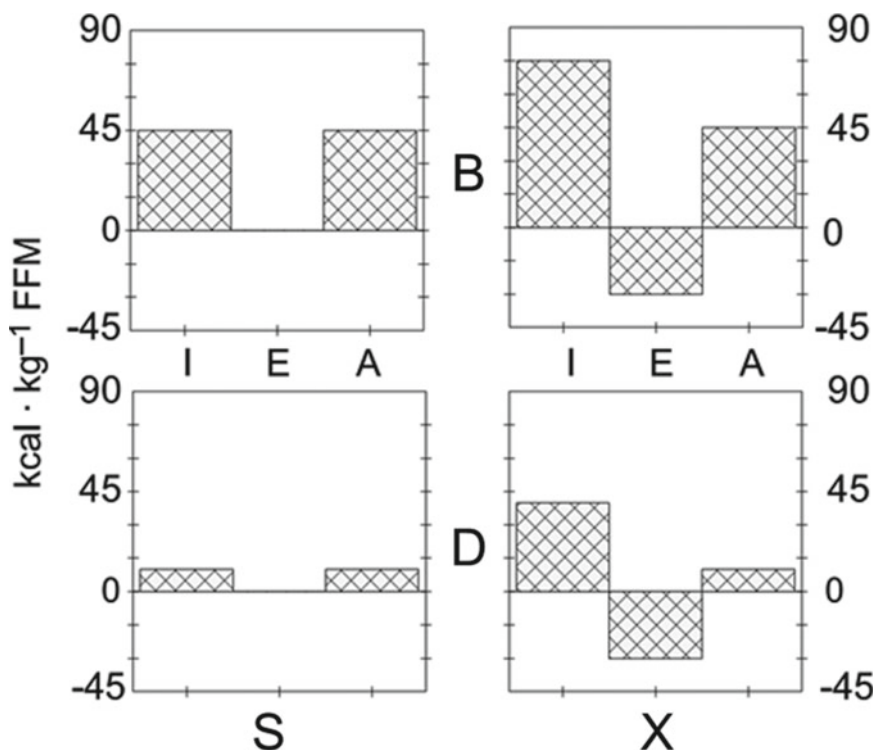


Fig. 1. Experimental design of Excalibur III. Dietary energy intake (I) and exercise energy expenditure (E) were controlled to achieve balanced ($B=45$ kcal/kgLBM/day) and deprived ($D=10$ kcal/kgLBM/day) energy availability ($A=I-E$) treatments. Deprived energy availability was achieved by dietary restriction alone in sedentary women (S) and by exercise energy expenditure alone in exercising women (X) (1 kcal=4.18 kJ) (adapted from (76), The Endocrine Society).

menstrual cycle. In sedentary women in the early follicular phase, the pulsatile pattern is characterized as high frequency and low amplitude. In regularly menstruating athletes, the pulses occur less often and are larger in amplitude, but they still at regular intervals. In amenorrheic athletes, LH pulses occur even less often and irregularly (43).

Therefore, in Excalibur III (75, 76), we investigated whether exercise has any suppressive effect on LH pulsatility beyond the impact of its energy cost on energy availability. The design of Excalibur III is illustrated in Fig. 1. For 4 days in the mid-follicular phase of two menstrual cycles, we controlled the energy availability of two groups of women. During one cycle, we administered a balanced energy availability of 45 kcal/kgLBM/day, and during the other cycle we administered a low energy availability of 10 kcal/kgLBM/day. One group of subjects performed no exercise during the two treatment periods. A second group performed the same large volume of high intensity exercise that we had utilized in Excalibur I (30 kcal/kgLBM/day at 70% VO_2max). We imposed balanced and low energy availabilities on the non-exercising group by feeding them 45 and 10 kcal/kgLBM/day, respectively. We imposed the same balanced and low energy availabilities on the group performing 30 kcal/kgLBM/day of exercise by feeding them 75 and 40 kcal/kgLBM/day, respectively.

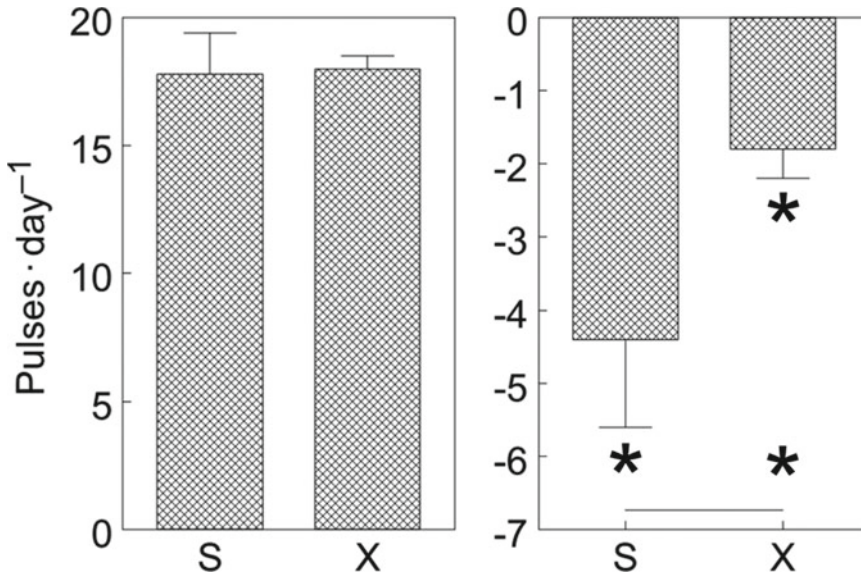


Fig. 2. Effects of low energy availability on LH pulsatility in Excalibur III. *Left:* Luteinizing hormone (LH) pulse frequency in sedentary (S) and exercising (X) women with the same balanced energy availability (45 kcal/kgLBM/day). *Right:* Reduction in LH pulse frequency caused by low energy availability (10 kcal/kgLBM/day) in sedentary (S) and exercising (X) women. * $p < 0.01$ (adapted from (75), The American Physiological Society, (76) The Endocrine Society).

At the end of each of these 4-day treatments, we admitted the women to a General Clinical Research Center and drew blood samples from them at 10-min intervals for 24 h. Later, we measured the amount of LH in each sample and used a special statistical computer program to detect and to calculate the frequency and amplitude of their LH pulses. We determined the effects of energy availability on these frequencies and amplitudes by contrasting data taken while performing the same exercise at different energy availabilities, and we determined the independent effect of exercise stress by contrasting groups exercising differently at the same energy availabilities.

We found that low energy availability reduced LH pulse frequency and increased LH pulse amplitude, while exercise stress had no suppressive effect on LH pulsatility beyond the impact of the energy cost of exercise on energy availability (Fig. 2). LH pulsatility was disrupted by extreme energy restriction alone and by extreme exercise energy expenditure alone. Dietary supplementation prevented the suppression of LH pulsatility by exercise energy expenditure. Others have shown that short-term fasting also reduces LH pulse frequency in sedentary women during the early follicular phase (80, 81), and that in lean women, ovarian function is also impaired during the ensuing menstrual cycle (81).

In Excalibur III, low energy availability also suppressed plasma glucose, insulin, insulin-like growth factor-I (IGF-I), leptin, and T_3 while raising GH and cortisol levels. All these effects are reminiscent of abnormalities observed in amenorrheic athletes (40–43, 59–61).

This contradiction of the exercise stress hypothesis has been confirmed by more prolonged experiments on animals. Amenorrhea was induced in monkeys by training them

to run voluntarily on a motorized treadmill for longer and longer periods while their food intake remained constant (82). Then their menstrual cycles were restored by supplementing their diets without any moderation of their exercise regimen (83). The exercise stress hypothesis was also contradicted in a novel animal model of the entire female athlete triad (84). In this modified activity stress paradigm, rats were habituated to voluntary wheel running for 90 days and then randomized to control and restricted diets for the next 90 days. Although both groups ran similar distances and expended similar amounts of energy in exercise, estradiol was suppressed, estrous cycling ceased, ovaries were atrophied, and the bone mineral content of the femur and tibia were reduced only in the underfed rats.

The suppression of LH pulse frequency by low energy availability in Excalibur III was actually *smaller* in exercising women than in non-exercising women with the same low energy availability (76). This result was unexpected and it suggested that LH pulsatility might actually depend on a more specific metabolic factor that is easily confused with energy availability, but which is less compromised by exercise energy expenditure than by dietary energy restriction.

Research in other mammals suggests that GnRH neuron activity and LH pulsatility are actually regulated by brain glucose availability (35, 38). The adult female human brain oxidizes about 80 g of glucose each day at a continuous rate. This must be provided daily by dietary carbohydrate, because the brain's rate of energy expenditure can deplete liver glycogen stores in less than a day (85). Moderate exercise oxidizes as much glucose in an hour.

In the non-exercising women in Excalibur III, low energy availability due to dietary energy restriction reduced carbohydrate intake by 77%. This reduction in carbohydrate intake was similar to the 73% increase in carbohydrate oxidation revealed by respiratory gas analysis in the exercising women during the balanced energy availability treatment. By contrast, carbohydrate oxidation increased only 49% in the exercising women under low energy availability conditions. This alteration in fuel selection conserved almost 70% of the brain's daily glucose requirement. Thus, exercise may compromise brain glucose availability less than dietary energy restriction, and this may account for the smaller disruption of LH pulsatility that we observed in exercising women than in dietary-restricted women. Thus, LH pulsatility may depend specifically on carbohydrate availability rather than energy availability in women, just as it does in other mammals.

EXCALIBUR IV

Excalibur IV (77) was designed to reveal whether refeeding reverses the suppression of LH pulsatility in women as quickly as it does in other mammalian species. In food-restricted female rats (12, 86) and ewes (87) and in fasted heifers (88) and male rhesus monkeys (89), a single ad libitum meal stimulates LH pulses within 2 h. Such observations have been interpreted to imply that the physiological signals produced by a single large meal are sufficient to activate the hypothalamic GnRH neurons that control LH pulsatility (90).

We suspected that the restoration of LH pulsatility by refeeding might be considerably slower in energetically disrupted women than in other mammals, because the human brain requires so much more energy than does the brain of any other mammal.

The brain competes against all other tissues of the body for energy and the adult human brain requires 20% of basal metabolic energy, compared to only 2% for most species and 8% for nonhuman primates (91). Therefore, we suspected that a single meal might not provide enough energy to activate GnRH neurons in energetically disrupted women.

To stringently test this hypothesis, we assayed LH in blood samples drawn from women at 10 min intervals for 48 h during the mid-follicular phase, first during 24 h on the fifth day of low energy availability treatments and then during 24 h of aggressive refeeding. A combination of moderate dietary energy restriction (25 kcal/kgLBM/day) and moderate exercise energy expenditure (15 kcal/kgLBM/day) was administered to impose a low energy availability of 10 kcal/kgLBM/day. The aggressive refeeding regimen was comprised of 15 meals providing a total of 85 kcal/kgLBM/day. Combined with the same exercise treatment, the energy availability during the 24 h of aggressive refeeding was 70 kcal/kgLBM/day.

Compared to measurements of LH pulsatility in 18 other women studied previously in our laboratory under balanced energy availability conditions and at the same phase of the menstrual cycle, low energy availability suppressed LH pulsatility unambiguously in five of the eight subjects treated in this experiment. Their LH pulse frequency was reduced 57% to 8.2 ± 1.5 pulses/24 h, well below the 5th percentile of LH pulse frequencies in energy-balanced women (14.6 pulses/24 h), while their LH pulse amplitude was increased 94% to 3.1 ± 0.3 IU/L, well above the 95th percentile of LH pulse amplitudes in energy-balanced women (2.5 IU/L).

Among these women, aggressive refeeding raised LH pulse frequency by only 2.4 ± 1.0 pulses/24 h, still far below the 5th percentile of LH pulse frequency in energy-balanced women. Meanwhile, the unambiguously elevated LH pulse amplitude was completely unaffected ($\Delta = 0.0 \pm 0.4$ IU/L) by aggressive refeeding. (Results were similar when all eight subjects were included in the analysis. Aggressive refeeding pushed the group as a whole to, but not past, the 5th and 95th percentiles of LH pulse frequency and amplitude, respectively.) Thus, as we had suspected, 24 h of a refeeding protocol much more aggressive than the ad libitum refeeding protocols commonly employed in animal experiments had very little restorative effect on LH pulsatility in our energetically suppressed women.

EXCALIBUR V

In an experimental protocol similar to that of Excalibur II, Excalibur V determined the dose–response effects of low energy availability on LH pulsatility in habitually sedentary, regularly menstruating young women (25). To do this, we administered balanced and one of three low energy availabilities (45 and either 10, 20, or 30 kcal/kgLBM/day) to healthy, habitually sedentary, regularly menstruating women for 5 days. The design is illustrated in Fig. 3.

We found that LH pulsatility was disrupted within 5 days below a threshold of energy availability at ~ 30 kcal/kgLBM/day (Fig. 4). This was, in fact, the same actual energy availability that we had reported as 25 kcal/kgLBM/day in Excalibur II (74), because between the two experiments we had changed the way we calculated energy availability. Prior to Excalibur III (76), we had calculated energy availability by

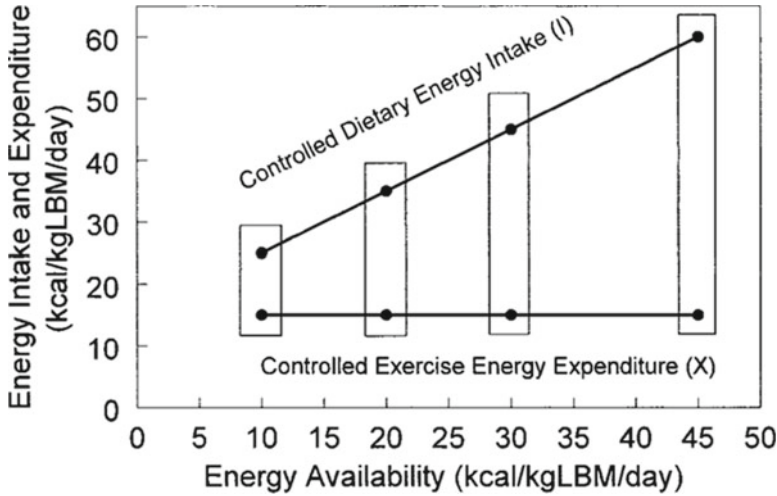


Fig. 3. Experimental design of Excalibur V. Women were assigned to contrasting energy availability treatments of 45 and 10, 45 and 20, and 45 and 30 kcal/kgLBM/day. All subjects performed a controlled exercise energy expenditure of 15 kcal/kgLBM/day in aerobic exercise at 70% VO₂ max under supervision while their dietary energy intake was controlled to achieve the intended energy availabilities. Reproduced, with permission, from (25), Copyright 2003, The Endocrine Society.

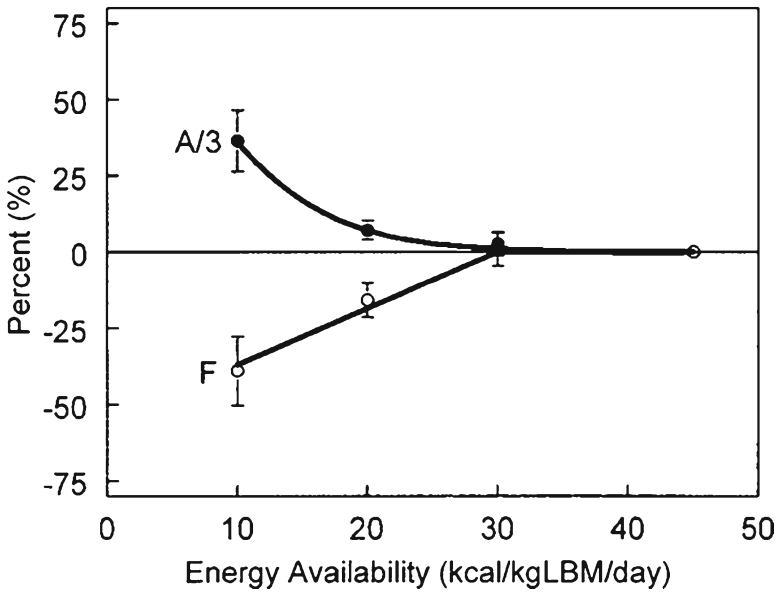


Fig. 4. Incremental effects of energy availability on LH pulse amplitude (A/3) and LH pulse frequency (F) in Excalibur V. Effects are expressed relative to values at 45 kcal/kgLBM/day. Effects on LH pulse amplitude have been divided by three for graphical symmetry. As energy availability declines from energy balance at approximately 45 kcal/kgLBM/day, effects begin at a threshold at approximately 30 kcal/kgLBM/day and become more extreme as energy availability is further reduced below 20 kcal/kgLBM/day. Reproduced, with permission, from (25), Copyright 2003, The Endocrine Society.

subtracting total energy expenditure during exercise from dietary energy intake. While we were designing Excalibur III, however, we recognized that if our exercising subjects had not been exercising, they would still have expended energy in their other routine activities during the same hours. Therefore, in Excalibur III and our later experiments we changed the way we calculated energy availability by subtracting from dietary energy intake only the portion of total energy expenditure during exercise that was directly attributable to the exercise itself. We achieved this by using an activity monitor to measure our subjects' energy expenditure in their normal daily activities during the same hours of the day when they would be exercising in our experiment. We then subtracted that energy expenditure in routine activities from their total energy expenditure during exercise to obtain the amount of energy expenditure during exercise that was specifically attributable to the exercise. In Excalibur II, our subjects' energy expenditure in routine activities on a non-exercising day during the same 3 h when they exercised in the experiment had amounted to 5 kcal/kgLBM/day. From the perspective of Excalibur V, therefore, the exercise energy expenditure of the subjects in Excalibur II was 5 kcal/kgLBM/day less than their total energy expenditure during exercise, and their energy availability was 5 kcal/kgLBM/day higher.

The disruption of LH pulsatility below 30 kcal/kgLBM/day in Excalibur V was consistent with many observational studies of amenorrheic runners, all of which indicated energy availabilities less than 30 kcal/kgLBM/day (92). It was also consistent with the only prospective study of the refeeding of amenorrheic athletes, in which menstrual cycles had been restored in runners by increasing their energy availability from 25 to 31 kcal/kgLBM/day (93). Energy availabilities below 30 kcal/kgLBM/day have also been reported in eumenorrheic athletes (92), 80% of whom display subclinical ovarian disorders in which the suppression of progesterone may also impair fertility (94).

In the same experiment, we also determined the dose–response effects of low energy availability on several metabolic substrates and hormones. Down to an energy availability of 30 kcal/kgLBM/day, the responses of insulin, cortisol, insulin-like growth factor (IGF)-I/IGF binding protein (IGFBP)-1, IGF-I/IGFBP-3, leptin, and T_3 maintained plasma glucose levels to within 3% of normal values. Below that threshold, however, plasma glucose levels fell and effects on LH pulsatility appeared, despite larger responses of the metabolic hormones.

Excalibur V also revealed the dose–response effects of low energy availability on biochemical markers of bone turnover (95). Urinary concentrations of N-telopeptide of type I collagen, a marker of the rate of whole body bone resorption, rose as estradiol concentrations declined, when energy availability was lowered to 10 kcal/kgLBM/day. By comparison, markers of bone formation declined at higher energy availabilities. Concentrations of serum carboxy-terminal propeptide of type I procollagen, a marker of bone type I collagen synthesis, and insulin declined linearly with energy availability. By contrast, concentrations of osteocalcin, a marker of bone mineralization, declined abruptly below 30 kcal/kgLBM/day together with IGF-I and T_3 , which modulates the hepatic synthesis of IGF-I in response to GH stimulation. Such uncoupling of bone turnover, with increased resorption and reduced formation, can lead to irreversible reductions in bone mineral density (96).

EXCALIBUR VI

The prevalence of amenorrhea has been reported to decline from 67% in marathon runners younger than 15 years of gynecological age to only 9% in those who were older (97). Meanwhile, in the general population, the incidence of menstrual disorders declines during the decade after menarche as fertility increases (98). Excalibur VI investigated whether these two observations might both be explained by a declining sensitivity of LH pulsatility to low energy availability as the energy cost of growth decreases (26). Calcium balance, which is an index of growth, does not decline to zero until 14 years of gynecological age (99).

In Excalibur VI, contrasting balanced and low energy availabilities (45 and 10 kcal/kgFFM/day) were administered to healthy, habitually sedentary, regularly menstruating, older adolescent women (5–8 years of gynecological age, ~20 years of calendar age) and young adult women (14–18 years of gynecological age, ~29 years of calendar age) for 5 days. Low energy availability suppressed LH pulsatility in the adolescents but not in the adults, even though metabolic and endocrine signals of energy deficiency (i.e., plasma glucose, β -hydroxybutyrate, insulin, cortisol, T_3 , leptin, IGF-1, and GH) were altered as much or more in the adults as in the adolescents (26).

This insensitivity of LH pulsatility to energy deficiency in adult women was subsequently confirmed by a corresponding insensitivity of ovarian function to energy deficiency (100). In that experiment, the energy availability of women 25–40 years of age was reduced to ~25 kcal/kgFFM/day for 4 months by a combination of dietary restriction (~600 kcal/day) and exercise (~200 kcal/day). This subthreshold energy deficiency reduced the body fatness of these reproductively mature women from 32 to 27%, but caused no more than a mild suppression of luteal function.

An adult reproductive system that is more robust against insults of energy deficiency may be explained by a greater availability of glucose to the brain in adults than in adolescents at the same energy availabilities. This might occur if peripheral tissues in full-grown adults do not compete as aggressively against the brain for available energy or carbohydrate. Alternatively, the sensitivity of sensors in the central nervous system to signals of energy deficiency may decline during adolescence. These possibilities remain to be investigated.

CONCLUSIONS ABOUT THE HYPOTHETICAL MECHANISMS OF FUNCTIONAL HYPOTHALAMIC AMENORRHEA IN FEMALE ATHLETES

We are unaware of any experiments that have determined the independent effect of body composition on the HPG axis. From the available experimental data, however, it would appear to be more likely that a lean body composition and disruption of the HPG axis are both effects of low energy availability than that a lean body composition disrupts the HPG axis. Our short-term experiments on women have demonstrated that exercise stress has no suppressive effect on LH pulsatility beyond the impact of the energy cost of the exercise on energy availability. These short-term 4–5-day experiments investigating the independent effects of exercise stress and low energy availability on LH pulsatility predicted and, as we expected, were later confirmed by long-term

experiments investigating the independent effects of exercise stress and low energy availability on estrus and menstrual cycles. Prospective controlled experiments on both humans and animal models have demonstrated that the factor disrupting the HPG axis in physically active women is low energy availability. These experiments suggest that women may be able to prevent or to reverse menstrual disorders by dietary reform alone without moderating their exercise regimen. As long as dietary energy intake is managed to keep energy availability above 30 kcal/kgLBM/day, there may be no need to interfere with endurance, strength, and skill training. Finally, the susceptibility of women to the disruption of reproductive function by energy deficiency appears to be substantially greater in those younger than 15 years of gynecological age.

CAUSES OF LOW ENERGY AVAILABILITY IN FEMALE ATHLETES

Effective treatment of low energy availability in athletes requires that the origin of the low energy availability be identified. Low energy availability behaviors appear to derive from four different origins (1, 101). Some athletes intentionally reduce energy availability in a rational, but misguided, pursuit of the body size, body composition, and mix of metabolic fuel stores that are thought to optimize performance in their particular sport. Complex objectives may include reducing fat mass while increasing muscle mass and maximizing glycogen stores. For such athletes who reduce energy availability excessively, nutrition education and guidance regarding appropriate, individualized intermediate and ultimate goals, schedules, and methods may be sufficient to modify their diet and exercise behavior.

In other athletes, low energy availability originates in an eating disorder. Eating disorders are clinical mental illnesses that are often accompanied by other mental illnesses (102, 103). Therefore, eating disorders require psychiatric treatment, often inpatient treatment, as well as nutritional counseling. Because the mortality of eating disorders is so high, sports organizations need to develop institutional methods for distinguishing undernourished athletes with eating disorders from those who do not have eating disorders. This distinction may not be obvious, since undernourished athletes who are only trying to optimize performance may practice many of the same disordered eating behaviors (e.g., skipping meals, vomiting, using laxatives) as athletes with eating disorders. Athletes with eating disorders are distinctive in their resistance to the efforts of coaches, trainers, nutritionists, and physicians to modify their behavior.

The third origin of low energy availability in athletes is the suppression of appetite by prolonged exercise. This effect is compounded by the appetite-suppressing effect of diets containing high percentages of carbohydrates, which are commonly recommended to athletes in endurance sports. Even though many studies on this subject have been published over the past 15 years (101, 104), appetite remains a largely neglected topic in the field of sports nutrition. Indeed, the word “appetite” appears only once (and then only in a discussion of fluid losses at high altitude) in the recently revised joint position stand of the American Dietetic Association, the Dietitians of Canada, and the ACSM on nutrition and athletic performance (105).

Briefly, food deprivation increases hunger, but the same energy deficit produced by exercise energy expenditure does not (106). The appetite-suppressing effect of prolonged exercise has been demonstrated in controlled experiments with protocols ranging from a few hours to 12 weeks (101). The effect is mediated by the orexigenic hormone ghrelin, which induces us to begin eating, and by several anorexigenic hormones (including peptide YY, glucagon-like peptide 1, and pancreatic polypeptide) that induce us to stop eating. Exercise does not stimulate an increase in ghrelin concentrations, but does stimulate increases in the concentrations of anorexigenic hormones. As a result, “there is no strong biological imperative to match energy intake to activity-induced energy expenditure” (107).

Meanwhile, the appetite-suppressing effect of diets containing high percentages of carbohydrates has been demonstrated in experimental protocols ranging from a week (108) to a month (109, 110). As the percentage of carbohydrates in the diet was reduced, ad libitum energy intake spontaneously increased. As a result, the actual amount of carbohydrate consumed was preserved even though the percentage of carbohydrates in the diet decreased from 67 to 55%. The mechanism of this effect has not yet been identified, but may involve the greater bulk and fiber content of carbohydrate-rich foods.

Importantly, the large effects of these two factors are additive (108) so that together they can reduce energy availability below 30 kcal/kgFFM/day in endurance athletes. To avoid inadvertent low energy availability, therefore, athletes in endurance sports need to be trained to eat by discipline (i.e., planned amounts of selected foods at scheduled times) instead of appetite.

The fourth apparent origin of low energy availability among female athletes is that young women undereat for social reasons unrelated to sport. Around the world, about twice as many young women as young men at every decile of body mass index perceive themselves to be overweight, and the numbers actively trying to lose weight are even more disproportionate (111). The disproportion even *increases* as BMI declines, so that almost nine times as many lean women as lean men are actively trying to lose weight! Indeed, more young female athletes report improvement of appearance than improvement of performance as a reason for dieting (112). As a result, social issues unrelated to sport may need to be addressed to persuade female athletes to eat by discipline *beyond* their appetites.

NEEDED RESEARCH

More short-term experiments are needed to resolve the ambiguity about whether LH pulsatility depends on energy in general or on specific macronutrients in particular. Clinical trials are needed to verify that women can prevent or reverse functional hypothalamic amenorrhea by dietary reform alone without moderating the exercise regimen, and to develop effective interventions that may be sport specific. In addition, more animal experiments using the new modified activity stress paradigm (84) are needed to explore the physiological and neuroendocrine mechanisms of the female athlete triad in more detail. Finally, more experiments like Excalibur III are needed to determine whether other stressors besides exercise have any suppressive effect on LH pulsatility beyond the impact of their energy cost on energy availability.

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12 Ghrelin Responses to Acute Exercise and Training

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INTRODUCTION

Energy homeostasis is regulated by a complex neuroendocrine system including central and peripheral tissues (1, 2). Therefore, the hypothalamus in the brain appears to centrally integrate the various metabolic and hormonal signals, and has an important role in the central responses to changes in energy balance (3). Important in this regulatory system is also the existence of several gut, pancreatic and adipose tissue hormones that communicate the status of body energy stores to the hypothalamus (1). Among the gut hormones secreted in response to nutrient ingestion, ghrelin, peptide YY, cholecystokinin and glucagon-like peptide-1 are involved in regulating both acute and chronic energy homeostasis (4, 5). Therefore, peptide YY, cholecystokinin and glucagon-like peptide-1 function as negative feedback signals, suppressing appetite and food intake once nutrients are ingested (5, 6), and peptide YY has received more attention among appetite and food consumption suppressing peptides (4, 6–9). In contrast, to date, ghrelin remains unique as the only known circulating hormone that stimulates appetite and

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food consumption (6, 10). This chapter focuses on the available information about the effects of acute exercise and chronic training on the secretion of ghrelin.

Ghrelin, a 28-amino-acid-residue peptide produced by distinct endocrine cells of the stomach, was first described as an endogenous ligand for the growth-hormone-secretagogue receptor (11). Ghrelin has been found to promote positive energy balance by increasing appetite and food intake (12, 13). The rise in circulating ghrelin before a meal is a physiological signal for hunger and the body's cue for meal initiation (14, 15). Therefore, it is interesting to note that the rise in ghrelin levels and hunger occurs independent of food and time of day cues (14, 15). Meal responses of ghrelin are related to acute caloric intake over a typical day of eating in normal-weight subjects (16). In addition, fasting total ghrelin concentrations are shown to be negatively correlated to 24-h caloric intake (17). Accordingly, ghrelin concentrations increase while fasting and decrease after caloric intake (14). The decrease in ghrelin release is related to the amount of calories ingested (15, 18). Ghrelin is responsive to diet- and exercise-induced changes in body mass (16, 19). In addition to total ghrelin, acylated and unacylated forms of ghrelin have been found (20, 21). The acylated form of ghrelin is thought to be essential for ghrelin biological activity (22), whereas unacylated ghrelin, which is present in greater quantities than acylated ghrelin, has been suggested to be biologically inactive (20). It has also been shown that short aerobic exercise has no effect on total ghrelin response to a liquid meal, whereas an increase was shown in the acylated form of ghrelin (23). However, there are no data showing that significant increases in total ghrelin do not increase acylated ghrelin in humans (1, 24). It has also been found that total ghrelin and acylated ghrelin are positively correlated (25, 26), and both forms of ghrelin potentially play role in energy balance (23). In addition, recent studies have demonstrated that unacylated ghrelin is also biologically active, when the increase in unacylated ghrelin was strongly associated with weight loss and reduction in central adiposity in young healthy men (27, 28). Accordingly, future studies are needed to better clarify the responses of total ghrelin and its specific forms in various conditions of energy balance.

GHRELIN DURING GROWTH AND MATURATION IN CHILDREN

It has been proposed that ghrelin is a hormone that could influence growth and physical development in children (29). Cross-sectional investigations conducted in healthy children have demonstrated that ghrelin levels peak around 2 years of age and then gradually decrease during childhood (30). The initiation of puberty has been reported to substantially decrease circulating ghrelin concentrations (29, 31). A significant negative correlation has been found between fasting ghrelin and age (29, 31), and pubertal development (29, 32). In contrast, there are studies that have reported no reduction in fasting ghrelin concentration with increasing age (33) or pubertal development (34). It is well known that puberty is characterised by increased appetite and food intake (29) and ghrelin is known to stimulate appetite (20, 23). However, this increased appetite and food intake in puberty is not associated with an increase, but with a decrease in circulating ghrelin concentration in healthy children (31, 35, 36). Whatmore et al. (29) suggested that there could be an increased sensitivity for appetite stimulation by ghrelin over puberty to sustain growth during this period. There are also results to suggest that elevated energy expenditure and, therefore, also an increased energy intake in physically

active children during pubertal development are linked to higher ghrelin levels in these children compared with physically inactive children (35, 36). Accordingly, regular physical activity may increase circulating ghrelin levels during puberty to stimulate appetite and food intake to cover higher energy expenditure (35, 36). However, further longitudinal studies throughout puberty in children with various physical activity levels are needed before any definitive conclusions can be drawn.

GHRELIN RELATIONSHIPS WITH ADIPOSITY AND ENERGY AVAILABILITY

Ghrelin concentration is also significantly reduced in obese individuals (37–39) and substantially elevated in patients with anorexia nervosa (15, 40, 41), likely as an adaptive mechanism (15, 30, 42). Consistent with these patterns, a negative relationship of ghrelin concentration with body mass (32, 43–45), body mass index (4, 32, 36), total body fat mass (31, 35, 36, 45) and visceral fat mass (46, 47) has been found. Long-term exercise intervention and diet-controlled investigations have showed that ghrelin levels increase in response to exercise-induced weight loss and not because of food restriction per se, acting via a negative feedback loop that regulates body weight (10, 19, 48, 49). It has been suggested that changes in ghrelin concentration appear to be most sensitive to changes in body weight resulting from an overall energy deficit, independent of specific effects of nutritional intake and/or physical exercise (10, 19, 48, 49). Accordingly, recent studies have showed that short- (50) and long- (7) term manipulations in food intake and exercise energy expenditure demonstrate a close relationship between ghrelin and energy availability. For example, Scheid et al. (7) measured ghrelin, energy balance and body composition parameters before and after 3-month intervention in exercising women and found that circulating ghrelin does not play a role in the adaptive changes associated with exercise training when exercise occurs in the absence of weight loss. However, fasting ghrelin concentration increases when body weight is lost and may respond to even smaller changes in energy availability. In addition, the change in ghrelin concentration was inversely correlated with the change in body weight, body mass index, fat-free mass and energy availability after diet- and exercise-associated weight loss (7). In contrast, recent study by King et al. (51) demonstrated that equivalent energy deficits induced by food restriction or physical exercise have markedly different effects on appetite, energy intake and ghrelin concentrations. While food restriction elicited a rapid increase in appetite and energy intake and these responses appear to be related to postprandial suppression of ghrelin, acute energy deficits induced by vigorous-intensity exercise session did not alter appetite or energy intake and may be related to the failure of acute exercise to induce compensatory ghrelin responses. These results together may suggest that changes in body weight are needed before any changes in circulating ghrelin could be seen in healthy normal-weight untrained individuals.

GHRELIN RESPONSES TO ACUTE EXERCISE

Acute exercise may induce acute negative energy balance. There are a number of studies, including in athletes, that have examined the influence of an acute bout of exercise on total ghrelin (35, 52–64) and on acylated ghrelin (65–68) concentrations.

Most investigations with healthy untrained individuals (52, 54, 61, 64) and also athletes (57, 58, 63) would suggest that exercise-induced acute negative energy balance may not be sufficient to alter total ghrelin response. However, some studies have reported that total ghrelin concentration increased (53, 55, 56, 69) or even decreased (59, 60, 62) as a result of short-term exercise session. In addition, recent studies have reported no change (51, 66, 67) or significant suppression (65, 68) in acylated ghrelin after acute exercise.

Earlier study by Dall et al. (54) reported no change in total ghrelin concentration after acute cycling exercise for 45 min at the level of anaerobic threshold (AT) intensity in middle-aged healthy men. In healthy physically fit male individuals, circulating ghrelin concentrations remained unchanged after acute submaximal running workloads (50, 70 and 90% of maximal oxygen consumption (VO_2max)) (64) and also after a single bout of treadmill running for 1 h at 73.5% of VO_2max (52). In endurance athletes, Kraemer et al. (58) showed that a progressively intense intermittent exercise trial with runners on a treadmill at different exercise intensities (10 min at 60%, 10 min at 75%, 5 min at 90% and 2 min at 100% of VO_2max) does not change total ghrelin concentration, whereas no significant effects of 30-min acute on-water sculling exercise performed either below or above the intensity of individual AT on total ghrelin concentration in elite male rowers were observed (57). Therefore, constant load sculling exercise above individual AT showed that total ghrelin increase after exercise reached almost statistical significance (57). In another study, ghrelin concentrations were increased as a result of 2-h endurance rowing training session performed at the intensity of 80% of individual AT in competitive male rowers (69). Therefore, it was argued that the reduced resting levels of total ghrelin may have influenced the significant exercise-induced increase in ghrelin concentration in these athletes (69). However, assuming that the energy balance drives the ghrelin response to prolonged rowing exercise with the estimated energy expenditure of 1,200–1,500 kcal, it was conceivable to see that the post-exercise ghrelin concentration was related to the amount of work performed ($r=0.75$; $p<0.05$) (69). Another study in our laboratory showed that maximal 6,000-m rowing ergometer test (mean performance time 19 min and 52 s), in which all major muscle groups were involved, also increased immediate post-exercise total ghrelin concentration in elite male rowers, whereas at 30-min post-exercise, ghrelin levels were already decreased to the pre-exercise level (56). These results suggest that in contrast to other acute exercise studies with limited energy expenditure (52, 54, 58, 64), the energy expenditure in these rowing studies (56, 69) was sufficient to invoke significant post-exercise increases in circulating ghrelin concentrations. In addition, the participants in these studies were well-trained rowers, with a relatively large body mass. Their rowing movement requires a relatively high recruitment of total muscle mass, that is more energy costly in comparison with running or cycling (1). This may imply that the response of circulating ghrelin to acute exercise depends on the amount of total work performed (1). Taken together, it could be argued at present that a certain threshold reduction in energy availability should be reached before any significant post-exercise increases in total ghrelin concentration occur, and that the amplitude of the total ghrelin increase could be linked to the energetic status induced by acute exercise stress (1).

If the body energy reserves are limited, an acute exercise session with higher energy expenditure may cause significant alterations in total ghrelin concentrations and in

well-trained athletes, which use less overall muscle mass during a single exercise session (1, 53). For example, Christ et al. (53) found that a 3-h aerobic exercise session on a cycle ergometer at 50% of maximal aerobic power caused a negative energy balance that resulted in a significant increase in post-exercise total ghrelin concentration in well-trained athletes after a low-fat diet (0.5 g.kg⁻¹ lipids per body mass) but not after a high-fat diet (3.5 g.kg⁻¹ lipids per body mass). However, to what extent exercise intensity may influence total ghrelin response to acute exercise has not yet exactly been determined, although it has been suggested that low- rather than high-intensity exercise stimulates ghrelin levels independent of exercise duration (20, 55). Specifically, Erdmann et al. (55) investigated the effect of exercise intensity and duration on ghrelin release, hunger and food intake in normal-weight healthy individuals. Bicycle exercise on an ergometer for 30 min at a low intensity led to an increase of circulating ghrelin concentration, while ghrelin concentration remained unchanged during 30 min of moderate-intensity exercise (55). Another group of individuals exercised at low intensity on bicycle ergometer for 30, 60 and 120 min, respectively. Ghrelin concentration increased significantly above baseline about 50–70 pg/mL for the respective bouts of exercise (55). Furthermore, food intake after 120 min of bicycling was significantly greater than the first exercise and control trials (55). These data suggest that low- rather than high-intensity exercise stimulates increase in circulating ghrelin concentrations independent of exercise duration (55). However, future studies are needed before any definitive conclusions can be drawn.

There are some studies that have suggested that acute exercise stress could also result in a suppression of total (59, 62, 70, 71) and acylated (65, 68) ghrelin concentration during the exercise and the recovery period. The studies with total ghrelin concentrations have used more intensive exercise bouts including resistance exercise protocol (59, 62, 70), and it has been suggested that glucoregulatory stress from the acute intense exercise could result in a suppression of ghrelin during the recovery period (58, 59). Indeed, studies that have utilised more intensive exercise bouts have demonstrated that maximal exercise-induced large increases in insulin (59) and growth hormone (59, 68, 71) levels may suppress circulating ghrelin concentrations during the recovery period. However, there are also studies that contradict the results of these investigations. For example, exercise-induced increases in both ghrelin and growth hormone values have been observed after exercise expended 800 kcal in postmenopausal overweight women (72) and also following a 3-h aerobic exercise session at an intensity of 50% of maximal aerobic power in athletes (53). It has also been argued that post-exercise ghrelin responses may be independent of changes in energy balance (6, 50) and that acute exercise stress increases energy intake only after some time post-exercise (6, 65). In addition, it has been suggested that acute exercise can cause a transient (1–2 h) suppression of appetite after exercise, and ghrelin may be related to this appetite suppression (6, 65). It has been demonstrated that moderate- to high-intensity exercise sessions may also suppress acylated ghrelin concentrations, which occurs concomitantly with appetite suppression (6, 65, 67, 73). However, this effect appears to be relatively brief and suppressed acylated ghrelin levels return quickly to at least control levels and may remain relatively constant for some time after exercise (6, 68). Accordingly, it is possible that acylated ghrelin may be important in determining changes in appetite resulting from exercise (51, 65, 67). However, given the diversity of the role of ghrelin in

human physiology (68, 74), it is possible that the transient suppression of circulating acylated ghrelin that may be observed during acute exercise may be entirely unrelated to appetite regulation (66, 67). At present, the physiological relevance of this response is not known (67, 68). Shiiya et al. (68) argued that the possible suppression of acylated ghrelin during acute exercise might also be due to a decreased synthesis and/or an increased utilisation of acylated ghrelin in peripheral tissues. Clearly, it is inconclusive how circulating ghrelin concentrations change in response to acute exercise, and there is a need for further investigations to elucidate the mechanisms regulating ghrelin synthesis and clearance during and after acute exercise (20, 68).

CHRONIC EXERCISE TRAINING AND GHRELIN RESPONSES

Ghrelin concentrations have been reported to increase after long-term exercise interventions in some (16, 19, 20, 47, 48, 75) but not all (45, 76) studies in previously untrained individuals. It appears that weight loss associated with long-term exercise plays an important role to increase total ghrelin concentrations (19, 47, 48). Unfortunately, data on total ghrelin responses to prolonged exercise training are mainly available from obese individuals (47, 48, 76, 77), whereas only limited data are provided for athletes (24, 78, 79). Most of the previous investigations have studied total ghrelin response to prolonged exercise training (16, 19, 20, 24, 47, 48, 75, 76, 78, 79), while only few intervention studies have measured acylated (23, 77, 80) and unacylated (27, 28) ghrelin concentrations separately. To the best of our knowledge, there are no published investigations that studied the response of different forms of ghrelin to prolonged training period in athletes.

Different investigators have found that total ghrelin levels increase during body weight loss, and that the loss of body weight is the most potential determinant of the increase in total ghrelin concentration (16, 19, 20, 47, 48, 75). For example, Leidy et al. (19) demonstrated that a 3-month energy deficit-imposing diet and 5-days-a-week exercise training intervention programme had no impact on circulating ghrelin concentration in weight-stable normal-weight women, even though the participants expended a mean of 486 kcal per exercise bout. In another group of healthy women, circulating ghrelin concentrations were increased, together with body weight loss, as a result of the same exercise programme (19). Therefore, body weight, body fat mass and resting metabolic rate significantly decreased before the increase in circulating ghrelin in the weight-loss group (19). It was suggested that ghrelin responds in a compensatory manner to changes in energy homeostasis in healthy young women and that ghrelin exhibits particular sensitivity to changes in body weight (19). In addition, Foster-Schubert et al. (48) reported that total ghrelin levels increased by 18% in overweight sedentary postmenopausal women who lost more than 3 kg body weight after 1-year aerobic exercise training programme. In addition, another study investigated two groups of obese prepubertal children who were randomly assigned to two interventional groups, either receiving dietary recommendations or engaging in physical exercise classes for 6 months (47). In both groups, circulating ghrelin levels showed a progressive increase during the study period, which was accompanied by significant reduction of overweight and negative energy balance (47). Children were also measured 1 year later, when they had regained weight—and ghrelin concentrations decreased towards baseline measures (47).

It was also found that baseline ghrelin had strong negative correlation with measures of central obesity in prepubertal obese children (47). In contrast to these findings, Ravussin et al. (45) found that neither positive energy balance caused by overfeeding nor negative energy balance induced by exercise had a significant effect on total ghrelin concentration over 100-day study period. The impact of negative energy balance on ghrelin concentrations at the end of study was smaller, due to the possible effect of accustomization (45). Similarly, Morpurgo et al. (76) found that circulating ghrelin concentrations were unchanged after a 3-week exercise training programme that reduced body weight in severely obese individuals. It could be speculated that the amplitude of total ghrelin response to negative energy balance in these studies could be linked to the energetic status of studied individuals, which is attributable to specific body fat and exercise characteristics.

In heavily exercising women, menstrual disturbances have been linked to an energy deficiency, where caloric intake is inadequate for exercise energy expenditure (15, 42). These menstrual disturbances, together with an energy deficiency, are largely attributable to athletic events where the emphasis is on the achievement of thin and lean physiques, which may require low body weight and body fat percent such as figure skating, gymnastics and long-distance running (15). There are data to suggest that exercising women with varying severities of menstrual disturbances are discriminated from each other based on their ghrelin concentrations (15, 40, 42, 81). It has been observed that the energy deficiency increases in severity across the continuum of menstrual cycle disturbances, such that physically active women with amenorrhea had the lowest resting energy expenditure relative to fat-free mass, together with the highest ghrelin levels, compared to the physically active women with subtle menstrual disturbances and non-athletic controls (40, 81). Increased ghrelin concentrations in women with amenorrhea may have a role in reproductive system (15, 42, 81). Accordingly, an inverse relationship between ghrelin concentration and gonadal steroids was observed, and it was postulated that circulating ghrelin levels may differentiate between athletes who will or will not develop functional hypothalamic amenorrhea (40, 42).

Unacylated ghrelin responses to prolonged exercise-intervention period have only been reported in overweight children (80) and in adult men (27, 28). For example, Cederberg et al. (27, 28) conducted a prospective study of 552 young men undergoing military service with a structured 6-month exercise training programme, and an overall increase in the level of unacylated ghrelin was observed. Therefore, an increase in unacylated ghrelin concentration was largest among those individuals whose body weight and waist circumference reduction was largest. It was suggested that an increase in unacylated ghrelin concentration was associated with improved insulin sensitivity (27) and with reduced body weight, fat mass and waist circumference (28). Therefore, the association of unacylated ghrelin with changes in central obesity was stronger than with total fat mass (28). In another study, a 12-week exercise intervention caused a trend for an increase in total and unacylated ghrelin concentrations with unchanged acylated ghrelin levels in 11-year-old overweight boys (80). However, the limitation of this study was that unacylated ghrelin was not directly measured, while total and acylated ghrelin concentrations demonstrated some variability (80). Taken together, further studies that directly measure total, acylated and unacylated ghrelin concentrations are needed before any further conclusions can be drawn about the exact roles of total ghrelin and its specific forms during body weight loss resulting from negative energy balance.

Only few studies have investigated total ghrelin response to exercise training in male athletes (1, 24, 78, 79). Specifically, ghrelin responses to a weight reduction period before competitions in bodybuilders (24), and a high-volume low-intensity endurance (78) and a high-volume low-intensity concurrent endurance and resistance (79) training periods in rowers have been studied. In our study with bodybuilders, 14 athletes were divided into seven competitors and seven control subjects and were tested three times: 11 weeks, 5 weeks and 3 days before the national championships (24). The competitors' group was in the negative energy balance at all three testing times, while there were no significant differences in the energy balance across the testings in the control group. Competitors were able to significantly decrease their mean body weight by 4.1 kg during the 11-week preparation for competition, which also resulted in significant decreases in body fat mass values, whereas no changes in body composition or ghrelin parameters over the 11-week study period were seen in the control individuals (24). The total ghrelin response in competitors' group was such that ghrelin levels increased in these well-trained athletes with relatively low body fat mass during the first weeks of energy restriction, but reached a plateau beyond which there was no further increase—despite continuing negative energy balance and body weight loss (24). Specifically, the energy deficit at about 536 kcal/day after the first 5 weeks of study period in the competitors' group was already sufficient to cause a significant increase in total ghrelin concentration, whereas no further increase in ghrelin levels was observed with the energy deficit reaching 978 kcal/day after 11-week preparatory period (24). The athletes in the present investigation were competitive bodybuilders with a mean body fat percentage of 9.6% at the beginning of the study and 6.5% at the end of the study. This indicated that total energy stores were quite limited in these athletes and that there was not extra accumulated energy available for them (24). Furthermore, Mäestu et al. (24) argued that ghrelin secretion might have reached its limits at some point, and the negative energy balance of more than 900 kcal/day and a significant body weight loss of 2.4 kg in the second 5 weeks (between weeks 6 and 11) were not sufficient to further the significant total ghrelin increase. According to the results of this study, it was concluded that circulating ghrelin levels increase in well-trained athletes with relatively low body fat mass, but reach a plateau beyond which there is no further increase in ghrelin concentration, despite continuing negative energy balance and body weight loss (24).

In studies with rowers, fasting and acute exercise-induced ghrelin concentrations were measured after a reference week with usual training volume, after 2 weeks of high-volume training and after a recovery week with reduced training volume (78, 79). Therefore, different training regimens were used in these studies. In the first study, 90% of the trainings were aerobic (rowing, running or cycling) and only 10% resistance type of exercise (78), while in the second study about 50% of the trainings were low resistance exercise and 50% aerobic type of trainings (79). These training periods are typical during preparatory period in rowers (82). It was interesting to find that fasting ghrelin concentrations were not increased after the 2-week period of extended training volume in both studies (78, 79), while a decrease in fasting ghrelin was observed after recovery week (79). These results may indicate that fasting ghrelin concentrations are not sensitive to temporarily increased training volume in rowers. Similarly, a 2-h submaximal rowing test demonstrated that there were no significant post-exercise increases in circulating ghrelin concentration after 2 weeks of high training volume, in contrast to

increased post-exercise ghrelin levels after reference (79) and recovery weeks (78). At present, the behaviour of ghrelin after 2 weeks of extended training period is difficult to explain, as it is conceivable to think that if caloric restriction is prevalent, an increase in ghrelin concentration would occur to provoke energy intake (78, 79). In addition, previous acute exercise studies with rowers have demonstrated that a 2-h submaximal rowing exercise bout significantly increases post-exercise circulating ghrelin concentration in rested well-trained rowers (69). Although energy intake and energy expenditure increased significantly, the negative energy balance after 2-week period of high training volume and energy restriction was about 408 and 455 kcal/day in concurrent resistance and endurance (79) and endurance (78) training studies, respectively. It could be postulated that during specific metabolic conditions resulting from the preceding high-volume training period with high energy expenditure, negative energy balance, temporarily restricted caloric condition in fasting state and probably relatively low body energy stores (i.e. low body fat percent) may all contribute to further exercise-induced effect of energy expenditure that leads to downregulation of ghrelin concentration (1, 78, 79). However, the mechanism of how the pre-exercise metabolic condition, that is, negative energy balance influences the behaviour of post-exercise ghrelin concentration, has not been established and needs further research (1, 78, 79).

CONCLUSIONS AND FUTURE DIRECTIONS

Ghrelin concentrations decrease during growth and pubertal development and are linked to the nutritional status, with lower levels in obese and higher levels in underweight individuals. Ghrelin has been reported to be responsive to diet- and exercise-induced changes in body weight. Accordingly, circulating ghrelin levels respond quickly to changes in energy balance, increasing with weight loss and decreasing with weight gain. There is some evidence to suggest that higher ghrelin levels in physically active children during pubertal development may likely be a compensatory mechanism to avoid body weight loss during growth and exercise training, when there is a need for higher energy consumption. In addition, elevated ghrelin levels have also been observed in female athletes with chronic energy deficiency, and ghrelin may differentiate between athletes who will or will not develop amenorrhea. To date, most of the studies have investigated the role of ghrelin in energy balance in different groups of obese individuals. However, further longitudinal studies throughout puberty, and also in adults, are needed to better describe the role of ghrelin in children with different physical activity and body composition parameters.

The current information regarding the role of circulating ghrelin concentration in energy balance during acute exercise and prolonged training stress seems to confirm that this peripheral mediator of energy balance at certain circumstances could be used to assess the physiological condition of the athlete. In well-rested athletes, an acute bout of exercise may increase ghrelin response when the exercise energy expenditure is at least 400 kcal. The amplitude of circulating ghrelin response could be linked to the energetic status induced by acute exercise, and at certain circumstances could be used to characterise acute exercise stress in endurance athletes. However, it appears that basal and post-exercise ghrelin response is not sensitive enough to represent changes in training volume and energy availability in athletes. There is also some evidence to suggest

that although ghrelin increases together with body weight loss in well-trained athletes with already relatively low body fat mass, there may be a plateau beyond which there is no further increase in ghrelin despite continuing negative energy balance and body weight loss. However, further studies are needed to describe the exact role of ghrelin at different training conditions in athletes representing different sport events.

Finally, it has to be taken into account that majority of the studies to date have measured only total ghrelin concentrations in blood, without measuring ghrelin's specific forms, acylated and unacylated, separately. It needs to be acknowledged that although a relationship between total and acylated ghrelin levels has been reported in various studies, different forms of ghrelin may not respond similarly to changes in energy availability in different conditions. Furthermore, it has recently been suggested that unacylated ghrelin may also have a role in body energy balance. Accordingly, further longitudinal studies in different populations with various body composition and physical activity patterns, including athletes, are needed to better understand the role of ghrelin and its specific forms in conditions of energy deficiency, surplus and balance.

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13

Hormonal Regulation of Fluid and Electrolyte Homeostasis During Exercise

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CONTENTS

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INTRODUCTION

In response to exercise, there are numerous alterations in fluid and electrolyte homeostasis. These perturbations occur immediately upon initiation of exercise and can persist for hours or even days after completion of exercise. The endocrine system plays an important role in the regulation of fluid and electrolyte homeostasis that must occur with exercise. Dysregulation of the endocrine system may limit exercise activity and, in some incidences, result in debilitating morbidities or death. This chapter emphasizes responses to exercise and reviews the importance and factors involved in the maintenance of fluid and electrolyte balance. Previous reviews will be used to address the basics of effected systems; however, emphasis is placed on new data and the current discussions about performance of work and exercise.

The term exercise is an ambiguous term covering a broad range of physical activities. The term is employed to define activities such as running and cycling, but is also used to cover the activities of daily living and work. Thus, when discussing responses to exercise it is important to clarify the type of activity, the level at which it is performed,

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and the duration. In defining the responses to exercise it is essential to understand the definitions of workload. The absolute workload is the level of exercise being performed, such as running on a treadmill at a defined speed. For individual subjects, this would produce a variable response depending on their level of fitness/training. Therefore, to compare exercise responses between subjects, relative workload is often employed as a normalization technique (1–3). Relative workload is expressed as a percentage of the maximum capability of the individual to perform that specific exercise and is often further standardized to the heart rate or oxygen consumption of the subject.

PHYSIOLOGIC RESPONSES TO EXERCISE

A variety of conditions results from alterations in fluid and electrolytes and affects the performance of exercise and work. The disruption of the balance of fluids and electrolytes correlates with limitation of work capacity; however, the range of changes tolerated may be extended with training and repeated exposures. In general the body can undergo one of several responses to exercise: dehydration, dysnatremia, hypovolemia, or hypervolemia. The following text will review each.

Dehydration is defined as a reduction in total body water (TBW) and an increase in plasma electrolyte concentrations. Heavy exercise and extreme heat are two of the most prevalent causes of dehydration, as both are associated with exercise and subsequent loss of fluid volume due to sweating and inadequate fluid intake (4–7). Current evidence suggest that dehydration resulting in a decrease of greater than 2% body mass will adversely affect exercise performance (6–8). However, decrements in self-paced exercise may not occur until a 4% loss in body weight (9). Regardless of the level of dehydration, a loss of TBW and an increase in plasma electrolyte concentrations are associated with limited work performance and, in extreme cases, death.

Dysnatremia covers the occurrence of both increases and decreases in plasma sodium observed with exercise (10–14). Siegel et al. noted an incidence rate of dysnatremia of 32.5% in 1,319 collapsed marathon runners (12). Of these, 85% were hypernatremic and 15% hyponatremic. Both of these conditions have been associated with deaths in competitive runners.

Hypovolemia is a decrease in blood volume in the absence of changes in plasma electrolyte concentrations. This can occur with exercise or hemorrhage (15), and follows periods of water submersion to the neck or the administration of diuretics commonly used in the treatment of hypertension (16, 17). Hypovolemia necessitates an increase in heart rate at submaximal workloads and a more rapid increase in body temperature, both indicative of limited work performance (7).

In contrast, hypervolemia is the expansion of blood volume. There is extensive literature on the expansion of blood volume by increasing the red cell mass; however, within the scope of this chapter this term refers to expansion of the plasma volume. Plasma volume is expanded by exercise training and by acute excessive ingestion of fluids, hyperhydration (7, 18–20). Warburton et al. reviewed the literature on the effect of acute expansion of plasma volume and found minimal increases in maximum oxygen consumption, but there were negligible changes in exercise endurance (20).

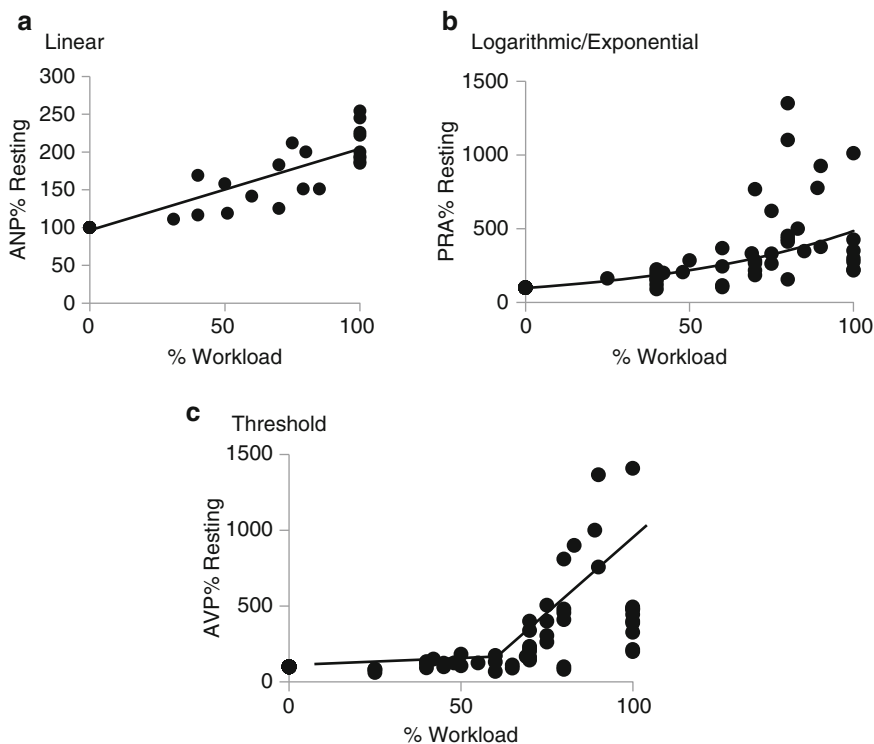


Fig. 1. The various patterns of response of hormones to exercise. The individual *dots* represent the response from independent studies of exercise on a cycle ergometer with varying workloads. The variance represents differences in how the exercise was performed, state of hydration of the subjects, and difference in assay techniques. With these confounders the patterns in response to exercise are still present. The linear example is demonstrated by the response of atrial natriuretic peptide (ANP; (a) 11 studies), the logarithmic/exponential increase by plasma renin activity (PRA; (b) 20 studies), and the threshold response by vasopressin (AVP; (c) 23 studies).

MODULATION OF HORMONES IN RESPONSES TO EXERCISE

Workload Intensity

The response of hormones to exercise is closely related to the amount of relative work performed. There are three basic patterns of hormones during exercise. The first is an increase proportional to the increase in relative workload. For example, with each increase in workload, there is a constant increase in the plasma hormone concentration of atrial natriuretic peptide (ANP) (Fig. 1a). The second pattern is a logarithmic/exponential increase such as that reported for plasma renin activity (PRA) (Fig. 1b). With increasing workloads, the level of hormone increases at an exponentially faster rate. The third pattern is related to an onset of an increase at a given threshold; this is observed for vasopressin (Fig. 1c). A threshold response for exercise is usually associated with the onset of anaerobic metabolism and a relative workload of about 70%. This has also been associated with the increase in stress-related hormones such as cortisol and adrenocorticotrophic hormone (ACTH). These patterns of increased hormone concentrations are consistently observed in studies of acute exercise when the response is expressed relative to the workload of the task performed.

Exercise Duration

The duration of exercise is also a confounding factor in the response of hormones to exercise. Extended time, rather than intensity, may have a greater influence on the levels of hormones during exercise. This is especially true of hormones involved in the regulation of fluid and electrolyte homeostasis. As exercise progresses, there is an increased metabolic heat necessitating sweating and therefore the loss water and electrolytes. The increase in aldosterone, which regulates sodium balance, is increased twofold with acute maximal exercise (i.e., running on a treadmill) and returns to baseline levels within an hour. Extended exercise times elicit similar changes in plasma volume and sodium concentrations, but aldosterone concentration increases three to four times the basal levels and remains elevated for over 24 h (21). The greater and enduring response to exercise of longer duration is postulated to be due to additional regulators associated with the “stress” of exercise (10, 22). Of note, hormone concentrations may vary over time with exercise of long duration, such as during a marathon or ultra-endurance events. For example, ANP is increased by a factor of ten during the first 10 km of a marathon but subsequently decreased to levels only fivefold greater than baseline (23). In addition, the conditions under which recovery is conducted, access to fluids or cool down exercise, are influential in the postexercise responses and need to be clarified (24, 25). Recently, Hew-Butler et al. compared the hormonal responses to maximal exercise with a mean duration of 10–60 min of exercise at a treadmill speed equivalent to 60% of the maximum (26). With maximal acute exercise, significant increases were reported for vasopressin (fivefold) and aldosterone (twofold), while at submaximal effort, only aldosterone was increased (3.3-fold). Thus, both the length of time and intensity of the workload must be considered when studying the regulation and function of hormones in response to exercise.

Training

The level of training of a subject may influence the hormonal response to exercise (27, 28). While much of the variance between subjects at absolute workloads may be due to differences in the relative workload being performed, there are still aspects of training that change the response. Individuals undergoing persistent heavy bouts of exercise training may have alterations in resting levels. In subjects doing daily long-distance runs, plasma aldosterone concentrations are elevated compared to controls (29). However, for the majority of hormones regulating fluid and electrolyte homeostasis, training does not appear to be as an important of a factor as the intensity and duration of exercise in the response of these hormones.

Hydration Status

The initial hydration status of a subject may influence subsequent responses to exercise. Fluid intake during the performance of exercise is also an influencing factor. Dehydration or hyperhydration alters initial hormone levels; however, the subsequent response to exercise appears independent. Geelen et al. found that following dehydration, ingestion of fluid caused a rapid and pronounced reduction in vasopressin and an increase in norepinephrine that was independent of changes in plasma osmolality and

volume (30). No changes were noted in epinephrine, aldosterone, PRA, or ANP. Additional investigations reported that the greater the volume consumed, the more pronounced the decrease in vasopressin and increase in norepinephrine (24, 31–33). This suggests an oropharyngeal reflex may be present and mediated by the sympathetic nervous system.

Khamnei et al. evaluated the effect of the combination of exercise and postexercise fluid intake on vasopressin (24, 32). Subjects exercised at 50% of their maximum oxygen uptake for 30 min. Exercise resulted in a 45% increase in vasopressin which was sustained after exercise in the absence of fluid intake. In contrast, when a large volume of fluid was ingested after exercise, control levels of vasopressin were obtained within 3 min. These findings suggest fluid intake may have a profound effect on hormonal responses during exercise, independent of changes in plasma volume and osmolality. Hew-Butler has put forth the hypothesis that inappropriate increases in vasopressin during prolonged exercise in the presence of adequate fluid intake may be a contributing factor to hyponatremia and subsequent morbidity (10, 22). This line of research awaits additional well-controlled prospective studies to fully identify underlying mechanisms.

Sex

Sex of the subject is another factor with demonstrated differences. In women, the phase of the menstrual cycle in which exercise is performed may alter the hormonal responses. Resting aldosterone levels are increased during the mid-luteal phase of the cycle, and the response to exercise is amplified (34). Further work by Stachenfeld and coworkers has demonstrated the effect of progesterone and estrogen on the levels and responses of hormones that are important in fluid and electrolyte homeostasis (35, 36). In patients with coronary heart disease, basal levels of vasopressin were elevated in men; however, in responses to a 6-min walk test that increased vasopressin, ANP, norepinephrine, and epinephrine, there were no differences between males and females (37). Following exercise in well-trained subjects to decrease body mass by 3%, women had a lower PRA and faster recovery of aldosterone and slower recovery of vasopressin compared to men (38). Overall, there are minimal differences reported between male and females in resting hormone levels, and differences in response to exercise are not fully delineated (39).

Health Status

The initial health of the subject is an influencing factor in the hormonal responses to exercise, and offers insights to the pathophysiology of various disease processes, and in some cases a means of diagnosis and/or rehabilitation. The presence of disease represents a shift in homeostasis that requires alteration in the responsiveness of hormones important in fluid and electrolyte homeostasis. In age-matched subjects, Shim and coworkers reported that subjects with an exaggerated blood pressure response to exercise, which is indicative of a greater risk for hypertension and prevalence of cardiac hypertrophy, had elevated levels of angiotensin II at rest and an augmented increase in response to exercise (40). However, there were no significant differences in norepinephrine, epinephrine,

PRA, or aldosterone at the end of exercise. Kjaer et al. studied patients with congestive heart failure (CHF) and compared them with healthy subjects at 50 and 75% of their maximum workloads on a cycle ergometer (41). Basal levels of ANP, brain natriuretic peptide (BNP), vasopressin, and PRA were elevated in patients with CHF. In response to exercise, ANP, arginine vasopressin (AVP), norepinephrine, and epinephrine were all increased in both groups. Even though higher absolute levels were observed in subjects with CHF, when expressed as a percent of basal concentrations group, differences were negated. BNP was increased with exercise only in patients with CHF.

Coiro et al. assessed the response of vasopressin to exercise to exhaustion on a bicycle ergometer in subjects with diabetes and controls, and further segregated the groups as smokers and nonsmokers (42). Baseline vasopressin concentrations at rest (2.1–2.6 pg/mL) were not different between groups. In all groups, there was a significant increase in vasopressin in response to exercise. While smoking was not identified as a contributing factor, there was a greater increase in vasopressin in subjects with diabetes (12–13 pg/mL) than controls (7–8 pg/mL). The difference between diabetic and normal subjects could not be attributed to cardiovascular or respiratory responses.

Other Influencing Factors

Other confounders, such as position of exercise and age, have been identified to influence hormonal responses to exertion. Wolf et al. compared supine and upright exercise on a cycle ergometer at a relative workload of 40–50% for 20 min. With supine exercise, the response of PRA and aldosterone to exercise was increased by 90% and 49%, respectively, in contrast to upright exercise (43). These differences occurred in the absence of difference between the types of exercise in plasma osmolality or blood pressure. Perrault et al. found ANP concentrations to be increased and vasopressin, PRA, and norepinephrine to be reduced, during supine exercise on a cycle ergometer in comparison to exercise in an upright position (44). During the performance of a marathon, subjects with a mean age of 47 years had an increase in ANP to 104 pg/mL compared to 43 pg/mL in younger subjects with a mean age of 28 years (23). In addition, differences in hormone concentrations reported in response to exercise may be in part explained by the differing methods of measurement. The presence of such confounders in the comparison of the hormonal responses to exercise has not been systematically addressed, partially limiting our interpretation of the role of hormones in fluid and electrolyte homeostasis during exercise.

HORMONE RESPONSES TO EXERCISE

The hormones of consequence to fluid and electrolyte balance in exercising humans are those involved in the regulation of thirst, and function of the kidneys and sweat glands. The essential hormones are the catecholamines, vasopressin, the renin-angiotensin-aldosterone system, and natriuretic peptides. While these hormones have a variety of functions, the focus of the present review will be on their responses to exercise and impact on fluid and electrolyte homeostasis during and following exercise. Circulating levels of these hormones are altered during exercise as a function of changes in secretion,

metabolism, and volume of distribution. The most common measurement of these hormones in association with exercise is the circulating concentrations, which will be the focus of the present effort.

Catecholamines

Catecholamines, specifically norepinephrine and epinephrine, are derived from increases in sympathetic nervous system activity and the adrenal glands (45, 46). The kidneys are also suggested as a source of norepinephrine (47). Levels of circulating catecholamines respond rapidly upon the onset of exercise in order to redistribute blood flow to meet metabolic demands (2, 48, 49). In response to exercise, there is a progressive increase in circulating norepinephrine levels from 1.3 to 3.0 nmol/L at rest to 12.0 nmol/L following maximal exercise (45, 50–52). The increase in epinephrine occurs later in the course of exercise and can rise from resting levels of 380–655 pmol/L to concentrations over 3,000 pmol/L. The increase in the ratio of norepinephrine to epinephrine demonstrates activation of the sympathetic nervous system and is attributed to active spillover from the muscles during exercise (45, 52–54). With continued exercise, there is an attenuation of the increase in the ratio of norepinephrine to epinephrine, which is indicative of an increase in the release of epinephrine predominately from the adrenal medulla under the control of hypothalamic mediation in addition to the sympathetic nervous system. Following exercise, plasma levels of catecholamines return to resting levels in a matter of minutes, as they have a short half-life due to degradation, and reuptake by the sympathetic nervous system. Recent studies that inhibited the reuptake of norepinephrine have demonstrated an increase in the time necessary to complete work equal to 30 min of exercise at 75% of maximal workload (55, 56). These studies suggest clearance from the circulation of norepinephrine plays a role in fatigue.

Vasopressin

AVP is also known as vasopressin or antidiuretic hormone (ADH). It is a neurohypophysial hormone synthesized in the hypothalamus and stored in the posterior pituitary (57, 58). Vasopressin is a pressor that alters peripheral resistance, but its greatest effect is on the reabsorption of water in the collecting tubules of the kidneys. Secretion of vasopressin is regulated by alterations in plasma osmolality and blood pressure. Circulating concentrations of vasopressin in humans are 1–4 pg/mL (57, 59–62). With maximal exercise, vasopressin concentrations of 4–24 pg/mL are reported. Maximum conservation of water by the kidneys is observed at vasopressin levels of 10–20 pg/mL. With progressive increases in exercise, elevation of vasopressin is not observed until 70% of maximum workload is attained, i.e., the anaerobic threshold (Fig. 1c). Animal experiments have demonstrated an increase in activation of hypothalamic neurons that is indicative of increased vasopressin content (production) and of performing above the anaerobic threshold (63). Thus, the response of vasopressin appears to be associated with the onset of anaerobic metabolism, which is also related to increases in “stress hormones” such as cortisol and ACTH. An increase in vasopressin may persist for over 60 min after exercise or longer if access to fluids is restricted. Of note, at low workloads of about 25% of the anaerobic threshold, vasopressin decreases have been reported.

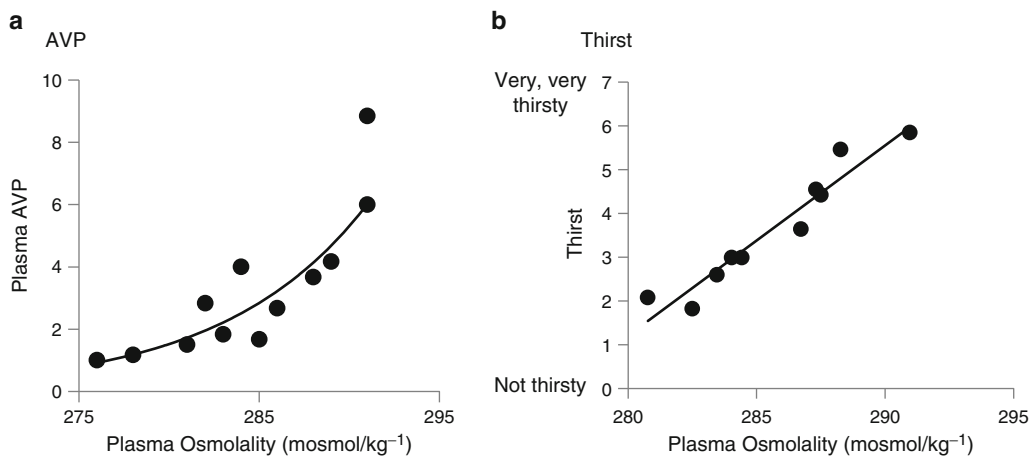


Fig. 2. (a) Levels of vasopressin and (b) subjective assessment of thirst in association to plasma changes in osmolality during moderate exercise. Measurements were from subjects with different levels of fitness, under various levels of hydration (redrawn from Merry et al. (27)).

A variety of factors have been demonstrated to mediate the increase in vasopressin with exercise, including the increase in osmolality and reduction in intravascular volume; however, the increase in plasma osmolality appears to be the primary mediator (Fig. 2) (59, 64, 65). In subjects exercising at 65% of maximum while running on a treadmill, there was a progressive increase in vasopressin with progressive workloads (66). In subsequent tests which involved dehydration that decreased body weight by 3 and 5%, resting vasopressin levels were increased in association with the decrease in blood volume; however, in response to exercise, further increases in vasopressin were related to the magnitude of the increase in osmolality. Brandenberger et al. evaluated rehydration during exercise giving subjects no fluids, water, or an isotonic solution. Intake of water reduced osmolality, but did not alter plasma volume (67). Consumption of the isotonic solution did not change osmolality, but increased plasma volume. Both methods of rehydration decreased the rise in vasopressin levels with exercise, as well as those of PRA and cortisol. Others have reported similar findings (32, 44, 68). The independence of the increase in osmolality and blood volume, and the regulation of vasopressin in response to exercise, is similar to that reported with dehydration. Coiro and colleagues have demonstrated that the increase in vasopressin during exercise to exhaustion may be attenuated by blockade of 5-HT₃ serotonergic receptors and administration of somatostatin, supporting another means of mediating the increase in vasopressin during exercise (69). Recently, Hew-Butler et al. have questioned the relationship of vasopressin and plasma osmolality during exercise. In subjects participating in an ultramarathon, they observed 3.9-fold increase in plasma vasopressin, no significant change in plasma sodium, and a significant decrease in plasma volume (10, 22). They also evaluated cyclists during a 109 km race and observed nearly identical changes (70). In subjects participating in an ultramarathon they observed a 3.9-fold increase in plasma vasopressin in the absence of a significant change in plasma sodium though plasma volume was significantly decreased. These authors and others hypothesize that under

conditions of prolonged exercise, the osmotic regulation of vasopressin is overshadowed by non-osmotic stimuli, of which, the reduction in blood volume plays a minor role (14, 71, 72). The increase in AVP was associated with elevations in cortisol, oxytocin, and BNP, which underscores the relationship of AVP release with “exercise stress.” Irrespective of the means, vasopressin is elevated by more than fourfold during acute exercise to exhaustion or intense prolonged exercise.

Renin-Angiotensin-Aldosterone

The renin-angiotensin-aldosterone systems are closely coupled, and increased in response to exercise. Renin is released from the kidney in response to sympathetic nerve stimulation, as well as norepinephrine spillover, resulting in increased plasma concentrations (17, 45, 52, 73–76). Renin then converts angiotensinogen to angiotensin I, which is subsequently transformed to angiotensin II in the lung. Angiotensin II promotes the release of aldosterone from the adrenal gland.

With exercise, all aspects of this system are increased and play a variety of roles in the regulation of fluid and electrolyte homeostasis (3, 45, 60, 77, 78). At rest, PRA has levels in the order of 0.15–0.55 ng angiotensin I/mL/h and with maximal exercise increases to levels of 1.11–1.67 ng angiotensin I/mL/h. There is an exponential increase in renin activity with increasing workloads; significant differences are reported at levels of 60–70% of maximum (Fig. 1b). The increase in PRA with exercise is positively associated with the increase in angiotensin II. Basal levels of angiotensin II are 15–25 ng/L, with values of 130–160 ng/L achieved with maximal exercise. Aldosterone release is regulated by angiotensin II, as well as ACTH and the plasma levels of sodium and potassium. Aldosterone concentration increases from resting levels of 80–830 pmol/L to concentrations of 250–3,330 pmol/L with maximal exercise. Blockade of the conversion of angiotensin I to angiotensin II does not attenuate the response of aldosterone to maximal exercise, which supports the theory that other pertinent regulatory factors are involved (79, 80). The elevation of aldosterone may persist for days after exercise, and levels are dependent upon the sodium and water intake (21). In the postexercise period, the increase in aldosterone may be the product of increased water intake, which reduces the plasma sodium concentration or the persistent elevation of aldosterone—which is due to activation of the ACTH. Irrespective of the cause, the increase in aldosterone due to exercise plays a role in the conservation of sodium in sweat glands and kidneys (Fig. 3).

Natriuretic Peptides

Peptides demonstrated to elicit a natriuresis have been deemed natriuretic peptides. These include ANP, BNP, urodilatin, and adrenomedullin. These peptides appear to participate in the regulation of fluid homeostasis by protecting against volume and pressure overloads. Though these peptides have been extensively studied over the past 30 years in patients with disease such as heart failure, pulmonary hypertension, and chronic renal disease, their response to and role during exercise are not well defined. Additionally, well-designed studies in control subjects or during competitive events have yet to be undertaken.

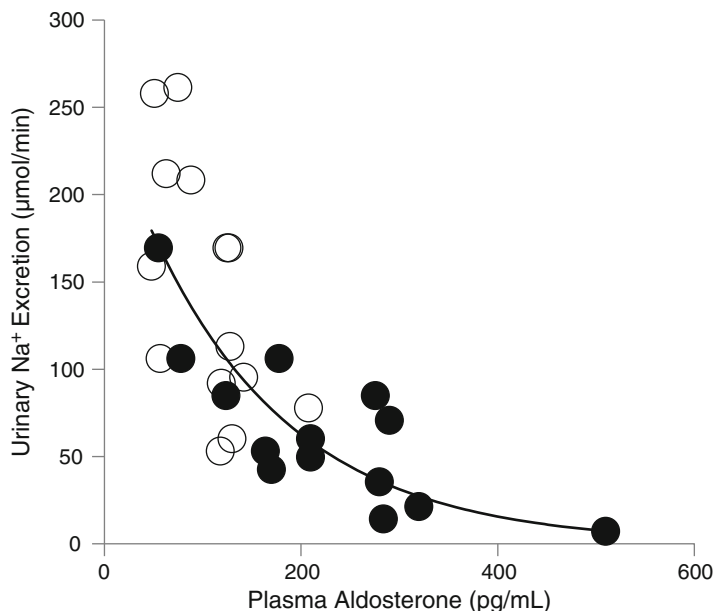


Fig. 3. Plasma aldosterone concentrations were compared to the urinary excretion of sodium at the end of a 2-h run (*closed circles*) and following 48 h of recovery with food and water ad libitum (*open circles*). With exercise, there was an increase in aldosterone, and over the recovery period there was a decrease (adapted from Wade et al. (29)).

ATRIAL NATRIURETIC PEPTIDE

ANP is increased with exercise in a linear response (Fig. 1a). Resting plasma levels of 10–49 pg/mL are increased to over 100 pg/mL with acute maximal exercise (22, 25, 39, 44, 81, 82). In response to long-duration exercise, there is initially a pronounced increase, a subsequent fall, and then a re-elevation of levels, persisting until completion of the exercise (23). Resting levels are obtained within hours of cessation of the activity (77). The primary stimulus for the increase in ANP with acute exercise is an increase in atrial stretch due to an increase in venous return (62). However as exercise progresses, atrial pressure decreases as blood flow is redistributed (cardiovascular drift) to meet the metabolic demand of active tissues and to dissipate the thermal load (48, 49). The response of ANP to extended exercise may be increased if water is ingested, suggesting a fluid volume change directly on the heart mediating release (83, 84). Recently, pronounced increases in ANP with exercise have been associated with increases in cardiac troponin levels, suggesting myocardial damage during heavy exercise could be a contributing factor to increases in ANP (85). In cardiac transplant patients, ANP levels are elevated and the response to exercise is accentuated. This suggests that in normal subjects with naturally innervated hearts, there may be neural inhibition of ANP release (44, 86, 87). Support for this hypothesis is the observation in patients with hypertension, that chronic beta-blockade substantially increases the ANP response to exercise (88). Sodium intake appears to also affect the ANP response to exercise (89, 90). During submaximal cycle ergometer exercise when subjects were on a low-sodium diet, ANP increased from 42 to 59 pg/mL, in contrast to a high-sodium diet where the increase was

from 72 to 119 pg/mL. Thus, the increase in ANP with exercise appears to be related to a number of factors: stretch of the atrium due to volume changes, neurological inputs, and sodium intake.

BRAIN NATRIURETIC PEPTIDE

BNP, as its name implies, was first identified in the brain and subsequently identified in other tissues, specifically in the heart (91, 92). BNP is collocated with ANP in the heart and appears to have similar paths of regulation and actions. BNP is not consistently altered in normal subjects in response to acute exercise (41, 83, 93–95). However, with long-duration exercise, such as a 100 km ultramarathon, BNP levels were increased from resting values of 3.3 to 18.8 fmol/mL at the end of the race. The response of BNP to exercise is altered by a number of conditions (96). When subjects performed submaximal exercise on a low-sodium diet, an increase in BNP was not noted; however, on a high-sodium diet a significant increase was seen. A similar finding was reported with the presence or absence of fluid intake in the course of exercise (83). If subjects did not ingest water, there was no response to exercise, but if fluid was provided, BNP was increased with exercise. In hypertensive subjects, the increase in BNP with exercise was the same with or without beta-blockade, in contrast to the greater increase in ANP with beta-blockade (88). This suggests that while similar mechanisms, such as atrial stretch, fluid intake and sodium status, modify the response of both BNP and ANP to exercise, and the neurological component present in the regulation of ANP is not an important factor for BNP.

URODILATIN

Urodilatin, a natriuretic hormone derived in the kidneys, has been suggested to play a role in the renal handling of sodium (97, 98). Schmidt et al. assessed the response of urodilatin and ANP during bicycle ergometer exercise at 60% of maximum for 1 h (99). Plasma ANP concentrations increased and the excretion of urodilatin decreased; i.e., the hormones had a negative correlation. The decrease in urodilatin was associated with a reduction in the percent of the filtered sodium load excreted. As urodilatin increased, the amount of sodium lost also increased. These findings suggest a possible role in the regulation of sodium homeostasis during exercise that needs to be investigated further.

ADRENOMEDULLIN

Adrenomedullin is reported to have natriuretic and diuretic effects. Adrenomedullin is produced in the vascular endothelium and in smooth muscle cells. In humans, plasma concentrations are responsive to changes in blood volume (100, 101). Furthermore, changes in adrenomedullin are correlated with changes in ANP and BNP in patients. In normotensive subjects, adrenomedullin concentrations in response to submaximal exercise of short duration were not altered, even though ANP and BNP levels were increased. In contrast, during maximal exercise, Tanaka and colleagues found adrenomedullin to be increased by 45% compared to at rest, and to be negatively associated with systolic blood pressure (102). Piquard et al. also reported that with acute maximal exercise, adrenomedullin increased from resting levels of 15 to 29 pmol/L at the end of exercise (103). Yet others have found adrenomedullin to be increased with submaximal exercise and decreased with maximal exercise (104, 105). Therefore, further investigation is warranted to elucidate the responses and actions for adrenomedullin during exercise.

FLUID AND ELECTROLYTE REGULATION

The management of fluids and electrolytes is a careful balance between loss of salts and water through sweat, shifts between body compartments, and conservation by the kidneys and replenishment through ingestion (106). While some losses are tolerated during exercise, once critical levels are exceeded there are decrements in performance. In order to avoid these reductions, a series of compensatory mechanisms are activated that have to work in concert to maintain the milieu, to optimize performance, and to avoid subsequent morbidities and mortality.

Total Body Water

During exercise there is a loss of TBW, predominately via sweating and in part from increased respiratory loss. The reduction of TBW is tolerated until a critical level is attained. The loss of TBW during exercise is equivalent to the reduction in total body mass over the period of exercise performance. Though this assumption has been questioned, there is still a strong relationship between the decrease in TBW and body mass (107–109). During long-duration exercise, the reduction in TBW may exceed 5% of body mass. In a 70 kg person, this would equate to fluid loss of 3,000–4,000 mL (6, 8, 110). In laboratory experiments, a reduction of more than 2% body mass has been shown to decrease performance (110). In contrast during competitive endurance events, a reduction of greater than 4% body mass was demonstrated to have a decrement in performance (9). Of note, even with free access to water, a loss in TBW during exercise is observed. This water loss, in the presence of fluids to ingest, is referred to as voluntary dehydration (18, 111). Voluntary dehydration represents about 20–30% of the total loss of body water during an activity, as 70–80% is replaced by supplemental intake over the period of exercise. During a marathon the average body mass loss was 2.3% even though fluids were available. Interestingly, in subjects finishing under 3 h, the loss was 3.1%, from 3 to 4 h 2.5%, and over 4 h 1.8% (112). The ability to tolerate a greater decrease in TBW was inversely associated with finish time. These observations suggest that individuals who are successful in these events are able to tolerate a greater TBW loss and still perform at a high level. The loss of fluids sustained in the course of exercise is usually replaced in the subsequent 24 h (21, 29, 60, 113). Irrespective of the TBW loss tolerance, at some point the loss of TBW will impact the performance of an individual.

The loss of TBW during exercise is not equally distributed throughout the body or between body fluid compartments. Over the course of exercise there is a redistribution of fluids among the various compartments of the body, with a pronounced reduction in plasma volume (59, 61, 65, 114). The reduction in plasma volume during maximal acute exercise is 8–12%, resulting in a 5–7% decrease in blood volume. This shift of fluids from the vascular space to the extravascular space has been attributed in part to increases in endothelial permeability, which could possibly be modified within specific tissues by angiotensin II, vasopressin, and norepinephrine (115–117). The decrease in blood volume is compensated for by an increase in cardiac output and a redistribution of blood flow (48, 49, 118, 119). During the performance of exercise the redistribution of fluids within the vascular compartment is required to meet the metabolic demands of active tissues and to dissipate the thermal load resulting from the increase in metabolism.

This redistribution of flow is the result of increases in local vascular resistance, which is in part due to hormonal regulation, predominately by catecholamines, angiotensin II, and vasopressin.

Sweating

The principal means of fluid and electrolyte loss during exercise is in sweat. Sweating is essential to dissipate the increased thermal load incurred by the elevation of metabolism with exercise (120). The density of sweat pores is highly variable among subjects, as is the magnitude of sweat produced due to the subjects' level of training and prior adaptation and acclimation to a hot environment (90, 120–123). The rate of fluid loss by sweating can be as high as 1,500 mL/h (6, 18, 108, 124). The magnitude of fluid loss in sweat is hormonally mediated by vasopressin (125, 126). Circulating levels of vasopressin are positively associated with the rate and composition of sweat during exercise. The rate of sweating during exercise is coupled with the changes in plasma osmolality and volume, the primary mediators of vasopressin; thus, it has been difficult to separate cause and effect (118, 119, 127, 128). However, local subcutaneous injection of vasopressin alters the rate and composition of sweat from glands exposed to an increase in local skin temperature (129). Plasma vasopressin concentrations have been associated with sweat sodium concentration and osmolality, suggesting vasopressin promotes water conservation in the sweat gland (125, 130). In addition, studies involving a possible role of catecholamines on sweat rate have resulted in conflicting findings (131, 132). However, in a study of the effect of fluid intake, it was shown that the ingestion of a large volume, >3 L, was associated with an increase in sweating, reduction in plasma concentration of norepinephrine, and an increase in skin blood flow. In contrast, the opposite effects were seen with ingestion of a small volume, >0.5 L, during long-duration submaximal exercise in the heat. Therefore an increase in catecholamines appears to be associated with a decrease in skin blood flow that results in a decrease in sweating.

Sweat is composed of a significant amount of electrolytes (90, 120, 121, 133, 134). Thus, during exercise the predominate means of the loss of electrolytes is through sweat. The concentration of sodium in sweat ranges from 20 to 135 mmol/L, potassium from 3 to 35 mmol/L, and chloride from 10 to 100 mmol/L, in contrast to “normal” plasma concentrations (sodium 135–145 mmol/L, potassium 3.5–5.0 mmol/L, and chloride 96–106 mmol/L) (135). While the levels of electrolytes in sweat are lower than in plasma, the losses are significant. At a sweat rate of 1.5 L/h at a sodium concentration of 60 mmol/L, a total of 90 mmol would be lost or 3% of total body sodium. As noted above, however, the concentrations of electrolytes in sweat are highly variable. Electrolyte concentrations of sweat are decreased as a result of training and heat acclimation (65, 90, 121). The lower concentrations reduce the tonicity of the sweat and therefore facilitate evaporation and cooling. In a comparison of 10 min of acute maximal exercise to 60 min of submaximal exercise (60% of maximum workload), minimal differences in the electrolyte concentrations were noted: sodium 70 vs. 77 mmol/L, potassium 7.7 vs. 4.8 mmol/L, and osmolality 171 vs. 172 mOSM/L for maximal and submaximal exercise, respectively. The reductions in the sodium concentration of sweat appear to be in part mediated by aldosterone (121, 136).

Fluid and Electrolyte Intake

Consumption is the primary means of replacing the fluid and electrolytes losses incurred during the course of exercise (18, 137, 138). In the performance of long-duration exercise, 80% of the fluid lost in sweat is replaced by voluntary ingestion if free access to fluids is provided (108, 137). The extent to which volume losses are replaced is dependent upon the composition of the ingested fluid (137–141). In humans during extended exercise, the volume of fluid replacement appears to be closely regulated. In contrast, the replacement of electrolytes does not appear to be as closely titrated, and is a by-product of normal nutrient intake. Takamata et al. suggested that 6–24 h after heavy exercise, salt appetite is increased in association with a decrease in plasma osmolality and sodium concentrations resulting from fluid intake (113). Leshem et al. monitored salt intake after exercise and found a voluntary increase of 50% in the amount of salt added to food (142). Passe et al. assessed the acceptance of hypertonic saline fluids during exercise and reported an increase in palatability of a 60 mmol/L sodium solution, suggesting a relationship between sensory reception, hedonic response, and drink composition in the replacement of electrolytes post exercise (143). Replacement of electrolytes may be coupled with hunger and increase in salt appetite. In animal models salt appetite is strongly associated with angiotensin II; however, this proposed relationship has yet to be definitively demonstrated in humans (11, 144, 145).

As previously noted, the replacement of fluids is closely controlled over the course of exercise and thus readily adjusted for following exercise. This tight regulation is modulated by thirst, the subjective sensation to seek and drink fluids (144–147). The subjective sensation of thirst can persist for hours after exercise (113). As described earlier there is a level of voluntary dehydration that can be tolerated in the performance of long-duration exercise but the majority, about 80%, of the fluid loss is replaced by drinking. The residual loss associated with the level of voluntary dehydration is usually replaced within 24 h (21, 29, 113). This process is associated with a variety of factors, such as the increase in plasma osmolality and reduction in blood volume, both of which are closely tied to the regulation of numerous hormones. Immediately after exercise Takamata et al. found the subjective evaluation of thirst to be immediately reduced upon ingestion of fluids yet increased hours later in spite of plasma osmolality being reduced (113). This increase in thirst was associated with an elevation of aldosterone and presumably angiotensin II (91, 147). If the replacement fluid is water, plasma osmolality and sodium concentration can be decreased before blood volume loss is corrected, thus presenting conflicting regulatory mechanisms resulting in a reduction in thirst (148, 149). Merry et al. reported the subjective sensation of thirst to be associated with an increase in osmolality during moderate exercise under various levels of hydration in subject with different levels of fitness (Fig. 2) (27). Osmolality was also related to an increase in vasopressin, suggesting a possible association between vasopressin and thirst. Keneflick et al. assessed the response of thirst during 1 h of walking at 50% of maximum on a treadmill in temperate (27°C) or cold (4°C) environments (150). In the cold environment the sensation of thirst was reduced by 40% and associated with lower levels of vasopressin, even though plasma osmolality was increased. The authors speculated that peripheral vasoconstriction increased central blood volume that was sensed as an actual increase in blood volume. This hypothesis is supported in part by the observation that

immersion and dehydration, which increase and decrease central blood volume, respectively, alter thirst via volume-induced stimulation of the cardiopulmonary baroreceptors. Stimulation of these baroreceptors by an increase in volume results in decreased vasopressin and PRA and increased ANP (151). In contrast dehydration causing a reduction in volume elicits the opposite responses (33). The specific roles of these hormones in the regulation of thirst during and following exercise have yet to be clearly defined.

The ingestion of fluid during the performance of exercise has been advocated to sustain performance (4, 6, 110). To determine fluid replacement by water ingestion during exercise, Robinson et al. had subjects perform two bouts of exercise, one with and another without fluids, on a cycle ergometer for 1 h at 85% of their maximum oxygen uptake (133). The subjects ingested 1.5 L of water to replace the fluid loss due to sweating, which resulted in a 60% decrease in the loss of body mass. The ingestion of fluid did not alter sweat rate, the increase in body temperature, or perceived exertion. Though plasma osmolality and sodium concentrations had a greater increase in the absence of water intake, no differences in vasopressin or angiotensin II were reported. These findings were confirmed by McConell et al. who stated that ingestion of fluids had little benefit on exercise of 1 h (152). However, others have consistently shown hypohydration to impair performance. There is an absence of data as to whether someone exercising should drink “as much as tolerable,” “to replace the weight lost during exercise,” or “ad libitum;” thus, Noakes et al. had also questioned the effects of fluid hydration during exercise (153). The role of hormones in this debate is even more difficult to evaluate. Rehydration is shown to attenuate the response of atrial natriuretic hormone, vasopressin, and PRA to exercise (24, 32, 66). Furthermore, the role of these hormones in the modulation of thirst during exercise is confounded. At present the data supports maintenance of an adequate hydration status to avoid the adverse effects of dehydration. The means of achieving this, and the levels needed, have yet to be defined.

In light of the present state of data in this area, an understanding of the function of hormones in the regulation of thirst is essential. Hew-Butler has reviewed the role of vasopressin in fluid balance and its possible role in dysnatremia, specifically exercise-associated hyponatremia (10). Hyponatremia with exercise may result from water retention associated with excess fluid intake, sodium loss predominately via sweat, or more likely a combination of these factors. Put forth is the hypothesis that non-osmotic-mediated AVP release from the pituitary increases circulating levels of vasopressin leading to retention of water, even if fluid intake does not exceed recommended guidelines. This inappropriate fluid retention/overload could be a contributing factor of hyponatremia and its subsequent sequelae. The efforts from this group, in the lab and in the field, provide insights as to the contribution of vasopressin and other hormones to the regulation of fluid and electrolyte homeostasis (10, 22, 26, 70, 71).

Renal Function

The action of hormones in the regulation of kidney function is well defined due to their role in the pathophysiology of hypertension. While extensive studies have been directed at the study of hormones on kidney function during exercise, the contribution of the kidneys to fluid and electrolyte balance is limited (59, 60, 154–157). Zambraski described the limited contribution of the kidney noting that in a normal individual the

kidneys produce about 1 mL of urine a minute or 60 mL/h (53). This is in comparison to the loss of fluid from sweat on the order of 1,000 to 1,500 mL/h, during moderate to heavy exercise. Zambraski estimated that during exercise the renal conservation of water would only account for 4% of the loss of water and about 8% for the sodium (53). Thus, the conservation of fluid by the kidney is hampered by the limited amounts of water and electrolyte excreted in the basal state. Nevertheless, the hormonal influences on the kidney provide insights into their role in the overall maintenance of fluid and electrolyte homeostasis during and following exercise (53, 60, 158).

RENAL BLOOD FLOW

At rest the kidney receives about 20% or approximately 1,000 mL/min of the overall cardiac output. During exercise renal blood flow is reduced in relation to the intensity and duration of exercise. With mild to moderate exercise (50–70% of maximum workload) there are negligible changes, but with maximal exercise flow is decreased by 40–60% from the normal (45, 48, 53, 158–160). The reduction in renal blood flow persists for over 1 h after completion of the exercise. This reduction is caused by vasoconstriction of afferent arterioles, associated with an increase in sympathetic nerve activity and circulating levels of norepinephrine derived from spillover from the kidney (45, 47, 53, 159, 161). In animal models upon initiation of exercise, there is an immediate reduction in renal blood flow which increases over time to a steady state associated with the level of exercise (162). This immediate decrease suggests the predominance of the neural regulatory component in the initial phase of exercise. The reduction in renal blood flow decreases the volume of fluid and electrolytes delivered to the glomeruli of the kidney and in turn contributes to regional shifts in renal blood flow within the kidneys.

GLOMERULAR FILTRATION RATE

The amount of fluid moving across the membrane of the glomeruli of the kidney is termed the glomerular filtration rate. The movement of fluid is the product of the drive pressure across the membrane and oncotic pressure of the plasma. As noted above there is an increase in afferent arteriole resistance with exercise; however, this is accompanied by an increase in efferent arteriole resistance facilitating filtration. The increase in efferent arteriole resistance is controlled by angiotensin II. Changes in the rate of glomerular filtration are related to the intensity and duration of exercise and may persist for up to 24 h after exercise (163, 164). Minimal changes in filtration are observed with exercise of less than 50% of maximum. With acute maximal exercise or long-duration exercise above 70% of maximum, the rate of filtration may be decreased by 50–70%. With heavy exercise there is also an increase in the permeability of the glomerular membrane as demonstrated by the occurrence of an increase in protein excretion (53, 165). This alteration of permeability is suggested to be in part mediated by norepinephrine, vasopressin, and angiotensin II and results in an increase in the excretion of protein (53, 163, 166, 167).

URINE FLOW RATE

Urine flow rate is the product of the amount of fluid filtered (glomerular filtration rate) and the net reabsorption of fluid in the tubules. With exercise of low intensity, there is either no change or a slight increase in urine flow rate (39, 155). With acute maximal exercise or long-duration exercise eliciting voluntary dehydration, urine flow rates are decreased by 20–60% of the normal basal levels of 0.8 to 1.2 mL/min (53, 59, 60). This

minimal decrease results in the conservation of water in light of the losses due to sweating. The decrease in the amount of filtered water is predominately due to vasoconstriction of the afferent arterioles caused by norepinephrine (45, 48, 131, 161). Exercise also causes an increase in the osmolality of urine, indicative of an increase in the reabsorption of water (57–59). However, decreases have been reported in urinary osmolality indicative of an increase in free water clearance during heavy exercise (53, 59). Therefore the role of vasopressin in the control of water reabsorption in the collecting tubule during exercise has been questioned. There may be inhibition of vasopressin or the possibility of a “wash out” of the osmotic gradient in the medullary area of the kidney due to the redistribution of blood flow associated with the actions of angiotensin II. After exercise the reduction in urine flow persists and may contribute to the rectification of fluid loss along with increased drinking (21, 113).

RENAL HANDLING OF ELECTROLYTES

At the normal rate of glomerular filtration, the amount of fluid equivalent to the TBW is filtered in 5–6 h. The filtrate contains electrolyte concentrations equivalent to those of plasma. Over the course of traversing through the kidneys, 80–99% of the filtered load of electrolytes is reabsorbed. This reabsorption is hormonally mediated for sodium and establishes an electrochemical gradient for the handling of other electrolytes and an osmotic gradient for the handling other solutes. With acute exercise, the decrease in the excretion of electrolytes is predominately due to the reduction in glomerular filtration rate (21, 113, 156). During and following long-duration exercise, the reabsorption of sodium is regulated by aldosterone (21, 113). With daily heavy exercise there is a persistent increase in aldosterone, which is strongly associated with an increase in the reabsorption of sodium (Fig. 3) (21).

In summary, with exercise, kidney function changes and is regulated by a number of hormonal systems. The major alterations effecting fluid and electrolyte homeostasis are a decrease in renal blood flow and an increase in the reabsorption of sodium. There are several fallacies as to the contribution of these changes in kidney function to the net maintenance of fluids and electrolytes. The primary misunderstanding is the quantitative contribution of the kidney to fluid balance and the roles of hormones in these changes.

SUMMARY

Exercise elicits increases in a number of hormones important in the regulation of fluid and electrolyte homeostasis. The action of these hormones may persist for hours and days after completion of the exercise. While increases in hormone levels are noted, the regulation and actions of these hormones are often not well defined, specifically in relation to the changes in fluid and electrolyte balance during exercise. There are issues as to the influence by the type and duration of exercise on hormonal responses that are not often accounted for. Recent efforts employing multifactorial analysis are just beginning to define some of these factors. In addition, the role of hormones in the etiology of the detrimental effects of exercise, such as dehydration and dysnatremia, are beginning to be addressed. Finally, evidence is mounting to show that exercise plays a vital role in fluid and electrolyte homeostasis. Observations of the hormonal responses to exercise will lead to a better understanding of both exercise physiology and related disease processes.

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14

Hormonal Regulations of the Effects of Exercise on Bone: Positive and Negative Effects

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INTRODUCTION

Exercise is generally thought to have a positive effect on bone; numerous studies indicate that bone mass is increased with training. Increasing physical activity levels throughout the life-span has been recommended for preventing the development of osteoporosis (1). It has become evident, however, that too much exercise in combination with deficient energy intake may be detrimental to bone in some individuals. This may be owing to hormonal changes that occur when the body attempts to conserve energy or when an individual is under excess stress.

The “[Negative Effects of Exercise on Hormonal Regulation of Bone](#)” section of this chapter covers the negative effects of high levels of exercise on hormones that regulate bone metabolism. In females, adequate estrogen levels are necessary for maintenance of skeletal integrity. The secretion of estrogen is reduced with extremes of exercise training. A number of studies indicate that this may be prevented if dietary intake is sufficient to compensate for energy expenditure. Testosterone may be similarly affected in males, again with detrimental effects on the skeleton. Extreme exercise training may

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also have effects on the calciotropic hormones, but results of studies are mixed, with some indicating negative effects and some indicating positive effects on bone.

The “[Positive Effects of Exercise on Hormonal Regulation of Bone](#)” section of this chapter covers the positive effects that exercise may have on the hormones that affect bone mass. Exercise and estrogen may have synergistic effects on bone (i.e., their combined influences may be greater than the addition of their influences alone). Exercise may also stimulate the release of anabolic hormones (testosterone, growth hormone, insulin-like growth factor), and these may have positive effects on bone. Finally, exercise may have a positive effect on the calciotropic hormones to increase calcium balance and prevent bone resorption.

NEGATIVE EFFECTS OF EXERCISE ON HORMONAL REGULATION OF BONE

Negative Effects of Exercise on Reproductive Hormone Status

ESTROGEN AND PROGESTERONE

Adequate estrogen and progesterone production is necessary for maintenance of bone mineral status in women. Estrogen has several effects that lead to positive influences on bone: It increases the efficiency of intestinal absorption of calcium (2), and it affects bone-resorbing and bone-forming cells (osteoclasts and osteoblasts) to suppress bone turnover (3). The effects of progesterone on bone are not as well understood, but it may increase bone formation by influencing osteoblastic activity, or it may play a role in the coupling of bone resorption with formation (4). Although exercise is generally thought to be of benefit to bone, excessive exercise may have negative effects on reproductive hormone status in some females.

Athletic Amenorrhea. Intense training in some female athletes may lead to development of amenorrhea with decreases in progesterone and estrogen production, and a corresponding decrease in bone mineral content (5). Athletic amenorrhea is most common in sports that require lower body mass or activities that involve subjective judgment, including long-distance running (5), gymnastics (6), rowing (7), figure skating (8), and ballet (9). For some of these athletes, local positive effects of weight-bearing exercise or muscle pull on bone may override the negative effect of decreased estrogen and progesterone. Amenorrheic or oligomenorrheic gymnasts have elevated bone mass at most sites compared to amenorrheic runners and eumenorrheic controls (6, 10), and amenorrheic rowers have higher lumbar spine bone mass compared to amenorrheic runners and dancers (7). Amenorrheic dancers may (11) or may not (9) have an elevated bone mass at weight-bearing sites. Amenorrheic dancers have an increase in stress fractures (12), implying that weight-bearing exercise may not have a protective effect. Most studies of amenorrheic runners indicate a decrease in bone density, even at weight-bearing sites (13), and this is associated with an increased rate of stress fracture (14, 15).

Etiology of Athletic Amenorrhea. The development of amenorrhea with exercise training has been attributed to many factors, but the leading candidates are: (1) a decreased energy availability owing to greater energy expenditure of exercise than dietary energy intake, or (2) abnormal levels of stress hormones (i.e., cortisol) owing to chronic stress of exercise. Both factors may negatively effect gonadotropin-releasing hormone pulse generation

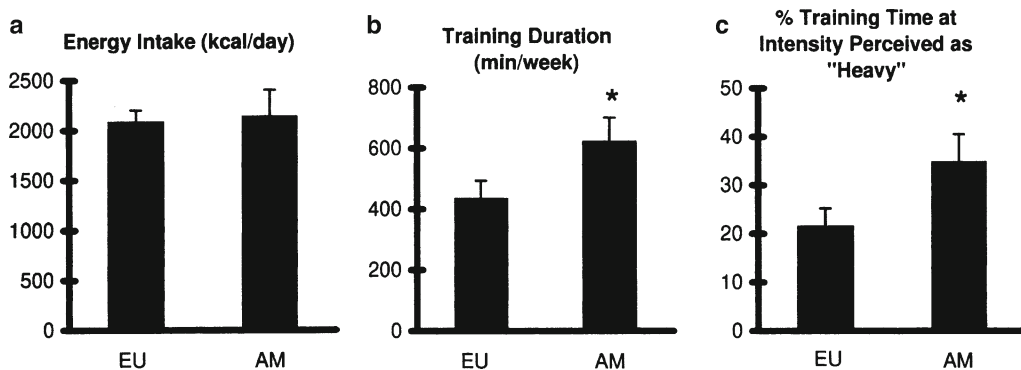


Fig. 1. *EU* eumenorrheic athletes; *AM* amenorrheic athletes. This is one form of experimental evidence for the “low energy availability” hypothesis proposed by Loucks et al. (16) in the development of amenorrhea with exercise training. For an equivalent level of energy intake, amenorrheic athletes were found to have a greater training volume and intensity when compared to eumenorrheic athletes (data taken from ref. (17)). This implies that amenorrheic athletes have a lower energy availability (where energy availability = energy intake — energy expenditure). *Amenorrheic means are significantly different from eumenorrheic means ($p < 0.05$).

at the hypothalamus, which in turn results in decreased release of follicle-stimulating and leutenizing hormone from the anterior pituitary. The evidence for both hypotheses has recently been outlined by Loucks et al. (16) (Figs. 1 and 2). The “low energy availability” hypothesis is supported by findings that amenorrheic and eumenorrheic athletes in general have lower dietary energy intakes than expected for their activity levels (17, 18) and that amenorrheic athletes have endocrine profiles (i.e., altered thyroid status (17, 19)) similar to groups characterized by chronic energy deficiencies (20). The “stress hormone” hypothesis is supported by findings that amenorrheic athletes have alterations in the hypothalamic–pituitary–adrenal axis (21, 22) and that alterations in either corticotropin-releasing hormone or cortisol levels in animals negatively affect gonadotropin secretion (23, 24). In support of the “low energy availability” hypothesis, it has recently been demonstrated that short-term induction of menstrual cycle changes with exercise can be prevented by adequate dietary compensation (16). It is therefore suggested that athletes may be able to reverse menstrual disorders by increasing their dietary energy intake, without decreasing their exercise levels (16).

Treatment and Reversal of Athletic Amenorrhea. Amenorrheic athletes have been able to reverse menstrual disorders and increase bone mass successfully by reducing training volume and increasing consumption of calcium (25, 26) or by taking hormone-replacement therapy (27). With resumption of menses in these athletes, bone mineral density increased by an average of 6–14% over 15.5–30 month at the lumbar spine (25–28) and 4% over 24–30 month for the femoral neck (27). The frequency of stress fractures in one report was also reduced following reversal of menstrual disorders (26). The greatest increase in bone mineral density was in those athletes that gained weight (25, 26, 28), implying that they had achieved a more positive energy balance. Despite these increases, however, bone density still remained below control levels in many of the formerly amenorrheic athletes (25, 28). Recently, a follow-up of 8 year duration determined that despite return of normal menses or use of oral contraceptives, the bone mass of former

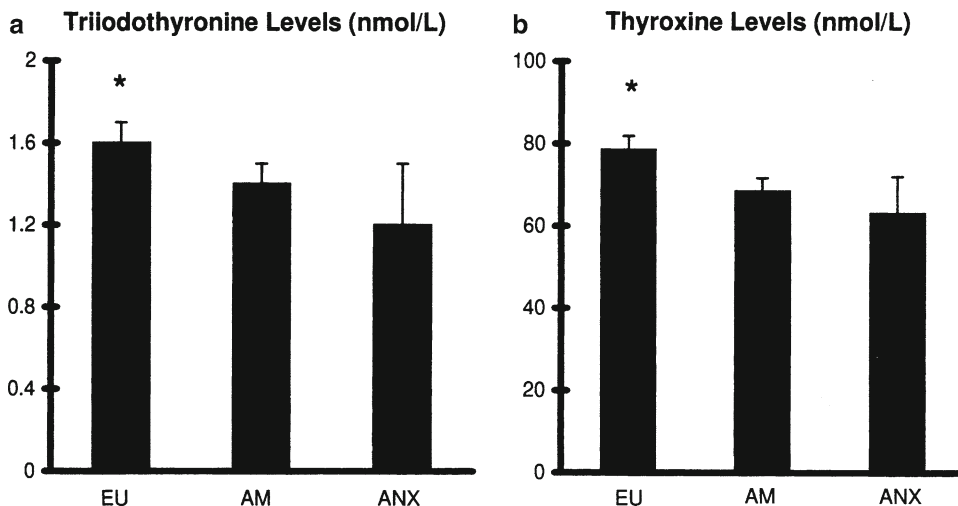


Fig. 2. *EU* eumenorrheic athletes; *AM* amenorrheic athletes; *ANX* anorexics. This is a second form of experimental evidence for the “low energy availability” hypothesis proposed by Loucks et al. (16) in the development of amenorrhea with training. Amenorrheic athletes have endocrine profiles (altered thyroid status) similar to a group of subjects (anorexics) characterized by chronic energy deficiencies (data taken from refs. (17, 20)). *Eumenorrheic means are significantly different from amenorrheic and anorexic means ($p < 0.05$).

oligomenorrheic or amenorrheic athletes remained below normal (29). This suggests that early treatment and reversal of menstrual disorders may be necessary to prevent an irreversible bone loss (29).

TESTOSTERONE

Just as excessive exercise may affect reproductive hormone status in females, excessive exercise may negatively affect testosterone levels in males (30, 31). Adequate testosterone levels in males may be necessary for intestinal calcium absorption (32), and for stimulation of osteoblasts and bone formation (33).

Similar to the etiology for disruption of the hypothamic–pituitary–gonadal axis in females, disruption in this axis with excessive exercise in males may be owing to decreased energy availability (31) or production of stress hormones (34, 35). When caloric balance is shifted to allow for increased body weight, testosterone levels are returned to normal (31).

In contrast to studies of females, with males there is less evidence for a link between reductions of reproductive hormones and a corresponding reduction in bone mass. Testosterone levels are altered with excessive training in a number of studies (30, 31), and excessive training is associated with reduced bone mass in others (36, 37); however, in studies in which bone mass was reduced, testosterone levels were found to be normal (36, 37). We are aware of only one case study where excessive training was associated with hypogonadism, decreased bone mass, and skeletal fragility in a single male subject (38). Treatment of this subject with clomiphene citrate (an analog of the nonsteroid estrogen, chlororriani-sene) simulated gonadotropin secretion, and testosterone levels returned to normal (38). Further research is needed to determine if other male athletes with low testosterone levels may be at a risk of losing bone mass and developing skeletal fracture.

Negative Effect of Exercise on Calcitropic Hormones

The calcitropic hormones (parathyroid hormone, calcitonin, and to an extent, 1,25-dihydroxyvitamin D₃ (vitamin D)) are involved in the maintenance of optimal blood calcium levels, which is vital for the regulation of neuromuscular excitability. Low blood calcium levels stimulate the secretion of parathyroid hormone from the parathyroid gland. Parathyroid hormone stimulates osteoblasts to release a factor that stimulates osteoclastic bone resorption, causing movement of calcium from bone to blood (39). Calcitonin, a much less powerful hormone, has an opposing effect. Calcitonin is released from cells of the thyroid gland (which are distinct from the cells that release thyroxine and triiodo-L-thyronine) in response to high blood calcium levels and inhibits the release of calcium from bone. The major hormone-like action of vitamin D is a stimulation of active intestinal calcium absorption. Excessive exercise may alter the regulation of the calcitropic hormones, with a negative effect on bone mineral homeostasis. The effects of excessive exercise on parathyroid hormone, calcitonin, and vitamin D metabolism are covered in the following sections.

NEGATIVE EFFECTS OF EXERCISE ON PARATHYROID HORMONE

The response of parathyroid hormone to acute exercise bouts is quite variable with studies reporting an increase in release (40), a decrease in release (41), and no change (42). Although parathyroid hormone increases bone resorption (39), in some cases, it may paradoxically have an anabolic effect on bone (43). Whether parathyroid hormone has catabolic or anabolic effects may depend on the amount released and whether its release is continuous or intermittent. Continuous release of parathyroid hormone induces bone loss, whereas intermittent administration may increase bone mass through stimulation of osteoblasts (44). The responses of bone to changes in parathyroid hormone levels with exercise training have therefore been inconsistent, with some studies demonstrating positive effects on bone and some demonstrating negative effects. The studies demonstrating negative effects are discussed below, with studies demonstrating positive effects discussed in the “[Parathyroid Hormone](#)” section.

Chronic high-intensity exercise training may cause an increase in the continuous release of parathyroid hormone (40), and this may negatively affect bone mass in individuals who overexercise. The continuous release may be related to alterations in catecholamine levels with chronic exercise training. Catecholamines stimulate parathyroid hormone release in animal experiments (45), and release of parathyroid hormone correlates with the intensity (46) or volume (40) of exercise. Assuming that catecholamine release is greater with higher exercise intensities and volumes, this could be a mechanism by which parathyroid hormone is continuously released with repeated exercise bouts. Two longitudinal exercise training studies have found reductions in bone mineral density and elevations in bone turnover associated with elevations in basal parathyroid hormone levels (47, 48). Training in both studies could be described as moderate in intensity and is the type of training usually associated with increases in bone mass. Indeed, one of the studies found a decrease in parathyroid hormone with further training and an increase in bone mass (48). As bone turns over in a cycle of resorption and formation, initial measurements may have occurred during the bone resorption phase of the cycle. The influence of elevated basal parathyroid hormone levels on bone mass as a

result of training is still unclear, since elevated parathyroid hormone levels with training have also been associated with increases in bone mass (49). More work is needed in this area to clarify these relationships.

Aside from changes in basal parathyroid hormone levels, excessive training may also alter the set point for which parathyroid hormone is released in response to changes in blood calcium levels. Grimston et al. (42) assessed the changes in parathyroid hormone release in response to exercise or exercise combined with calcium ingestion in groups of female runners with normal and low bone mineral density. The osteopenic group had an elevated parathyroid hormone release compared to the group with normal bone mineral density, implying that the set point for parathyroid hormone release was altered in the osteopenic group. There was also a negative correlation between parathyroid hormone release and bone mineral density in this group. It is suggested that low estrogen levels that may have been present in some female runners may amplify the effect of parathyroid hormone on bone turnover, as it does in postmenopausal women (50). More study is needed on the effects of excessive training and the relationship between altered estrogen levels and the set point of parathyroid hormone release.

NEGATIVE EFFECTS OF EXERCISE ON CALCITONIN AND VITAMIN D

A limited number of studies have shown that excessive endurance training in female athletes may result in alterations in calcitonin release and vitamin D levels. Grimston et al. (42) demonstrated that female runners characterized by low bone mass had decreased calcitonin release in response to elevated blood calcium levels following exercise, which differed from subjects with normal bone mass, who had increased calcitonin release. This may prevent the beneficial effect of calcitonin on preventing bone resorption in the subset of runners with low bone mass.

Vitamin D levels were found to be lower in runners with low bone mass in one study comparing amenorrheic athletes to eumenorrheic athletes and controls (14), although levels were still within a normal range. It is not clear whether this lowered vitamin D level would significantly alter intestinal calcium absorption and contribute to the lower bone mass of amenorrheic athletes.

POSITIVE EFFECTS OF EXERCISE ON HORMONAL REGULATION OF BONE

Interactions Between Exercise and Estrogen for Increasing Bone Mass

Exercise and estrogen replacement have been shown to be successful therapies for increasing bone mass in postmenopausal women. Recently, it has been shown that when the two are combined, their effects on some bone sites may be synergistic (i.e., greater than the addition of each therapy alone). This implies that exercise may augment the effects of estrogen or vice versa. In postmenopausal women, exercise and estrogen replacement therapy have an additive (51) or synergistic (52) effect on bone mass of the spine and a synergistic effect on whole-body bone mass (51, 52). Animal studies have demonstrated either additive (3) or synergistic (53) effects with the two therapies. The exact mechanism by which exercise and estrogen synergistically increase bone mass is unknown.

Table 1
Effects of anabolic hormones on bone and the influence of exercise^a

<i>Hormone</i>	<i>Effect of hormone on bone</i>	<i>Effect of exercise on hormone level</i>
Estrogen	↑ Ca ²⁺ absorption (2); ↓ bone turnover (3)	Extreme training with low energy intake: ↓ release (5–9, 11–14, 17–19)
Progesterone	↑ Bone formation (4); couples formation to resorption (4)	Same as above
Testosterone	↑ Ca ²⁺ absorption (32); ↑ bone formation (33)	Extreme training: ↓ release (30, 31) Acute exercise ↑ release (57, 60, 62) Chronic exercise ↑ release (62, 69) or ↔ (75)
Growth hormone	↑ Bone formation (55); ↑ production of active form of vitamin D (83)	Acute exercise ↑ release (59, 61, 64, 65) Chronic exercise: ↔ (75)
IGF-1	↑ Bone formation (56, 77)	Acute exercise ↑ release (59, 61, 64, 65) Chronic exercise ↑ release (66, 68) or ↔ (75, 76)

^aReferences to individual points are shown in parentheses. ↑=increase; ↓=decrease; ↔=no change; IGF-1=insulin-like growth factor

Effects of Exercise on Anabolic Hormones

A number of anabolic hormones have been shown to increase following acute exercise sessions, and basal levels may be elevated in response chronic training. These anabolic hormones include testosterone and growth hormone. Insulin-like growth factor 1 (IGF-1), or somatomedin may also be considered an anabolic hormone that increases with exercise; however, its synthesis in the liver or other sites, including bone, may be mediated by growth hormone (54). Each of these anabolic hormones have been shown to stimulate bone growth or formation by proliferation or activation of osteoblasts (33, 55, 56). This section will review the effects of acute and chronic exercise on release of anabolic hormones and the resultant effect on bone mineral.

ACUTE EFFECTS OF EXERCISE ON ANABOLIC HORMONES AND BONE METABOLISM

Single exercise sessions may result in increases in blood levels of anabolic hormones in both men and women (Table 1). In men, a single bout of weight-lifting exercise has been shown to result in increases in testosterone (57), growth hormone (57, 58), and IGF-1 (59) levels. A single bout of cycle ergometer exercise similarly results in elevations of testosterone (60), growth hormone, and IGF-1 (61). The response occurs in young and old men, but seems to be attenuated with aging (58). Increased testosterone levels have been attributed to hemoconcentration (57), increased testicular production (60), and reductions in clearance rates from hepatic and adipose tissue (59). Increased production of IGF-1 has not always followed the same pattern as growth hormone changes (59, 61); therefore, their release may be independent. Similar responses to acute exercise occur in women. In response to a single bout of weight-lifting, increases have been noted in blood testosterone (62), growth hormone (63), and IGF-1 (64) levels. Increases in testosterone levels in females have been attributed to production by the adrenal cortex (62).

A single study has related the increases in anabolic hormones with acute exercise to changes in markers of bone turnover (65). In a group of men and women, arterial and venous blood was sampled during and following repeated one-leg, knee-extension exercise. Exercise resulted in an increase in arterial serum concentrations of growth hormone, and there was an exercise-induced uptake of growth hormone over the thigh and a release of IGF-1. Levels of biomarkers for bone formation and resorption were increased during exercise. This release of anabolic hormones and increase of bone turnover may result in increased bone synthesis with training, but firm conclusions can only be drawn from longitudinal study of these responses.

EFFECTS OF EXERCISE TRAINING ON ANABOLIC HORMONES AND BONE MASS

Cross-sectional studies suggest that the higher bone mass of some chronically trained athletic groups may be related to higher basal levels of anabolic hormones. Young strengthtrained women were shown to have higher bone mass and higher levels of IGF-1 concentrations than aerobically trained women and sedentary controls (66). Among aerobically trained females, testosterone levels were found to be a significant determinant of bone density, independent of estrogen levels (67). Finally, endurance-trained postmenopausal women were found to have higher bone mineral density adjusted for body weight, along with higher IGF-1 levels and a trend toward higher growth hormone levels than sedentary controls (68). Although cross-sectional and correlative studies cannot imply cause and effect relationships, results suggest that various forms of training may lead to enhancement of basal anabolic hormone levels and stimulation of bone formation.

Longitudinal studies have demonstrated that basal levels of testosterone are elevated in males following 2 year of training (69). Luteinizing and follicle-stimulating hormones were also elevated, suggesting that training may have influences at the pituitary or hypothalamic levels, which led to the increase in testosterone levels (69). Baseline testosterone levels were also found to be higher following 2 month of resistance training in females when compared to nonexercised controls (62). It was suggested that increased testosterone levels may have been owing to either gonadal or adrenal responses (62).

Various longitudinal training studies geared toward increasing bone mass have been designed with protocols based on those found to increase anabolic hormone levels optimally during acute exercise sessions. Studies of acute exercise sessions have shown that the anabolic hormone response is greater during resistance training sessions that involve the greatest total work (i.e., a greater training volume) in both male (64, 70) and female (64) subjects. Based on this finding, one longitudinal study compared the effects of two training routines in young females: one where all exercises were combined on one training day, and another where exercises were split between successive training days (71). The authors hypothesized that the routine with all exercises combined on one day would produce a greater training effect, since the total work performed was greater within single exercise sessions. This routine produced a greater increase in legs' lean mass (71), but changes in bone mineral mass with both routines were not significant, most likely owing to the relatively short (20-week) training duration (72).

When total work output during acute weight-lifting sessions is held constant, the anabolic response is greater with sessions that employ higher loads and a lower frequency (repetitions) of movement than sessions that employ lower loads and a higher

frequency of movements (73). Specifically, sessions that involved exercises consisting of seven repeated lifts of heavy loads produced greater increases in growth hormone release than sessions that involved an equivalent amount of work, but 21 repeated lifts of loads that were one-third as heavy (73). A recent longitudinal 12-month training study compared the effects of training on bone using almost identical training protocols (exercises involving 8 lifts of heavy loads vs. exercises involving 20 lifts of lighter loads) (74). The high-load, low-movement frequency training was found to result in significant increases in bone mass, but the low-load, high-movement frequency training had no effect on bone mass (74). Thus, it has been shown that training using protocols known to enhance anabolic hormone secretion optimally result in enhancement of bone mineral synthesis.

One drawback to the abovementioned longitudinal studies is that changes in bone mass and changes in basal levels of anabolic hormones were not measured in combination. Studies in humans combining both measurements have demonstrated that beneficial effects of training on bone mass can be realized without changes in anabolic hormones. Sixteen weeks of resistance training of elderly men produced significant increases in femoral neck bone mineral density without changes in levels of testosterone, growth hormone, and IGF-1 (75). Likewise, 27 week of gymnastics training of young women produced significant increases in lumbar spine bone mineral density without a change in serum IGF-1 levels (76). One elaborate study of rats was more successful, demonstrating a beneficial effect of training on anabolic hormone level and bone (77). Following 9 week of treadmill running, exercised female rats had higher levels of serum IGF-1, bone-specific IGF-1, and long-bone formation rate compared to nonexercised controls. IGF-1 concentrations in both long-bone and vertebral extracts were highly correlated with their respective bone formation rates as measured by histomorphology. This indicates that IGF-1, locally produced in stressed bone, may be a mediator between increased mechanical strain and the signal for increased bone formation (77).

Positive Effects of Exercise on Calcitrophic Hormones

PARATHYROID HORMONE

Parathyroid hormone stimulates bone resorption to maintain homeostasis when blood calcium levels are low (39). With chronic exercise training, parathyroid hormone levels may be lowered. This has been associated with higher bone mineral values: Cross-sectionally, male and female endurance-trained athletes have been found to have lower serum parathyroid hormone levels associated with higher bone mineral density when compared to inactive controls (68, 78). Rats endurance-trained by treadmill exercise also have lower parathyroid hormone levels and higher bone mass compared to untrained rats (79). The mechanism for the lowering of parathyroid hormone levels with training is not known.

In contrast to the above studies, an increase in basal levels of parathyroid hormone has been found following a resistance training program that increased bone mineral density in postmenopausal women (49). As mentioned in the “[Negative Effects of Exercise on Parathyroid Hormone](#)” section, parathyroid hormone may have anabolic effects on bone through stimulation of osteoblasts, if released in an intermittent fashion (44).

Table 2
Effects of calciotropic hormones on bone and the influence of exercise^a

<i>Hormone</i>	<i>Effect of hormone on bone</i>	<i>Effect of exercise on hormone level</i>
PTH	↑ Bone resorption when continuously released (39)	Acute exercise: ↑ (40), ↓ (41), ↔ (42)
	↑ Bone formation when intermittently released (44)	Chronic exercise ↔ (47–49) or ↓ (48, 68, 78, 79)
Calcitonin	↓ Bone resorption	Extreme training: ↓ (42) Acute exercise: ↑ (41) Chronic exercise: ↔ (68)
Vitamin D	↑ Ca ²⁺ absorption	Extreme training: ↓ (14) Chronic exercise ↑ (68, 79, 80, 82)

^aReferences to individual points are shown in parentheses. PTH=parathyroid hormone; ↑=increase; ↓=decrease; ↔=no change

Further research is needed to determine the exact direction of changes in basal parathyroid hormone levels in response to different training protocols and whether these changes can be considered beneficial or detrimental to bone.

CALCITONIN AND VITAMIN D

Few studies have looked at the effects of exercise on calcitonin levels. One study showed that calcitonin levels increased in response to exercise (41); this may prevent bone resorption. However, chronic exercise training does not appear to alter serum calcitonin levels (68).

Exercise training may have beneficial effects on vitamin D levels, resulting in increased intestinal calcium absorption and increased bone mass (Table 2). Cross-sectional studies indicate that vitamin D levels may be elevated in endurance-trained (68) and resistance-trained (80) individuals. This is associated with a higher bone mass in these individuals compared to inactive controls (68, 81). Rats trained by treadmill exercise have an increase in vitamin D levels, increased calcium balance, increased intestinal calcium absorption efficiency, and increased bone mass compared to untrained rats (79, 82). Increases in growth hormone release with exercise training (68) may simulate the production of the active form of vitamin D (83).

DIRECTIONS FOR FUTURE RESEARCH

Extremes of exercise training produce negative effects on bone owing to decreases in estrogen (in females) or testosterone (in males) production. Short-term studies have shown that alterations in hormonal release can be prevented if adequate dietary energy intake is maintained during periods of heavy exercise (16). Longer-term studies need to be performed to determine whether increasing energy availability can prevent reductions in reproductive hormones that may occur with participation in large amounts of exercise.

Another area open for research is assessment of precise treatments that may reverse athletic amenorrhea. Cross-sectional studies on small numbers of subjects have indicated that reduction in training volume and weight gain may reverse amenorrhea (25, 26),

but larger-scale studies would be beneficial. Although hormone replacement therapy has been shown to increase bone mass in amenorrheic athletes (27), the effects of treatment with low-dose oral contraceptives have not been tested, although it has been recommended (84).

There is an abundance of studies that have compared the effects of different exercise regimes on anabolic hormones during acute exercise sessions; however, few investigators have compared the effects of different training regimes on long-term changes in bone mass within the same study (74). Research is needed to develop exercise prescriptions for optimal enhancement of long-term hormone profiles that result in bone formation.

SUMMARY

Extremes of exercise training may negatively affect hormonal profiles that influence bone mass. Dietary energy compensation for energy expended during exercise has been shown to prevent changes in reproductive hormone profiles in the short term. Longer-term studies are needed to assess whether increased dietary intake can prevent the occurrence of amenorrhea without a decrease in training volumes.

When hormone replacement therapy is administered to postmenopausal women and combined with exercise training, estrogen and exercise may act synergistically to increase bone mass at some skeletal sites. At other sites, the effects of estrogen and exercise are additive.

The effects of exercise on the calciotropic hormones are mixed. Parathyroid hormone may be increased or decreased with exercise training. Parathyroid hormone stimulates bone resorption, but intermittent release may stimulate bone formation. Tightly controlled animal experiments demonstrate a decrease in parathyroid hormone and an increase in vitamin D with training, resulting in positive calcium balance and increased bone mass.

Resistance training involving high volumes of work within single sessions and heavy loads may optimally stimulate release of anabolic hormones (testosterone, growth hormone, and IGF), but the long-term effects of changes in hormone profiles and their effects on bone have not been studied in detail.

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15

Interrelations Between Acute and Chronic Exercise Stress and the Immune and Endocrine Systems

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INTRODUCTION

Interaction between the endocrine and immune system is necessary to regulate our health. However, under some conditions, stress hormones can overstimulate or suppress the immune system, resulting in harmful consequences (1). Stress is often considered negative, yet it is an intrinsic part of everyday life. Stress is not clearly defined; it is context-specific and depends on the nature of factors that challenge our body. Internal stimuli will elicit different stress reactions compared with external stimuli (1). Similarly, some stressors will induce responses that may benefit survival, whereas others will cause disturbances that may endanger our health. Stress also depends on how our bodies perceive and respond to stressful stimuli (1).

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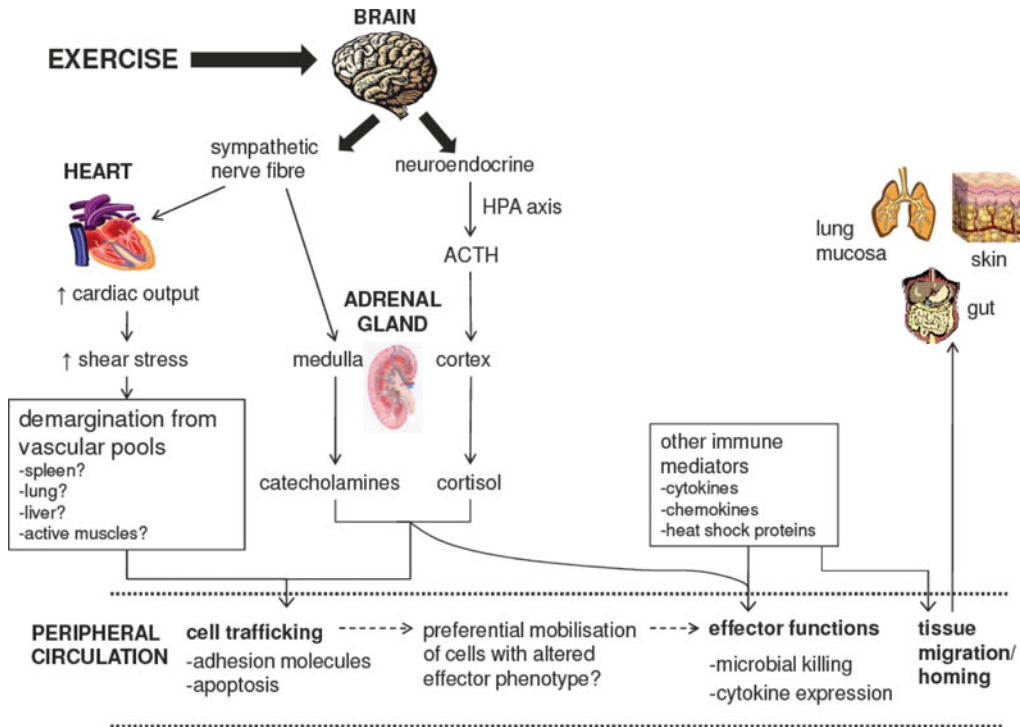


Fig. 1. Potential mechanisms by which stress hormone interact with the immune system during exercise. Reprinted, with permission, from TOM-Systemdruck GmbH, Walsh N et al., Position statement. Part one: Immune function and exercise. *Exercise Immunology Review* 2011;17: 6–63.

Several important factors determine whether stress hormones stimulate or inhibit the immune system. These factors include (1):

- The effects of stress on the distribution of immune cells in the body
- The duration of stress
- Hormone concentrations
- The timing of stress hormone exposure relative to the activation status of immune cells (i.e. naïve vs. activated, early vs. late activation)

Exercise is a reproducible and quantifiable model of stress, and is useful for studying the interactions between the endocrine and immune systems. Exercise stimulates the secretion of a variety of stress hormones, but catecholamines, cortisol and growth hormone are most closely linked with exercise-induced changes in immune function. Research on the interactions between endocrine and immune systems following acute exercise and chronic training is important. Regular exposure to mild short-term stress can potentially enhance immune function and lead to various health benefits. Conversely, prolonged exposure to the chronic stress of intense training may inhibit certain immune functions that are required for health maintenance. This chapter describes the regulatory roles of stress hormones on immune cell counts and activity during acute exercise and following chronic exercise training. Figure 1 summarises the immunoendocrine interactions during exercise and their potential functional significance.

MECHANISMS OF INTERACTION: IN VITRO EVIDENCE

Stress hormones modulate immune function directly by binding to cognate receptors on immune cells and indirectly by modulating the production of cytokines (e.g. IFN- γ , IL-1 β , IL-6, TNF- α) (2). Glucocorticoid receptors are expressed on monocytes and B lymphocytes, whereas glucocorticoid receptor expression is much lower on CD3⁺ T cells and neutrophils (3, 4). β_2 -adrenoreceptors for catecholamines are expressed on (in descending order) natural killer (NK) cells, monocytes, B lymphocytes and T suppressor lymphocytes (5). Macrophages (6) and neutrophils (7) also express β_2 -adrenoreceptors. Within T lymphocyte subpopulations, β_2 -adrenoreceptors are mainly expressed in naïve CD4⁺ T cells, T helper 1 and T helper 2 cells (8–10). mRNA for α -adrenoreceptors is expressed by activated T cells (11) and in peripheral blood mononuclear cells of patients with juvenile rheumatoid arthritis but not healthy individuals (12). Although B lymphocytes, monocytes and neutrophils all express growth hormone receptors (13–15), growth hormone most likely exerts its effects on the immune system by binding to prolactin receptors, which are expressed on monocytes, B and T cells (16). Immune cells also express receptors for other stress hormones, including substance P (17), neuropeptide Y (18), corticotrophin-releasing hormone (19) and serotonin (20).

Glucocorticoids regulate the activity of immune cells by binding to glucocorticoid receptors, which in turn suppresses the transcription factors activator protein 1 (AP-1) and nuclear factor κ B (NF κ B) (21). Glucocorticoids inhibit AP-1 transcriptional activity by preventing the oncoproteins c-Fos and c-Jun from binding to the AP-1 consensus binding site in DNA (22). Glucocorticoids inhibit NF κ B transcriptional activity through two mechanisms. Firstly, glucocorticoids can induce expression of the inhibitory protein I κ B, which then prevents NF κ B from translocating to the nucleus where it initiates transcription (23). Secondly, physical interaction or cross-talk between glucocorticoid receptors and NF κ B can suppress transcription (24, 25). By suppressing the transcriptional activity of AP-1 and NF κ B, glucocorticoids regulate various immune functions, including cytokine production (21). In particular, glucocorticoids inhibit monocyte production of the type 1 cytokines IL-12 and IFN- γ , which in turn favours the production of the type 2 cytokines IL-4 and IL-10 by CD4⁺ lymphocytes and peripheral blood mononuclear cells (26–29). Type 1 cytokines regulate the activity T cytotoxic cells, NK cells and macrophages which defend against intracellular pathogens. Type 2 cytokines regulate the activity of B lymphocytes, eosinophils and mast cells, which defend against extracellular pathogens (30). The type 1/type 2 cytokine balance determines the balance between cell-mediated vs. humoral immunity and the risk of various immune-related disorders (31). For information on the effects of glucocorticoids on other aspects of immune function, readers are referred to other more comprehensive reviews (21, 32).

Binding of catecholamines to β_2 -adrenoreceptors can inhibit IL-2 and IFN- γ and stimulate IL-4 and IL-10 production by T cells and peripheral blood mononuclear cells (26, 33, 34). Similar to glucocorticoids, catecholamines can therefore induce a shift towards type 2 cytokine production. The combined effects of glucocorticoids and catecholamines on IFN- γ , IL-4 and IL-10 production by peripheral blood mononuclear cells are in fact additive (26). However, there are some inconsistencies in the literature concerning the effects of β -agonists on cytokine production. Some studies report that T helper 2 lymphocytes do not respond to β -agonist stimulation (9, 35), but more recent

data indicate that activated T cells do produce cytokines following β -agonist stimulation (10). The effects of β -agonists on cytokine production may also be dose-dependent. Low concentrations of β -agonists (i.e. 1–10 nM) stimulate cytokine production, whereas high concentrations (i.e. 100 nM to 10 μ M) inhibit cytokine production by T cells (10). Downstream from cyclic AMP, β -agonists inhibit cytokine production by T cells by blocking the calcium-/calmodulin-dependent protein phosphatase calcineurin and p38 mitogen-activated protein kinase, but not NF κ B (10, 36). For information on the effects of catecholamines on other aspects of immune function, readers are referred to other more comprehensive reviews (21, 31, 37).

In comparison with glucocorticoids and catecholamines, less is known about the effects of growth hormone and prolactin on the immune system. The actions of growth hormone and insulin-like growth factor-1 (IGF-1) do not overlap entirely, but growth hormone exerts many of its actions through IGF-1. Neither growth hormone nor IGF-1 is essential for immune function, but growth hormone influences various aspects of immune cell development and activity (38). Growth hormone inhibits apoptosis of CD4⁺ T cells following treatment with dexamethasone (39). Growth hormone, through binding to its receptor on the surface of T cells, may activate phosphatidylinositol 3 kinase (which regulates cell proliferation) and NF κ B (which controls apoptosis through the anti-apoptosis protein Bcl2) (40). IGF-1 also stimulates macrophages to produce reactive oxygen species (41) and increases NK cell activity (42). Prolactin is also not essential to normal immune function (38), but it can promote lymphocyte proliferation (43) and haematopoiesis (44).

Interactions between the neuroendocrine and immune systems are bi-directional. Pro-inflammatory cytokines released from immune cells (e.g. IL-1 β , IL-6 and TNF- α) mediate communication between the immune system and the central nervous system. Cytokines can alter activity of the central nervous system through humoral, neural and cellular pathways (45). Cytokines can pass the blood–brain barrier directly (46). Alternatively, immune cells can pass across the blood–brain barrier and release cytokines into the central nervous system (47). Cells comprising the blood–brain barrier also secrete various cytokines (48). Cytokines may signal the central nervous system by stimulating afferent nerves, although this concept remains somewhat controversial (49). One theory proposes that cytokines target the blood–brain barrier during systemic inflammation, whereas they target afferent nerves during localised inflammation (49). Cytokines can pass back across the blood–brain barrier into the circulation following intracerebroventricular injection of lipopolysaccharide (LPS) (50). Cytokines interact with components of the central nervous system, resulting in behavioural changes. Specifically, cytokines alter neurotransmitter function, neuroendocrine activity, neural plasticity and neural circuitry. These actions can induce fever, changes in appetite, fatigue and depression (45).

Stress Hormones and Leukocyte Mobilisation In Vivo

A number of studies have investigated the effects of stress hormones on circulating leukocyte numbers by infusing variable doses of stress hormones in healthy humans over 30 min up to 5 h. Cortisol raises the number of circulating neutrophils, whereas it suppresses the number of lymphocytes, and does not alter the number of Leu⁺ NK cells

(51, 52). By contrast, adrenaline increases the number of circulating total lymphocytes and NK cells (51, 53–55). The number of circulating monocytes also rises 1–2 h following infusion of adrenaline (53, 55, 56).

In contrast with NK cells, the effects of adrenaline and the β -agonist isoproterenol on circulating T lymphocyte subpopulations are somewhat variable. In response to these agents, the number/percentage of circulating CD4⁺ T helper cells decreases (54, 56, 57) or increases (53, 58), whereas the number/percentage of circulating CD8⁺ T cytotoxic cells increases (53, 54, 58), decreases (57) or remains unchanged (56, 59). The number/percentage of circulating B lymphocytes decreases (53) or remains unchanged following infusion of adrenaline or isoproterenol (54, 56, 59). More recent research indicates that adrenaline increases the number of circulating CCR7⁻CD45RA⁺CD8⁺ effector T cells, CD4⁻CD8⁻ γ/δ T cells, CD3⁺CD56⁺ NK T-like cells, CD16⁺CD56^{dim} cytotoxic NK cells, and CD14^{dim}CD16⁺ pro-inflammatory monocytes. These cells most likely originate from marginated pools on the endothelial surface of blood vessels (60). In addition to these findings, γ/δ T cells and T cells expressing chemokine receptors (CXCR2, CXCR3 and CCR5) are mobilised into the circulation following psychological stress. These responses correlate with cardiac activation (61, 62).

The effects of noradrenaline on circulating leukocytes are also variable. One study has reported that noradrenaline raised the number of circulating neutrophils, monocyte, lymphocytes and CD16⁺ NK cells (58, 63). Another study found no changes in the numbers of these cell types or T lymphocyte subpopulations following treatment with noradrenaline (54). These inconsistent findings may be due to differences between these studies in noradrenaline dose, and in the duration of hormone infusion and blood sampling times relative to the period of infusion.

Combined treatment with cortisol and adrenaline increases the number of circulating neutrophils for up to 12 h (52). Growth hormone infusion in humans (2 IU) increases neutrophil number, but does not alter blood mononuclear cell subpopulations (64).

Stress Hormones and Leukocyte Function In Vivo

Several of the studies described above have also examined changes in immune cell function following infusion of stress hormones in healthy humans. Cortisol does not alter Leu⁺ NK cell activity (51) or neutrophil chemotaxis or production of reactive oxygen species (65). By contrast, adrenaline increases the activity of CD16⁺ NK cells (53, 55). Similarly, noradrenaline infusion in humans (16 $\mu\text{g}/\text{min}$ for 1 h) also increases CD16⁺ NK cell activity (63). The effects of catecholamines and isoproterenol on lymphocyte proliferation vary. Isoproterenol reduces lymphocyte proliferation (54), whereas adrenaline and noradrenaline have no effect (54, 57). This disparity may be due to variable changes in lymphocyte subpopulations in response to these agents. Adrenaline increases the number of T cells that express IFN- γ , IL-2, IL-4 and TNF- α (53). Adrenaline and noradrenaline infusions also raise the plasma concentrations of IL-6 and IL-1 receptor antagonist (IL-1ra) under normal resting conditions (66–68). In contrast, adrenaline infusion prior to experimental endotoxemia reduces subsequent changes in the plasma concentrations of IL-6, IL-8 and TNF- α (69). Hydrocortisone treatment immediately prior to experimental endotoxemia does not alter subsequent changes in plasma IL-6 concentration, but attenuates plasma TNF- α concentration and increases plasma IL-10

concentration endotoxemia (70, 71). Conversely, IL-6 and IFN- γ increase the plasma concentrations of cortisol and ACTH cortisol (72, 73), while infusion of LPS increases the plasma concentrations of adrenaline and cortisol (59).

To summarise, glucocorticoids, catecholamines and growth hormone bind to specific receptors on the surface of immune cells. This hormone-receptor binding mediates leucocyte trafficking and functional activity. In vitro, glucocorticoids and catecholamines induce a shift in the balance of type 1/type 2 cytokines towards greater production of type 2 cytokines. Growth hormone regulates immune cell activity through IGF-1, and can inhibit apoptosis of T lymphocytes. In vivo, cortisol mobilises neutrophils, but reduces the number of circulating lymphocytes and does not alter circulating natural killer cell numbers. Catecholamines increase the total number of circulating lymphocytes, monocytes and natural killer cells. They also stimulate natural killer cell activity. By contrast, the effects of catecholamines on circulating lymphocyte subpopulations and lymphocyte activity are more variable. By crossing the blood-brain barrier, immune cells and cytokines can alter the function of the central nervous system.

IMMUNOENDOCRINE RESPONSES TO ACUTE EXERCISE

Exercise immunologists have used various approaches to investigate the interaction between the endocrine and immune systems during exercise. On a basic level, some research has assessed the correlation between changes in stress hormones and immunological variables following exercise. Other research has examined the interactions between the endocrine and immune systems by using different exercise workloads, carbohydrate and caffeine supplementation, thermal stress or drugs. A small number of studies have also investigated how exercise-induced immune changes alter the activity of the central nervous system.

Correlations Between Stress Hormones and Immunological Variables

McCarthy et al. (74) first provided evidence that following brief, intense exercise, the number of circulating lymphocytes correlated positively with the plasma concentrations of adrenaline ($\rho=0.67$, $p<0.05$) and noradrenaline ($\rho=0.68$, $p<0.05$). Plasma adrenaline concentration also correlates positively with the number of circulating neutrophils after short, intense exercise (74, 75) and endurance exercise (76). Rhind et al. investigated the relationships between stress hormones and immune cells following exercise. Stepwise multiple linear regression indicated that plasma adrenaline concentration accounted for some of the variation in CD3⁺ T cells, CD4⁺ T helper cells, CD8⁺ T cytotoxic cells and CD3⁻/CD16⁺/CD56⁺ NK cells (77). Plasma noradrenaline concentration also explained some of the variation in CD3⁻/CD16⁺/CD56⁺ NK cells and CD19⁺ B cells (77). Steensberg et al. (78) discovered that following 2.5 h running at 75% $\dot{V}O_{2max}$, the number of T helper 2 cells that produce IL-2 and IFN- γ decreases below pre-exercise values, and this response is inversely correlated with plasma adrenaline concentration. Brenner et al. (79) used stepwise multiple linear regression to examine stress hormones and immune cells following cold exposure. Plasma noradrenaline concentration accounted for some of the variation in CD3⁺ T cells, CD8⁺ T cytotoxic cells and CD19⁺ B cells, whereas plasma adrenaline concentration was only linked with changes in CD19⁺ B cells (79).

The relationship between plasma cortisol concentration and the number of circulating immune cells is more variable. Some studies report no relationship (74, 80) or an inverse relationship (81) between plasma cortisol concentration and the number of circulating neutrophils after exercise. Other studies suggest that cortisol does mediate neutrophil mobilisation following exercise (76, 77, 82, 83). The association between plasma cortisol concentration and the number of circulating monocytes following exercise is also inconsistent (77, 81). It does seem, however, that plasma cortisol concentration accounts for some of the variation in CD4⁺ T helper cells and CD19⁺ B cells following exercise (77). These inconsistent findings may be due to variation in blood sampling points used to examine the association between plasma cortisol concentration and the number of circulating immune cells. In contrast with adrenaline, cortisol mobilises neutrophils into the circulation in a more delayed and prolonged fashion (51, 52). Recent evidence indicates that plasma cortisol concentration correlates strongly with lymphocyte apoptosis after resistance exercise (84). Although growth hormone can mobilise neutrophils at rest (64), there is no clear evidence to indicate that growth hormone regulates the number of circulating neutrophils following exercise (81).

Several studies suggest that stress hormones also regulate cytokine responses to exercise. The plasma concentrations of adrenaline, noradrenaline, cortisol and growth hormone correlate with the plasma concentrations of IL-6, IL-1ra, IL-12 and TNF- α following exercise in both thermoneutral and hot conditions (85–87). The plasma concentrations of noradrenaline and cortisol also correlate with plasma IL-6 concentration following cold exposure (79, 88). It is unclear whether hormones or cytokines are the driving factor behind these relationships. Stress hormones and cytokines regulate body temperature during exercise, albeit through distinct mechanisms (89). Adrenaline may stimulate a small rise in plasma IL-6 concentration during exercise (68). Alternatively, the correlation between plasma adrenaline and IL-6 concentrations following exercise may be purely coincidental, because both adrenaline and IL-6 regulate muscle glycogen depletion during exercise (90, 91). IL-6 release from skeletal muscle during exercise correlates with arterial IL-6 concentration (92). Treatment with the glucocorticoids hydrocortisone and dexamethasone reduces plasma IL-6 concentration during exercise (85). However, IL-6 stimulates cortisol release at rest (72). Further research is required to clarify the interactions between IL-6 and cortisol during exercise.

Exercise Workload, Stress Hormones and Immunological Variables

Stress hormones are released into the circulation as the intensity of exercise increases. Plasma adrenaline, noradrenaline and growth hormone concentrations rise in an exponential manner with increasing intensity (93–95). By contrast, plasma cortisol concentration only increases above exercise intensities of $>60\% \dot{V}O_{2\max}$ (76, 96, 97). Based on these hormone responses, a number of studies have compared immunological responses to exercise of variable intensity and duration.

Foster et al. (93) first provided evidence that catecholamines influence leukocyte mobilisation as a function of exercise intensity. The number of circulating granulocytes and lymphocytes increased with workload. Using the β -antagonist propranolol, they demonstrated that during exercise, catecholamines regulate changes in lymphocytes, but not granulocytes (93). Compared with moderate-intensity exercise, the number of

circulating monocytes is similar, while CD4⁺ T helper cells, CD8⁺ T cytotoxic cells and T cell proliferation decrease below pre-exercise values after high-intensity exercise (82, 97, 98). Conversely, the number of CD19⁺ B cells is higher after high- vs. moderate-intensity exercise (82). The number of circulating NK cells and NK cell activity is similar immediately after moderate- and high-intensity exercise, while NK cells and activity decrease below pre-exercise values 2 h after high-intensity exercise (98). These studies did not evaluate the relationship between stress hormones and these intensity-dependent immune changes. However, it seems likely that stress hormones play a more dominant role in mediating immune changes during high-intensity exercise. The plasma concentrations of IL-6, IL-1ra and IL-10 are also higher following high- vs. moderate-intensity exercise (76, 92, 99, 100). As discussed above, adrenaline may stimulate a minor rise in plasma IL-6 and IL-1ra concentration during exercise (66, 68), but it is more likely that IL-6 stimulates IL-1ra and IL-10 late in exercise (72).

Carbohydrate Supplementation, Stress Hormones and Immunological Variables

Cortisol and adrenaline play key roles in mediating metabolism during exercise (90, 101). Many studies have used carbohydrate supplementation to manipulate stress hormone responses and examine the mechanisms of exercise-induced changes in immune cell counts and activity.

With the exception of a few studies (102–104), carbohydrate consumption during endurance exercise generally reduces the plasma concentrations of adrenaline, cortisol and growth hormone (105–112). This decrease in the release of stress hormones most likely accounts for the decline in the number of circulating neutrophils and monocytes following carbohydrate ingestion during exercise (102, 103, 107, 109–111, 113). By contrast, although carbohydrate supplementation attenuates plasma cortisol concentration, in general it does not prevent the post-exercise decline in the number of circulating lymphocytes, lymphocyte subsets or NK cells (110, 114–118).

The effects of carbohydrate supplementation on other exercise-induced changes in immune cell function are variable. Despite changes in stress hormones, not all studies demonstrate that carbohydrate consumption maintains or increases neutrophil and monocyte function (102, 103, 107, 109, 113, 119). Most research indicates that carbohydrate supplementation does not prevent the post-exercise decrease in lymphocyte proliferation (114, 118, 120). However, Lancaster et al. (115) found that consuming carbohydrate reduces plasma cortisol concentration and helps to maintain the number of IFN- γ ⁺ CD4⁺ and CD8⁺ T cells and IFN- γ production by these cells during exercise. The metabolic stress of low muscle glycogen appears to increase plasma cortisol concentration and the number of circulating leukocytes, but does not alter lymphocyte proliferation during exercise (121, 122). Carbohydrate supplementation increases IL-2- and IFN- γ -stimulated NK cell activity, but not IL-4- and IL-12-stimulated NK cell activity (116, 117). These effects on NK cell activity are independent of changes in plasma cortisol concentration (116, 117). Nieman et al. (123) discovered that carbohydrate ingestion during exercise reduced plasma cortisol concentration but did not alter salivary immunoglobulin A concentration (when adjusted for saliva protein concentration and secretion rate). However, changes in salivary immunoglobulin A concentration

were negatively correlated with plasma cortisol concentration, and this relationship predicted the incidence of upper respiratory illness in the 2 weeks after exercise (123).

With a few exceptions (103, 106, 112), most research shows that carbohydrate attenuates the rise in plasma concentrations of IL-6, IL-10 and IL-1ra (but not IL-8 or TNF- α) following exercise (105, 108–111). These cytokine responses to consuming carbohydrate during exercise may be partly linked to changes in catecholamine release. Carbohydrate supplementation does not influence leukocyte mRNA expression of IL-6, IL-8, IL-10 and IL-1ra or monocyte intracellular production of IL-6 and TNF- α following exercise (105, 106). Carbohydrate ingestion attenuates the release of IL-6 from skeletal muscle during exercise, but the effects of carbohydrate on mRNA expression of IL-6 and IL-8 in skeletal muscle following exercise are variable (110, 111, 124, 125).

Caffeine Supplementation, Stress Hormones and Immunological Variables

Although caffeine is a well-known stimulant of the central nervous system, only a small number of studies have focused on its effects on stress hormones and immune responses to exercise. Ingesting 6 mg caffeine 1 h before endurance exercise consistently raises plasma adrenaline concentration (126–130). Compared with a placebo treatment, caffeine supplementation does not alter the number of circulating neutrophils following exercise or neutrophil production of reactive oxygen species (129, 130). The number of circulating CD3⁻/CD56⁺ NK cells is greater compared with a placebo treatment, whereas changes in the number of activated NK cells expressing CD69 are variable after exercise and caffeine ingestion (131, 132). Changes in the total number of circulating lymphocytes after exercise and caffeine intake are also variable (129, 130). The numbers of circulating CD4⁺ T helper cells and CD8⁺ T cytotoxic cells are lower, while the numbers of these cells that express the activation marker CD69 are greater after exercise and caffeine intake compared with a placebo treatment (126). Caffeine supplementation also increases the concentration and secretion rate of salivary immunoglobulin A and the plasma concentration of heat shock protein 72 after exercise compared with a placebo treatment (127, 128). This variation in the effects of caffeine on exercise-induced immune changes may be due to differences in exercise protocol, blood sampling times and the habitual caffeine intake of the study participants.

Thermal Stress, Stress Hormones and Immunological Variables

Some researchers have compared changes in stress hormones and immunological variables following exercise in hot vs. cold/thermoneutral conditions. Several studies have examined responses to exercise in hot vs. cold water. This approach appears to be more effective than comparing responses to exercise in hot vs. cold/thermoneutral ambient conditions, because water is a more effective conductor of heat than air. For detailed discussion on the effects of thermal stress on the endocrine and immune systems, interested readers should consult the comprehensive review by Walsh and Whitham (89).

Plasma stress hormone concentrations are higher following exercise in hot vs. cold water, and these responses most likely account for the higher numbers of circulating neutrophils and lymphocytes following exercise in hot water (77, 81, 133–135).

However, not all research supports a link between stress hormones and the number of circulating leukocytes following exercise in hot conditions (136, 137). This relationship may vary depending on the demands of exercise. Within the lymphocyte subsets, CD3⁺ T cells, CD34⁺ T helper cells, CD8⁺ T cytotoxic cells and CD3⁺/CD16⁺/CD56⁺ NK cells (but not CD19⁺ B cells) are higher at the end of exercise in hot vs. cold/thermoneutral conditions (77, 122). By contrast, the number of circulating CD3⁺ T cells is lower 2 h after exercise in hot vs. thermoneutral conditions (122).

The effects of thermal stress on neutrophil function following exercise are also variable, with reports of an increase (138), a decrease (137) or no change (122, 134). Thermal stress during exercise increases lymphocyte proliferation per cell (despite higher plasma cortisol concentration) (122), whereas it does not alter NK cell activity per cell (122, 139). The plasma concentrations of IL-10, IL-1ra, IL-12 and TNF- α are consistently higher after exercise in hot vs. cold/thermoneutral conditions, whereas changes in the plasma concentrations of IL-6, IL-8 and granulocyte-colony-stimulating factor (G-CSF) are less consistent (86, 134, 136–138).

Drugs, Stress Hormones and Immunological Variables

Several studies have used drugs to manipulate stress hormone responses to exercise and examine the resultant immunological responses. The findings of these studies are equivocal, possibly because of variation in the exercise protocols, treatment periods and drugs used in these studies.

As described previously, Foster et al. (93) treated men with a single dose of the non-selective β_1 -/ β_2 -antagonist propranolol 10 min before incremental exercise. They discovered that during exercise, propranolol reduced the rise in the number of circulating lymphocytes, but not neutrophils or plasma catecholamine concentrations. This finding suggests that catecholamines may not regulate leukocyte mobilisation directly during incremental exercise. Instead, catecholamines may work indirectly by increasing blood flow, which strips leukocytes from the endothelial surface of blood vessels in marginal pools such as the lungs. Murray et al. (140) conducted a follow-up study in which they treated men and women with propranolol or the selective β_1 -antagonist metoprolol for 1 week prior to an incremental exercise test. Neither drug reduced post-exercise plasma catecholamine concentrations compared with the control trial. However, compared with the control trial, propranolol (but not metoprolol) reduced the total number of circulating lymphocytes, numbers of CD4⁺ T helper cells and CD8⁺ T cytotoxic cells and NK cell numbers and activity, and reduced the post-exercise decline in lymphocyte proliferation (140). These findings suggest that circulating catecholamines may not mobilise lymphocytes into the circulation. Instead, these cells may be mobilised from the spleen in response to direct activation of β_1 -/ β_2 -adrenergic receptors in the spleen (141).

Starkie et al. (142) treated men with the selective α_1 -antagonist prazosin and the non-selective β -antagonist timolol or placebo 2 h prior to 20 min cycling at $\sim 78\% \dot{V}O_{2\max}$. Plasma catecholamine concentrations were higher, whereas plasma cortisol concentration was lower after exercise in the drug trial compared with the placebo trial. Starkie et al. (142) attributed the greater catecholamine response in the drug trial to reduced

clearance of catecholamines by β -receptors. The numbers of circulating lymphocytes and monocytes increased during exercise in both trials, but were lower immediately after exercise in the drug trial compared with the placebo trial—despite the higher plasma catecholamine concentrations. This finding conflicts with other research showing that infusion of adrenaline or isoproterenol *raises* the number of circulating lymphocytes (51, 53, 54). One possible explanation for this difference is that the drugs used in the study by Starkie et al. (142) may target different adrenergic receptors on lymphocyte compared with adrenaline or isoproterenol. The numbers of circulating IFN- γ^+ CD3 $^+$ T cells, IL-2 $^+$ CD3 $^+$ T cells and IFN- γ^+ CD3 $^-$ /CD56 $^+$ NK cells increased during exercise in both trials. However, the numbers of these cells were lower after exercise in the drug trial compared with the placebo trial. IL-2 production by CD3 $^+$ T cells and IFN- γ production by both IFN- γ^+ CD3 $^+$ T cells and IFN- γ^+ CD3 $^-$ /CD56 $^+$ NK cells decreased during exercise similarly in both trials. These findings suggest that α - and/or β -adrenergic receptor stimulation does not regulate cytokine production by T cells and NK cells during exercise.

Mazzeo et al. (143) treated women with prazosin or placebo for 3 days before cycling for 50 min at 50% $\dot{V}O_{2max}$. Prazosin reduced plasma IL-6 concentration after exercise compared with the placebo. Papanicolaou et al. (85) treated men with hydrocortisone, dexamethasone or a placebo 4 h before 25 min running at 78% $\dot{V}O_{2max}$. Both hydrocortisone and dexamethasone attenuated plasma IL-6 concentration after exercise compared with the placebo.

EVIDENCE FOR INTERACTIONS BETWEEN THE CENTRAL NERVOUS AND IMMUNE SYSTEMS

As outlined above, considerable attention has focused on how stress hormones regulate immune responses to exercise. The immune system is also capable of altering the function of the central nervous system. Several studies have examined this issue, and it is likely that more research will be conducted in this area in the future. In mice, exercise-induced muscle damage stimulates macrophages residing in the brain to secrete IL-1 β into the surrounding tissue (144, 145). This response appears to increase perceptions of fatigue, reduce voluntary activity and delay recovery from exercise (146). In humans, Steensberg et al. (147) observed that at rest, the concentrations of IL-6 and the cellular chaperone heat shock protein 72 (HSP72) are two to three times higher in cerebrospinal fluid compared with plasma. Although exercise stimulates the systemic release of IL-6 and heat shock protein 72, their concentrations remain stable in cerebral spinal fluid, which indicates that they do not cross the blood–brain barrier (147). The brain releases small amounts of IL-6 into the systemic circulation during exercise, and this is independent of hyperthermia (148). The functional significance of this response is not certain. It may provide a signal to the liver to increase glucose output, or it may be a more general indication of increased neural activity during exercise (148).

CHRONIC INTERACTIONS BETWEEN THE ENDOCRINE AND IMMUNE SYSTEMS

Compared with the amount of research on acute exercise, fewer studies have examined interactions between stress hormones and immunological variables following chronic training. Most studies have simply documented the effects of intensified training on simultaneous changes in stress hormones and immune cell counts at rest and/or in response to acute exercise. Very few studies have specifically examined the relationship between changes in stress hormones and immune cell counts and function.

Several studies report no changes in resting plasma and urinary cortisol concentrations, immune cell counts or serum cytokine concentrations after intensified training (149–153). Robson-Ansley et al. (154) discovered no changes in resting plasma cortisol or the number of circulating neutrophil counts, but did find that resting plasma IL-6 concentration was persistently elevated following 4 weeks of intense training. Fry et al. (155) observed that resting plasma cortisol concentration decreased, while the numbers of circulating neutrophils, monocytes and lymphocytes did not change after 10 days of intense interval training. The number of circulating CD3⁺, CD4⁺ and CD8⁺ T cells and CD20⁺ B cells also remained unchanged, whereas the number of circulating CD56⁺ NK cells decreased and CD25⁺ T cells increased following 10 days of training (155). It is unlikely, however, that these changes in CD56⁺ NK cells and CD25⁺ T cells were related to changes in plasma cortisol concentration. Smith and Myburgh (156) reported no change in resting plasma cortisol concentration, but found that CD4⁺ and CD8⁺ T cell counts and CD16⁺/CD56⁺ NK cells decreased following 4 weeks of intense training. Makras et al. (157) observed an increase in urinary cortisol concentration, an increase in CD4⁺ T cell count and a decrease in neutrophil count at rest after 4 weeks of military training. Ortega et al. (158) found that neutrophil phagocytic activity was higher in female athletes compared with non-athletes. In the athletes, neutrophil phagocytic activity correlated positively with plasma cortisol concentration, whereas it correlated negatively with plasma ACTH concentration. Findings from the study by Cunniffe et al. (159) suggest that elevated salivary cortisol concentration with training may reduce salivary immunoglobulin A concentration, resulting in increase susceptibility to upper respiratory illness. Some of the variability among these studies may result from differences in the physical fitness of study participants, training loads and blood sampling times.

The effects of chronic training on cortisol and immune responses to acute exercise are also variable. Verde et al. (160) reported that changes in serum cortisol concentration, CD3⁺ T cell counts and lymphocyte proliferation after acute exercise were all attenuated following 3 weeks of intense training. Lancaster et al. (161) discovered that 2 weeks of intense training reduced plasma cortisol concentration but did not alter lymphocyte production of the type 1 cytokine IFN- γ or the type 2 cytokine IL-4. In contrast with these findings, other research indicates no effect of chronic training on exercise-induced changes in plasma and salivary cortisol concentration, immune cell counts or salivary immunoglobulin A concentration (150, 152, 162).

A small number of studies have examined changes in plasma or urinary catecholamine concentrations and immune cell counts following chronic training. Imrich et al. (150) found no changes in plasma catecholamine concentration or immune cell counts

at rest or in response to acute exercise following 6 weeks of training. Hooper et al. (163) reported that both the number of circulating neutrophils and plasma noradrenaline concentration were elevated in swimmers showing symptoms of overtraining compared with swimmers who were not overtrained after 6 months of training. However, it is unclear whether these responses were linked in any way. Mackinnon et al. (151) observed that urinary norepinephrine concentration decreased, whereas plasma noradrenaline and leukocyte counts at rest did not change following 4 weeks of intense training. Makras et al. (157) found that the ratio of adrenaline:noradrenaline in urine increased after 4 weeks of military training. This response correlated positively with CD4⁺ T cell counts and correlated negatively with neutrophil counts.

BIOLOGICAL SIGNIFICANCE OF INTERACTIONS BETWEEN THE ENDOCRINE AND IMMUNE SYSTEMS

Dhabhar (1) proposes the following analogy to explain the possible significance of acute stress on the immune system. Within minutes of the onset of stress, catecholamines stimulate the body's 'soldiers' (i.e. leukocytes) to leave their 'barracks' (i.e. spleen, lung, bone marrow, lymph nodes) and enter the 'boulevards' (i.e. blood vessels and lymphatics). As stress proceeds, glucocorticoids are released which stimulate leukocytes to exit the bloodstream and enter potential 'battle stations' (i.e. skin, lung, gastrointestinal and urinary-genital tracts, mucosal surfaces and lymph nodes) in preparation for immune challenges that may occur in response to stressful stimuli (1).

In the context of exercise, the factors that stimulate the release of stress hormones are most often non-harmful. These factors may include demands for (1) increased blood flow to contracting muscle (to deliver oxygen and nutrients) and skin (for thermoregulation), and (2) release of energy substrates from the liver and adipose tissue (e.g. glucose, fatty acids, amino acids) to support muscle metabolism. Interaction between the endocrine and immune systems during exercise can therefore be considered as rather non-specific. However, stress hormones may (incidentally) prime immune cells to respond to infectious pathogens and/or airborne pollutants that invade mucosal surfaces lining the respiratory tract.

Dhabhar (1) proposed that the effects of stress on the immune system and general health depend on the duration of exposure to stress. Acute and intense stress may enhance immune function, and mild stress of moderate duration may promote immunosurveillance, while chronic stress may cause immune dysregulation (1). Immunoprotection resulting from acute stress may lead to more effective wound healing, responses to vaccination and resistance to infection and cancer. Immunopathology resulting from severe acute stress or persistent stress may promote pro-inflammatory and autoimmune diseases. Immunosuppression resulting from chronic stress may reduce the effectiveness of wound healing and vaccination, and increase the risk of infection and cancer. By contrast, chronic stress may reduce the risk of pro-inflammatory and autoimmune diseases by suppressing aspects of immune function that contribute to such conditions (e.g. T lymphocyte activity, cytokine production) (1).

Both acute exercise (164) and chronic training (165, 166) increase antibody production in response to vaccination. Mild repeated stress resulting from chronic training also

improves the rate of wound healing (167), decreases the risk of upper respiratory illness (168) and reduces the prevalence and severity of various chronic diseases (169). Although more work is needed to define their precise role, it is likely that stress hormones mediate some of these benefits of exercise.

SUMMARY

A variety of non-harmful stimuli during exercise induce the release of stress hormones. These stress hormones influence many physiological systems, including the immune system. Stress hormones act to mobilise immune cells into the circulation, and can increase or decrease the activity of these cells. The precise nature of the interaction between stress hormones and immune cells likely depends on multiple factors, including the intensity and duration of exercise, the physical fitness of exercising individuals and environmental conditions. Some stress hormones (e.g. catecholamines) influence immune cell activity mainly during exercise, whereas others (e.g. cortisol) may have a more delayed effect on immune function during the later stages of exercise and/or after exercise. Nutritional interventions such as carbohydrate and caffeine supplementation can alter the secretion of stress hormones during exercise, but these alterations do not always result in changes in immune function. Immunoendocrine interactions during exercise may serve to promote some aspects of health. However, further research is needed to understand the biological significance of such interactions in more detail.

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Effects of Female Reproductive Hormones on Sports Performance

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INTRODUCTION

Over the past 30 years, the involvement of women and girls in physical activities and competitive sport has increased exponentially. This is largely a consequence of Title IX of the United States Educational Assistance Act (enacted in 1972), which mandated institutions receiving federal monies to provide equal access for women to funding for extracurricular activities, including opportunities for participation, financial resources for scholarships, and qualified coaching. Equally as important, there have been progressive changes in societal and cultural views worldwide toward the acceptance of female athletes into the sporting arena—historically and traditionally a male bastion. Women

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now compete at the highest levels in most sports, some of which were previously played by men only, such as ice hockey, wrestling, and rugby. The 2012 Summer Olympic Games in London marked the introduction of women's boxing as a full-participation sport.

Yet scientific knowledge regarding a woman's unique physiology—from childhood through puberty and adolescence, across the reproductive lifespan, and into the postmenopausal years—has not kept pace with this explosion in sport participation by girls and women. In particular, there still remains a multitude of unanswered questions about the effects of the female reproductive hormones—estrogen and progesterone—on various aspects of athletic performance. *Athletic performance* itself is a multifaceted entity—a complex and intricate kaleidoscope of cardiovascular, respiratory, metabolic, endocrinological, and psychological factors that all must interact to enhance and facilitate sporting success. The relative importance of each varies with the specific demands of the particular discipline.

Athletes and coaches have long postulated that altered athletic performance might result from the hormonal swings of the female menstrual cycle (MC). Early studies were retrospective and nonspecific, with substantial recall bias in terms of MC phase and status (1–3). Researchers have investigated the influence of the MC on substrate metabolism, cardiorespiratory function, thermoregulation, psychological factors, and musculoskeletal injury rates. The individual sex steroids estrogen and progesterone can have antagonistic, synergistic, or additive effects, and furthermore, their relative concentrations change during the course of an ovulatory menstrual cycle (4, 5). In addition, hormonal levels have been shown to increase with exercise (6–8). Both oral contraceptives (OCs) and hormone replacement therapy (HRT) can provide a stable and controllable hormonal milieu for training and competition. However, the combinations of exogenous hormones in these formulations introduce yet other potential modifying factors. This chapter will integrate what is already known in this somewhat controversial field of inquiry (9–16), with new emerging evidence and interesting future directions. The reader is referred to summative reviews (17–22) for further details on the individual investigations.

PHYSIOLOGY OF THE MENSTRUAL CYCLE

A well-defined, predictable pattern of hormonal fluctuations takes place over the course of an ovulatory menstrual cycle (23, 24). In eumenorrheic females, an average menstrual cycle (MC) lasts 28 days, but may range from 20 to 45 days. The three main phases—*follicular*, *ovulatory*, and *luteal*—are based on ovarian function and controlled by pituitary hormonal signals (Fig. 1) (4). Intricate feedback mechanisms involve the gonadotropins—luteinizing hormone (LH) and follicle-stimulating hormone (FSH)—and the female sex steroids. Some studies further divide the cycle into five discrete phases, including an early and late follicular phase and an early, middle, and late luteal phase (see Chap. 8 by Petit and Prior) (25). Varying concentrations of estradiol and progesterone differentiate the phases of an ovulatory MC: both are low during the follicular phase (FP), estrogen is high and progesterone low during the ovulatory phase (OP), and both are high in the luteal phase (LP).

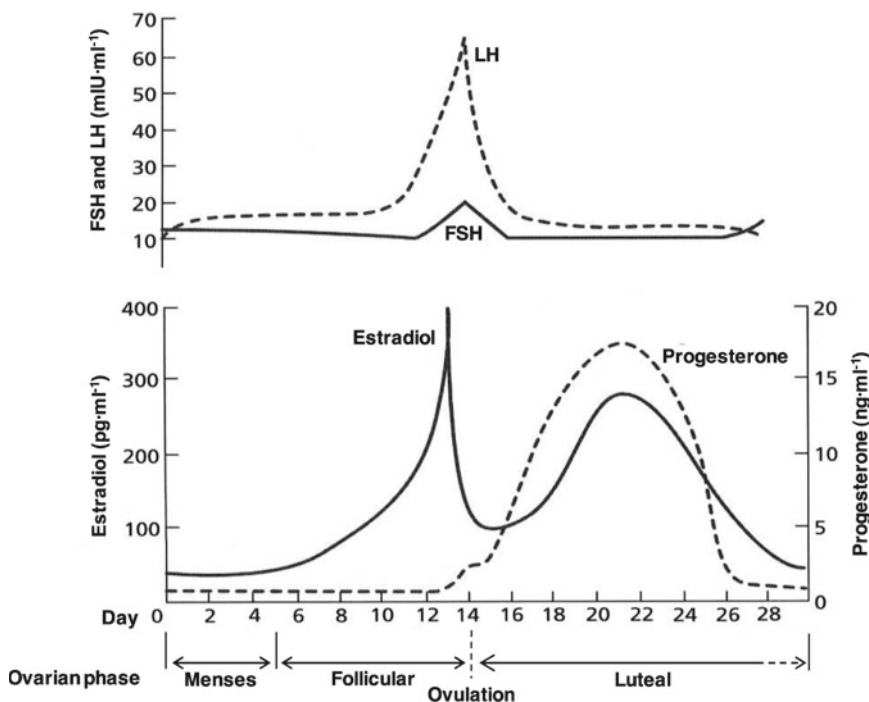


Fig. 1. Hormonal changes and phases of the menstrual cycle. *FSH* follicle-stimulating hormone; *LH* luteinizing hormone.

ORAL CONTRACEPTIVES

Oral contraceptive pills provide steady levels of exogenous estrogen and progestin, which act primarily by disrupting the normal hypothalamic-pituitary-ovarian (HPO) axis to suppress ovulation (Fig. 2). Current recommendations state that age alone does not constitute a medical reason for denying any contraceptive method including OCs and progestogen-only injectables, as this concern must be balanced against the advantages of avoiding pregnancy in adolescents below 18 years (26). OCs can be safely prescribed right up until the perimenopausal years in nonsmoking women. In addition to the prevention of pregnancy, OCs are sometimes utilized by female athletes to manipulate timing of menses around important competitions. However, it is estimated that the prevalence of OC usage in the athletic population matches that within the general community (27). Newer formulations such as Seasonale even make it possible to even completely withhold menstruation. This OC is administered continuously for 3 months, allowing only four *periods* or withdrawal bleeds per year, thereby decreasing premenstrual syndrome (PMS) and concomitant menstrual discomfort and pain, as well as blood loss and iron deficiency (28–30).

Contemporary low-dose combination pills have a three- to fourfold decrease in estrogen content and a tenfold decrease in progestin compared with earlier generation OCs, usually containing between 15 and 35 μg ethinyl estradiol (EE) and less than 1 mg progestin (31). In *monophasic* preparations, estrogen and progesterone doses are fixed

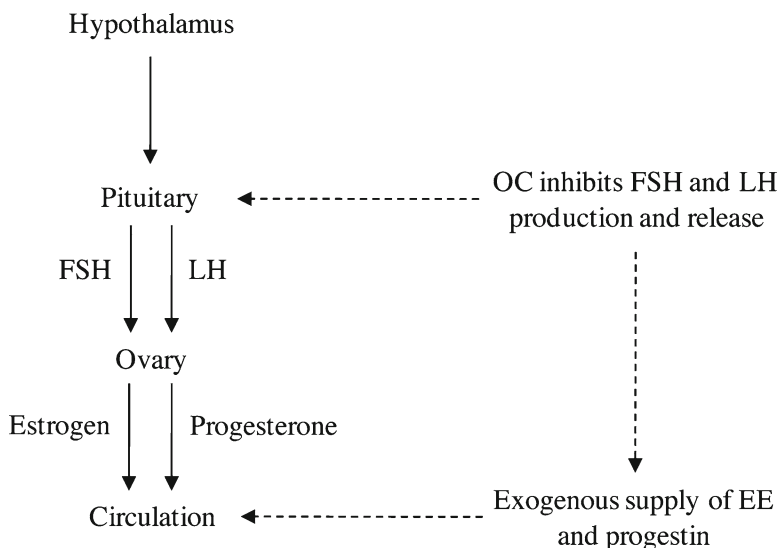


Fig. 2. Suppression of endogenous female sex steroid hormones by combined oral contraceptives (OCs). *FSH* follicle-stimulating hormone; *LH* luteinizing hormone; *EE* ethinyl estradiol.

over the entire pill cycle, while in *biphasic* or *triphasic* formulations, amounts vary to mimic normal physiologic cyclical patterns. Most OCs incorporate EE as the synthetic estrogen, although some older formulations contained mestranol.

Synthetic progestins, derived either from 19-nortestosterone or from 17-OH progesterone derivatives and 19-norprogesterone derivatives, have been chemically refined to decrease unwanted side effects. *First-generation* progestins include norethindrone and norethindrone acetate, norethynodrel, and ethynodiol diacetate. Levonorgestrel and norgestrel are *second-generation* drugs from the *gonane* group, with norgestrel the more potent and the more androgenic. The *estrane* group comprises norethisterone and its metabolites, as well as norethisterone pro-drugs: norethynodrel, lynestrenol, and ethynodiol acetate. *Third-generation* progestins are deliberately more selective for progestin receptors, with consequently a less negative impact on lipoproteins and atherogenesis of vessel walls. These include desogestrel (and its active metabolite 3-keto-desogestrel or etonogestrel), norgestimate, and gestodene. *Fourth-generation* progestins are dienogest (a hybrid progestin, with antiandrogenic actions) and drospirenone (derived from spironolactone, giving it anti-mineralocorticoid and progestogenic properties not found in most synthetic progestins). There are other new progestins, with varying pharmacological profiles and activities (32).

For women unable to tolerate these combined regimens or for those with medical contraindications to estrogen use, there are progestin-only preparations, such as progestin-only *minipills*, or injectable depot medroxyprogesterone acetate (DMPA) or Depo-Provera (33). Subdermal slow-release capsules containing levonorgestrel (Norplant) or etonogestrel (Implanon) provide rapidly reversible contraception for up to 5 years with only a 1% failure rate, but require special physician training on implantation and removal of the capsules. Increased and irregular intermenstrual bleeding

with progestin-only preparations may, in itself, be disruptive for athletic training and competition. There is little existing information on the use of these contraceptive methods in adolescent athletes and/or any associated effect on performance. Additional methods of long-acting reversible contraception include intrauterine devices (IUDs) containing either copper or levonorgestrel (Mirena), and the contraceptive vaginal ring or transdermal patch (34).

PHYSIOLOGICAL EFFECTS OF THE FEMALE SEX STEROID HORMONES

The female sex steroid hormones are all derivatives of cholesterol. Within the estrogen group of 18-carbon steroids, 17β estradiol is the major form, and estrone and estriol are less potent. In women, they are secreted primarily by the ovaries, and to a lesser extent by the adrenals (35). As discussed, synthetic forms include ethinyl estradiol and mestranol. The other major female hormones are the *progestins*: endogenous progesterone and first-, second-, third-, and now fourth-generation synthetic progestins in OCs. The various female sex steroids (endogenous and exogenous) exert a myriad of diverse and complex effects on multiple physiologic parameters, with the potential to influence athletic performance. Considerations for exercise performance in women therefore differ significantly from men (36).

Cardiovascular Function

All estrogens are known to have important actions on the cardiovascular system (37, 38). They alter plasma fibrinolytic activity and platelet aggregation, resulting in a detrimental increase in thrombosis. Conversely, they also confer protection against atherosclerosis by decreasing total cholesterol and low-density lipoprotein (LDL) levels and increasing high-density lipoproteins (HDL). Estrogen enhances vasodilation of the vascular smooth muscle of coronary arteries and peripheral vascular beds (39, 40), in turn increasing blood supply to the heart and muscles. At least theoretically, there is potential here for enhancement of cardiac function and aerobic performance. Estrogen controls production and release of nitric oxide (NO), now known to be the endothelium-derived relaxation factor (41), and can act as a calcium channel blocker (42, 43). In addition, estrogen is thought to be protective against inflammation (44, 45). Many of these actions are antagonized by progesterone, making this an extremely complex area of physiology (46).

Overall, estrogen is viewed as protective against cardiovascular disease and hypertension (47). Therefore, there is mounting concern that women with functional hypothalamic amenorrhea (and consequently low estradiol levels) have an increased risk of cardiovascular disease, through changes in the above mechanisms (48–54). Amenorrhea in female athletes is associated with endothelial dysfunction and an unfavorable lipid profile (55–57). One study showed that folic acid supplementation improved vascular function in amenorrheic athletes (58). By replacing estradiol with an exogenous form of estrogen, OCs may ameliorate this endothelial (and vascular) dysfunction and, by extension, potentially modify this increased risk (59).

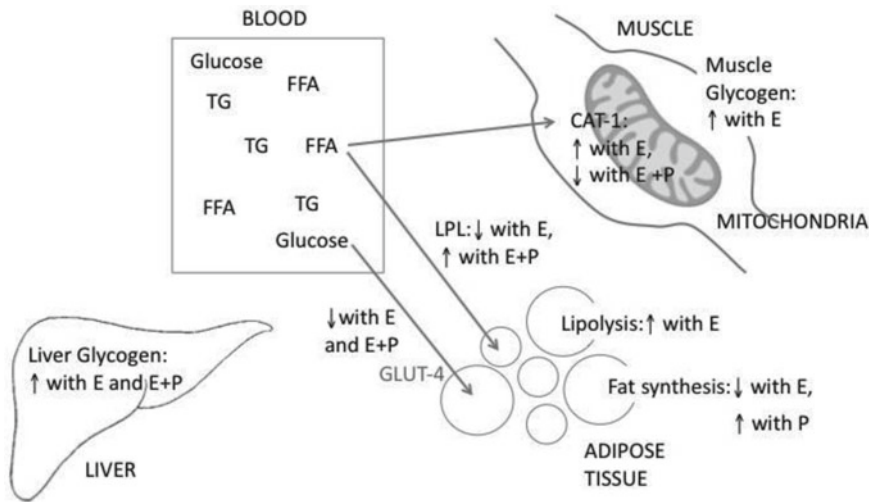


Fig. 3. Overview of the effects of estrogen (E) and progesterone (P) on pathways of carbohydrate and fat metabolism; ↑, increased flux through pathway; ↓, decreased flux through pathway; *TG* triglycerides; *FFA* free fatty acids; *LPL* lipoprotein lipase; *GLUT-4* glucose transporter type 4; *CAT 1* mitochondria carnitine acyltransferase 1.

Respiratory Function

Sex hormones are involved in central neural control of breathing (60), and can also affect the lungs and the airways. Investigations have consistently shown increased minute ventilation (VE) and augmented ventilatory response to hypoxia and hypercapnia during the LP, when hormone levels are highest (61, 62). Other researchers have documented changes in carbon dioxide (CO₂) sensitivity (63) and pulmonary carbon monoxide diffusing capacity (64). Similar changes in ventilation and respiration are seen in pregnancy (65, 66), due to elevated progesterone levels (see Chap. 17 by Bessinger). Different exogenous synthetic progestins in OCs may also have some influences on the respiratory system (67).

Substrate Metabolism and Energy Sources

Estrogen (and progesterone to a lesser extent) promotes glycogen uptake and storage in the liver and muscle (mostly type I fibers), and during exercise spares glycogen stores by shifting metabolism toward free fatty acids (FFA) (68–74). This occurs by increased lipid synthesis, enhanced lipolysis in muscle through induction of lipoprotein lipase (LPL), and greater utilization of FFA (Fig. 3). Such metabolic adaptations may be advantageous for women during ultraendurance exercise in comparison with their male counterparts (75, 76), by reducing reliance on muscle glycogen and increasing oxidative capacity (68). Women respond similarly to men to a protocol of carbohydrate (CHO) loading to supercompensate muscle glycogen stores, when fed the same relative amount of CHO (77).

Glycogenolysis (hepatic release of glucose) and gluconeogenesis help to control blood glucose levels along with peripheral glucose uptake. Progesterone may promote translocation of the GLUT-4 glucose transporter to the plasma membrane (73). It has been hypothesized that the drop in blood glucose represents an inability to maintain blood glucose homeostasis, rather than increased utilization (78). Paradoxically, however, both estrogen and progesterone suppress gluconeogenic output during exercise, which may potentially compromise performance in the latter stages of ultralong events, unless energy replacement supplements are adequate (22). Some have suggested that it may be more relevant to supplement energy intake during exercise with protein when progesterone is elevated, compared with estrogen, because progesterone promotes protein catabolism, while estrogen suppresses it (79). Similarly, estradiol and progesterone seem to have opposing effects in terms of lipid metabolism, with greater lipid oxidation when estradiol is used alone (Fig. 3). A detailed review of these complex metabolic processes, and the relative roles of hormones in regulating CHO and fat utilization at rest and during exercise, is beyond the scope of this chapter but is available elsewhere (73, 80).

Estrogen also acts in concert with growth hormone (GH), catecholamines, and insulin to regulate glucose metabolism (81) by decreasing insulin-binding capacity, resulting in deterioration in glucose tolerance and insulin resistance (82). Transdermal estradiol has been shown to change glucose metabolism, through decreased gluconeogenesis and changes in epinephrine secretion and glucose transport (83). Metabolic effects of progesterone include relative glucose intolerance and insulin resistance during the LP and pregnancy (when progesterone is high), with greater dependence on fat as a substrate, as shown by higher circulating FFAs (84), lower respiratory exchange ratios (RERs), and lower blood lactate levels during submaximal exercise (71, 85). Progesterone also appears to induce peripheral insulin resistance through actions on insulin receptors (81, 82), facilitated by the presence of estrogen. Female athletes with diabetes may need to be cognizant of such menstrual cycle influences, in order to optimize glucose control during training and competition.

There are varying concentrations and types of synthetic progestins in OCs, which also have the potential to impact hormonal responses and substrate utilization during exercise. The more androgenic progestins (norgestrel and levonorgestrel), in combination with ethynodiol diacetate, cause a decrease in glucose tolerance and an increase in insulin levels, but ethynodiol diacetate and norethindrone do not seem to have this effect. The newer *third-generation progestins* (i.e., desogestrel, gestodene, and norgestimate) have been designed to have less impact on glucose tolerance and insulin levels, as have *fourth-generation progestins* (86–88).

Deterioration of glucose tolerance and hyperinsulinemia are seen with DMPA, but norethindrone alone does not seem to have any significant effects. Approximately 25–50% of insulin-dependent diabetics will have an increase in insulin requirements while taking OCs, although there is great intraindividual variability (89). Low-dose monophasic OCs, limiting the amount of androgenic activity of the progestin, may be the best choice for women with insulin-dependent diabetes mellitus (IDDM). More information is needed on the impact of third- and fourth-generation progestins on glucose tolerance, insulin responses, and any clinical implications for exercising females.

Body Composition, Weight, and Bone Mineral Density

Other actions of estrogen include deposition of fat into the breasts, buttocks, and thighs, typically female characteristics. In terms of fluid balance and plasma volume, estrogen causes sodium and chloride retention, resulting in edema, weight gain, and an increase in blood pressure. Levels of both estrogen and progesterone are high in the end-luteal phase (i.e., immediately premenstruation). Through a complex feedback mechanism involving the renin-angiotensin system and aldosterone, progesterone in particular can cause fluid retention and increased body weight, possibly hindering performance (90). In one study, however, sodium retention during the LP did not correlate significantly with the typical premenstrual symptoms of subjective breast tenderness and bloating (91). In another 1-year prospective cohort study, monitoring of fluid retention during the MC in 62 healthy women revealed a peak on the first day of flow (when estradiol and progesterone levels are low), rather than premenstrually, possibly suggesting a lag of fluid dynamics in response to previous higher hormone levels (92). Administration of OCs has variable effects on weight, body composition (93), and fluid dynamics, depending on the relative androgenicity of the progestin used (94).

Importantly for bone mineral density (BMD), estrogen facilitates both gastrointestinal calcium absorption and calcium uptake into bone. Chronic estrogen-deficient states (such as menopause or amenorrhea and other forms of menstrual dysfunction) increase susceptibility to alterations in BMD. This can lead to either osteopenia or frank osteoporosis, with an increased risk for fractures, including stress fractures in younger individuals (a definite hindrance to training and competition!). The problem here is really twofold: lack of estrogen causes increased bone resorption, but equally and even more importantly, low energy availability (intentional or inadvertent) leads to menstrual dysfunction (95, 96). Low energy availability interferes with bone formation through the insulin growth factor-growth hormone (IGF-GH) axis (see Chap. 11 by Anne Loucks). This is most worrisome in the young adolescent athlete, who should be building up peak BMD during the critical years of rapid growth; even endurance training or weight-bearing exercises are not completely protective (97). There is an ongoing need to educate athletes, coaches, and physicians about this important issue (98).

Traditional postmenopausal HRT has been designed to counteract this process and replace the beneficial effects of estrogen in maintaining BMD. Because of the other energy-dependent mechanisms involved, it remains controversial whether or not administration of OCs is protective in younger women with functional hypothalamic amenorrhea (99–101), or if the length of previous exposure is a factor (102, 103). Influences of synthetic progestins on bone density are dependent on biochemical configuration and relative potency. Progesterone injections (DMPA) induce amenorrhea, with detrimental effects on BMD, which have been shown to be only partially reversible on discontinuation (104). It therefore behooves health-care providers to be aware of such potential effects, especially in younger female athletes, for whom stress fractures (especially in high-risk areas, such as the femoral neck) can be catastrophic. Little to no research has been done on BMD and contraceptive hormone-releasing patches, IUDs, or vaginal rings.

Thermoregulation

Progesterone has long been recognized to have thermogenic action. It causes an increase in basal body temperature (BBT) of 0.3–0.5°C during the LP (105–108) and during pregnancy (108), (see Chap. 17 by Bessinger) through a central action on the preoptic neurones (109). This central effect on body temperature also increases minute ventilation (110). There are numerous associated physiological mechanisms: altered skin blood flow (111, 112), a higher threshold for cutaneous vasodilatation, delayed onset of sweating (113, 114), and decreased thermal conductance (115). The increased BBT is postulated to result from an alteration or *shift* in set-point temperature related to the MC (116), but there may be individual differences in these temperature changes (117). Estrogen modifies the temperature effects of progesterone (118). Exogenous progestins in OCs can also affect centrally thermoregulatory mechanisms, and shift baseline core BBT and the threshold for the active vasodilator system to a higher internal temperature, with associated implications for exercise performance in the heat (119).

Psychological Factors: Estrogen and the Brain

There is increasing evidence that estrogen mediates different aspects of cognition, alertness, and mood, possibly through changes in the availability of neurotransmitters in the brain, such as serotonin. This is especially important during competition, where peak mental functions are required. Alterations in 5-hydroxytryptamine (5-HT) and serotonin pathways may also play a role in dysphoric PMS (120), and there may be associated changes in functional ability (121), which might increase risk for musculoskeletal injury. There have been reports of MC alterations in color vision performance, sleep-related memory consolidation, etc., but only with small numbers of subjects and mostly without hormonal verification. Brain size and morphology have been found to change over the MC, with significant grey matter volume peak and CSF loss at the time of ovulation, but with no correlation to estradiol or progesterone hormone levels (122).

It has also been proposed that estrogen benefits cognitive function and verbal memory in postmenopausal women, again through an effect on neurotransmitters, such as serotonin (123). Recent research has shown an inverse relationship between blood glutamate levels (which have neurotoxic properties) and levels of plasma estrogen and progesterone (124). Several current reviews explore these concepts in more detail (125, 126). The potential effects, if any, of a prolonged hypoestrogenic state on cognitive function in amenorrheic athletes are purely speculative and hypothetical at this time.

Researchers have linked gender differences in ischemic brain injury to estrogens (127), and have even examined clinical measures of concussion during the MC (128). Progesterone is also thought to possibly have neuroprotective benefits (129). There is potential for OCs to positively affect cognition, attention, mood, and various other psychological parameters, such as mental rotation and verbal fluency, depending on the specific chemical formulation (130–132). This is a fertile area for future research, especially since brain function under a varying human estrogenic environment is another source of modulation of exercise responses (133).

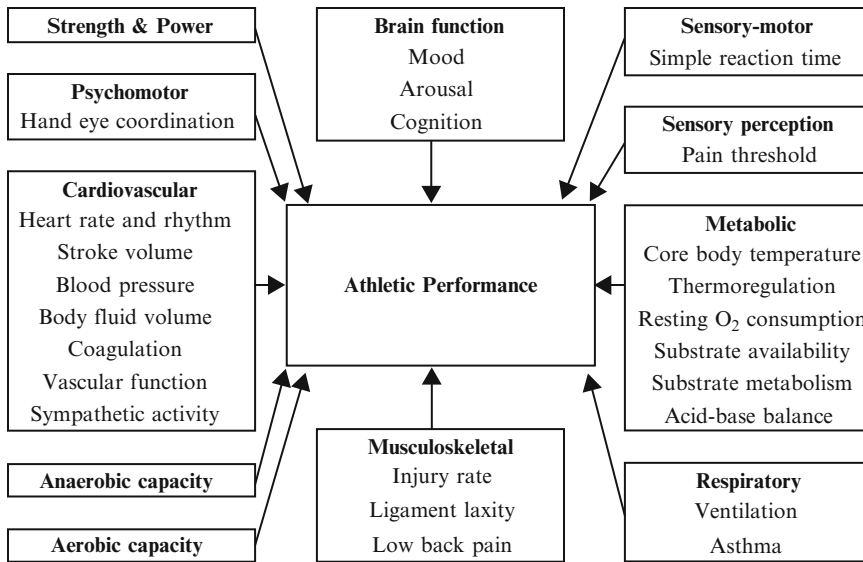


Fig. 4. Components of physical performance that may be affected by menstrual cycle fluctuations in endogenous hormones.

FEMALE REPRODUCTIVE HORMONES AND ATHLETIC PERFORMANCE

Physical fitness is frequently defined in terms of aerobic capacity, anaerobic capability, muscle endurance and strength, flexibility, and body fat percentage, but actual *athletic performance* is much more complex, with neuromuscular, sensorimotor, psychomotor, cognitive, and psychological functions all coming into play (Fig. 4). There are significant genetic inheritance effects on various components such as peak oxygen uptake and anaerobic power (134). Therefore, both *nature* and *nurture* (genetic factors and training) are involved in determining athletic prowess (135). Finally, the human spirit is of such tenacity that the ultimate determinate of success and athletic performance may actually reside within the brain—according to the central governor model (CGM) of Noakes (136, 137).

As detailed in previous sections, both estrogen and progesterone have significant effects on the metabolic, thermoregulatory, cardiovascular, and respiratory systems. Therefore, it is not unreasonable to conjecture that cyclical endogenous hormonal variations of the MC might influence performance. When comparing research findings, it is critical to consider the population being studied and the training status of the women (untrained, recreational, or elite athletes). Small subject numbers lead to inadequate power to detect significant differences, and individual variability could conceivably skew the results. Contradictory findings may be explained by the variety of testing protocols, differences in exercise intensity levels used, wide circadian variation in hormonal secretion, and discrepancies in the timing of testing, as well as the state of hydration, nutrition, and fitness, etc., of subjects. Much of the early research is fraught with methodological inaccuracies, the most significant being inconsistent definitions and documentation of MC phases (see Chaps. X and X by Hackney and Petit).

Cardiovascular Function and Athletic Performance

CARDIOVASCULAR FUNCTION AND THE MENSTRUAL CYCLE

Some physiological parameters can potentially be affected during the MC by the different cardiovascular effects of estrogen and progesterone. There are transient changes in plasma volume (PV), peaking within 2 days of the estimated day of ovulation and progressively increasing during the LP (138). Hemoglobin concentration may affect oxygen delivery to the tissues, but studies have shown no change (9), increased resting hemoglobin (139), or a decrease in hemoglobin in the LP (140). Also, in terms of actual oxygen utilization, increased body temperature during the LP causes a rightward shift of the oxyhemoglobin dissociation curve, while the concomitant LP increase in ventilation shifts the curve leftward due to an increase in pH, quite probably leading to no net effect.

Peripheral hemodynamics and renal function are affected through various mechanisms by both MC phase (141) and OC use, with additional effects during exercise (142). Measurements of tissue Doppler imaging derived myocardial performance index during the menstrual and the LP in 27 women aged 19–42 documented that endogenous estrogen improved combined systolic and diastolic function in both the left and right ventricles (143). Another study tested 19 eumenorrheic women with two incremental tests to voluntary exhaustion on a cycle ergometer and did not show any differences in cardiorespiratory variables, substrate metabolism (lactate), or performance (defined as power output and oxygen consumption or VO_2), but did find a greater ventilatory drive in LP (144).

Decreased estrogen levels increase peripheral resistance and decrease exercising muscle blood flow (37, 38, 43). Blood pressure is reduced and systemic vascular reactivity increased during the late FP, just prior to ovulation (145, 146). Both MC (147, 148) and OCs (148) alter sympathetic nerve activity during orthostatic stress in young healthy women.

Conversely, higher estradiol levels have been shown to lower cardiovascular responses to stress, most likely through effects on arterial wall tone and a decrease in beta-receptor sensitivity to catecholamines (149). Progesterone may act to increase cardiac excitability during the LP, as documented by a greater number and duration of episodes of paroxysmal supraventricular tachycardia (SVT) (150). A higher heart rate (HR) and rating of perceived exertion (RPE), at the same intensity of exercise, have been seen in many studies, suggesting greater cardiovascular strain during the LP. However, given the overriding adaptations of the cardiovascular system to exercise, it is not surprising to find few observed alterations in actual athletic performance, despite this increase in HR and RPE. Within the last decade, MC phase has been demonstrated to have an influence on timing of acute coronary events, with increases during the early FP (151, 152), possibly due to alterations in adrenergic control. Additionally, estrogen may have a beneficial effect on myocardial ischemia in women with coronary artery disease (CAD) (153). Given the increasing numbers of masters-level female athletes training and competing, some of these findings become more pertinent.

ORAL CONTRACEPTIVES AND CARDIOVASCULAR FUNCTION

Synthetic hormones also have significant cardiovascular effects. This was suggested by very early studies in subjects taking higher-dosage OCs (154, 155). Theoretically, such estrogen-mediated cardiovascular changes (e.g., increases in preload and stroke volume caused by increased PV) might augment effective cardiac

output (CO) and enhance performance. Higher CO has been found at rest and during exercise, with or without concomitant alterations in blood pressure and vascular volume, in women on several different higher-dosage combination pills (90, 156). It is likely that the newer formulations of synthetic progestins in OCs have less impact, as they have fewer side effects.

An echocardiographic study of 31 young women did not find any differences in resting left ventricular structure and function attributable to either hormonal variation over the MC, or with a combined OC pill (157), although estradiol levels were not measured directly. Studies have also found no significant alterations in cardiac index or pulmonary artery distensibility in inactive women taking OCs (154, 158). Heart rates at rest and during exercise generally do not change with administration of OCs; however, increases in *office* blood pressure (from 2 to 7 mmHg systolic and from 1 to 3 mmHg diastolic) have been documented during the first few months of use, diminishing over time and reversible on discontinuation of OCs. This finding has primarily been associated with low-dose monophasic OCs containing levonorgestrel, and not with triphasic preparations or OCs with the newer progestins. Detrimental changes in serum lipids are also minimized with the newer progestins, and are attenuated by regular exercise (159).

Exercise has an anticoagulation effect, and has been observed to act synergistically with some OCs to increase fibrinolytic activity. However, estrogen also enhances platelet aggregation and increases various coagulation factors, facilitating thrombogenesis; this effect is decreased with formulations containing 35 µg of EE or less (160). The newest progestins may be associated with a greater incidence of venous thromboembolism (161, 162), compounded in women with factor V Leiden thrombophilia. Because of the overall increased risk of cardiovascular disease in women using OCs, current prescribing guidelines should be carefully followed (163).

Respiratory Function and Athletic Performance

RESPIRATORY FUNCTION AND THE MENSTRUAL CYCLE

Minute ventilation (VE) and respiratory drive are generally increased under the influence of higher progesterone levels during LP (63, 140). Curiously, more recent research has suggested that estradiol mediates cyclic changes in angiogenesis and causes neovascularization in the pulmonary vascular bed, leading to decreased diffusing capacity (164). In one early study, a higher ventilatory rate during the LP was associated with greater oxygen demand, subjective dyspnea, and perceived exertion, and a decrease in maximal exercise response during the LP, but only in the nonathletes (62), suggesting that these responses may be overcome with increased training.

Conversely, another early study found no significant differences in $VO_{2\max}$, HR, time to exhaustion, or maximal ventilation between MC phases in five moderately trained women (165). Relative ventilatory threshold (VT), however, occurred at a significantly higher percentage of $VO_{2\max}$ in the early FP compared with the mid-luteal phase. Many others since then have documented either no MC variation in ventilation or mid-luteal elevation in resting minute ventilation (VE), but generally no changes in VE during submaximal exercise. These are well reviewed in a recent research publication (166). This latter group also did not find any MC effect on sensitivity to chemical stimuli,

either isocapnic hypoxic ventilatory response (iHVR) or hypercapnic ventilatory response. They concluded that any hormone-mediated influences are of insufficient magnitude to exceed the inherent variation in these chemosensitivity measures, and that feedforward and feedback mechanisms during exercise override the effects of naturally occurring changes in sex hormones. It appears that neither exercise VE nor physical performance in women at altitude is affected by MC phase (167), possibly for similar reasons, although some have found ventilatory alterations with higher work output, but no change in $\text{VO}_{2\text{max}}$ (168). Menstrual cycle-induced modulation of the ventilatory responses to exercise may be altered under acute hypobaric-hypoxic conditions (169).

There is also a documented link between female sex steroids and airway function: variously termed *perimenstrual asthma* (170), *premenstrual asthma (PMA)* (171), or *menstrual-linked asthma (MLA)*. Overall, an estimated 33–52% of asthmatic women report premenstrual worsening of asthma symptoms, and an additional 22% report their asthma to be more severe during menses (172). This can have significant clinical impact for female athletes with asthma (173–176).

The role of endogenous and exogenous hormones in asthma is postulated to involve various mechanisms (177). Airway hyperresponsiveness is exacerbated during premenstrual phase, due to the withdrawal of high levels of progesterone (a smooth muscle relaxant) and estrogen, or both. In terms of airway tone, estrogens increase the production of nitric oxide (NO), an endogenous bronchodilator, in the human bronchial epithelium (178). Estrogens also have anti-inflammatory effects, so they can modulate the increased airway wall inflammation found in MLA (179). Recently, a large cross-sectional population health questionnaire of women registered in an asthma database (540/1,260—43% response rate) found an 11% incidence of self-reported MLA (worse with menstruation) as well as an increased association with other autoimmune-type diseases, such as rheumatoid arthritis, eczema, and heart disease (180). In this study, women with MLA compared to women without MLA also reported more urgent/emergent asthma-related health-care visits per year, more emergency room visits, and higher asthma-related absenteeism, and used almost twice the number of β_2 agonist rescue doses per day.

The multifactorial etiology of MLA has implications for pharmacotherapy (181, 182). Short- and long-acting β_2 agonists are helpful (183), as are leukotriene antagonists (184). OC use has been reported to reduce the prevalence of current wheeze in women with a history of asthma (185), with a significant trend linked to duration of use (177); conversely, however, OC administration has also been found to exacerbate MLA (186).

ORAL CONTRACEPTIVES AND RESPIRATORY FUNCTION

Ventilatory changes similar to those of the LP of the MC, along with increased oxygen consumption (187), have also been documented in women taking the older higher-dose OCs, with some evidence for accommodation over time (188). However, studies using low-dose OCs did not show any associated ventilatory changes, but documented a slight decrement in aerobic capacity (189, 190). A recent pilot study of cardiorespiratory fitness in 12 endurance-trained runners suggested that OC use might attenuate MC-induced ventilatory changes as compared with non-OC users (191), thus allowing for more consistent cardiorespiratory fitness throughout the (artificial) cycle.

Substrate Metabolism

Estrogen and progesterone promote glycogen uptake and storage. Muscle biopsies have documented increased muscle glycogen storage during the LP (192), with greater fat use and oxidation at ovulation postulated to be due to higher estrogen levels. Glycogen repletion after exhaustive exercise has been noted to increase during the FP (68). Some investigators have found a lower RER during the LP, suggesting increased reliance on FFA for fuel (68, 139); however, most have not found this to be the case (113, 114, 193–195). This may be dependent on exercise intensity, which shifts the metabolic demands from aerobic to anaerobic fuel sources. For example, during the LP in nine eumenorrheic women, lower CHO use and oxidation rates, in association with greater lipid use and oxidation, were documented with 10-min submaximal treadmill exercise intensities at 35% and 60% $\text{VO}_{2\text{max}}$, but not at 75% $\text{VO}_{2\text{max}}$ (70).

Blood lactate generally increases in proportion to exercise intensity and the anaerobic contribution to glycolysis. A number of early studies with hormonal documentation suggested decreased lactate production during the LP (6, 78, 140), associated with an increase in endurance time (6, 139), but many others have not demonstrated any significant MC differences in lactate (144, 165, 193–197), or any enhanced LP performance. In a study of nine athletes, no differences were found in resting blood lactate or in running time to exhaustion, but recovery lactate levels were significantly lower during the LP (197). This suggests preferential metabolism of lipids and a reduction in CHO metabolism, but confounding factors are energy demand and nutritional status. A CHO-loading diet can supercompensate muscle glycogen stores in the early FP to values attained in the LP (198–200). There may also be mechanisms related to other hormones; for example, in a study of eight women performing three maximal exercise tests with simultaneous determination of lactate threshold during early and mid-FP, and mid-LP, there was no effect on lactate threshold, but there was a significant correlation between LT and the epinephrine breakpoint (201).

Theoretically, endurance performance could also be enhanced during the LP, through increased muscle glycogen stores (68, 192) and the effects of MC on CHO and lipid metabolism (84). Consistent with these findings, high estrogen levels during the LP are associated with sparing of muscle glycogen, in comparison to the FP (80). Hypothetically, such advantage might be lost in amenorrheic athletes (202). Dissociation of blood glucose homeostasis during exercise has also been shown to occur (78). An additional factor to consider is fluctuation with the time of day (203, 204). Contradictory research (especially for endurance performance) can be explained by differences in definitions and exercise protocols for endurance, variability in subject fitness, pre-exercise hydration, nutritional status, and initial muscle glycogen stores (205).

A different study found substrate oxidation and GH responses to exercise to be independent of MC phase and status (195). However, exercise-associated GH release is attenuated in amenorrheic athletes, due to decreased growth hormone-releasing hormone (GHRH) response to exercise, compared to eumenorrheic athletes. This is particularly relevant to adolescent female athletes, since the prevalence of athletic (hypothalamic) amenorrhea among these athletes is 4–20 times higher than the general population, most frequently also associated with energy deficiency. Reduced exercise-induced GH response in these athletes may be important, since it potentially indicates reduced effectiveness to training stimuli.

Glucose tolerance deteriorates during the LP (81), but it has been suggested that the cyclic changes in metabolic control are attributable to mechanisms other than the variations in insulin sensitivity, such as energy intake and caloric expenditure. Other investigators have not shown a substantial change in blood glucose over the MC (68, 195, 206). It may be more appropriate to look at E/P ratio (pmol/nmol) during the LP as well as the absolute magnitude of increase in ovarian hormones.

Substrate turnover studies using radioactive tracer isotopes have very precisely examined the kinetics of glucose and lipid metabolism, using glucose rate of appearance and glycerol appearance, respectively, and have found lower CHO utilization during the LP than the FP (207–209). Another group did not find any MC phase effect on glycerol or palmitate kinetics during 90 min of moderate exercise (210). This complex subject is well reviewed elsewhere (22).

Synthetic steroids in OCs appear to increase glucose flux, but not overall CHO and lipid oxidation rates, during moderate-intensity exercise, with greater metabolic effects than endogenous ovarian hormones (211, 212). Increased TG mobilization is not matched by an increase in oxidation but rather by reesterification. When CHO use predominates (such as in a postprandial state or during moderate-intensity exercise), whole-body plasma FFA turnover does not appear to be affected by either MC or OC, suggesting that the hierarchy of regulators of fatty acid oxidation is: exercise intensity > recent CHO nutrition > synthetic > endogenous ovarian hormones.

The interaction of OCs with energy metabolism systems is complex, and potential ergogenic or deleterious effects are not well defined. Although glucose production is determined primarily by insulin, other hormones, such as thyroxine, catecholamines (epinephrine and norepinephrine), cortisol, and growth hormone (GH), can have a counterregulatory effect. GH levels are increased by high doses of estrogen, hypoglycemia, and endogenous opioids, and decreased by progestins. At rest, fasting blood glucose, serum triglycerides, basal serum prolactin, and GH may be higher in women taking combination OCs. Other studies have shown lower blood glucose levels at rest and during exercise in OC users, and an increase in FFA levels during mild exercise (206). During prolonged exercise, women on OCs have been shown to have an elevated GH response to exercise and lower glucose and CHO use, with a shift more toward metabolism of FFA (165). Overall, there appears to be a possible glycogen-sparing effect of OCs, with the magnitude dependent on the specific chemical formulation. These alterations in substrate utilization, although minor, might still have an effect on aerobic performance, particularly in the elite female athlete.

Thermoregulation

Although some early studies did not show any cycle phase differences in response to short-term exercise or heat exposure (113, 114), others demonstrated that the normal hormonal fluctuations of an ovulatory MC may increase the propensity for heat stress during the LP (213–216). This can happen even at rest, i.e., with passive heat exposure (217), but can be somewhat ameliorated with acclimation and physical training (218). Short-term endurance training in previously untrained subjects improves heat loss responses by decreasing the threshold temperatures, with this effect occurring within a month of training, and disappearing within a month of cessation of training (219). The degree of

increase in sweating with training differs among body sites (chest, thigh, back, and forearm), and might be affected by MC phase.

The length of endurance performance in humans is related to a critical internal temperature (220), which is why precooling strategies before exercise, and in between bouts of exertion in the heat, are found to be physiologically advantageous (221, 222) and to decrease perceived exertion (223). A higher initial core body temperature during the LP may therefore theoretically increase the risk for heat accumulation when exercising in hot weather, thus decreasing time to fatigue (224, 225). An early study of seven eumenorrhic women (226) did not find any variation in sweat loss, sweat rate, and steady-state exercise metabolism rate during a 2-h cycle ergometer exercise at 30% of maximal O_2 concentration in the heat, during mid-FP, menstrual flow, midcycle (including ovulation), and mid-LP. However, this heat stress test was conducted *after* heat acclimation. Elevated skin temperature also has an adverse effect (227), as does a high starting ambient temperature (228).

Thresholds for shivering and sweating vary over the course of an ovulatory MC, with LP enhancement of the heat loss response, but with no associated impact on performance (113). Actual metabolic heat production does not seem to change from the FP to the LP, possibly due to decreased skin thermal conductance (115). A greater temperature threshold and larger gains for sweating have been reported during the LP compared with the FP (229), as well as a greater sweat rate (230). In the latter study, women exercising in a warm and humid environment (32°C and 80% humidity) with adequate water intake seemed able to adapt to the luteal phase increase of BBT, through reduced urinary volume and increased sweating rate.

Plasma volume (PV) dynamics during passive heat stress and exercise are also affected by MC phase (231). In women exercising at 80% $VO_{2\text{ peak}}$ on a cycle ergometer and passive heat stress of 50°C (dry environment), more fluid shifted out of the vasculature during the LP, and there was a greater fluid loss during exercise in the FP, yet the final PV was not different between phases. It has been proposed that the thermoregulatory vasodilator response is attenuated by increasing exercise-induced vasoconstrictor tone in proportion to exercise intensity (111). Therefore, under extreme conditions, and at high ambient temperature and humidity, there may be significant implications for women participating in prolonged endurance activities (e.g., marathons, ultramarathons, and triathlons) during the LP (216).

For unacclimatized game players, although the performance of intermittent, high-intensity shuttle running in the heat was unaffected by MC phase, it was influenced by OC use. Athletes on OC ran further (improved performance) on days 15–28 compared to days 1–14, despite higher rectal temperatures, HR, and GH, but lower plasma glucose. However, the numbers were small—7 women studied during the MC and 8 on OC (232). Interestingly, OCs may also impact thermoregulatory responses to exercise (229, 233–235) by increasing BBT by 0.2°C during the days on active OC pills, as compared with the days off OCs. There also appears to be an elevated HR during exercise (233). A potential advantage of OC use, then, is to make thermoregulatory responses more uniform across the cycle (at least for 21 days), thereby decreasing any cyclic performance alterations due to changes in core BBT. However, it appears that the administration of synthetic progestins in OCs still causes higher core BBT at rest and during exercise, similar to the influence of endogenous progesterone during the LP.

Physical Capacity, Strength, and Reproductive Hormones

PHYSICAL CAPACITY, STRENGTH, AND THE MENSTRUAL CYCLE

Physical work capacity during the MC has been investigated (196, 236, 237), but without hormonal documentation it is difficult to give much credence to these studies. However, there is a suggestion from these early studies that HR response to repetitive lifting and an isometric endurance lift were both greater by 7–10 beats per minute in the postovulatory phase, possibly as a result of the elevation in temperature (237, 238). Superior achievement in hip strength (flexion and extension) and standing broad jump during the premenstrual phase (1), isometric handgrip endurance of forearm contraction during the ovulatory phase (239), maximal voluntary contraction of handgrip during the FP (240), and handgrip strength during menses (241) have all been reported in the past, but most investigators have not noted any significant MC effects (3, 242–244). A prospective study with hormonal measurements did not find any variations in isokinetic strength of knee flexion and extension between FP and LP (245).

Muscle strength decreases with the onset of menopause, and it is thought that estrogen may have an inotropic effect on muscle strength (246, 247). Estradiol promotes secretion of GH, a known anabolic hormone. Some early research suggests that estrogen increases the ability of muscles to contract by about 10%, with a peak in strength just before ovulation (239, 248). Contrary to this finding, women being treated with gonadotropin injections for in vitro fertilization did not have any change in maximal strength and fatigability of the first dorsal interosseous muscle, despite the iatrogenic acute and massive fluctuations in estrogen (249). One group did not find any variation in either muscle strength or endurance with changing hormone levels across three MC phases (250). Others have documented maximal muscle contraction during the ovulatory phase, but without any significant changes in muscle strength, fatigability, or electrically stimulated contractile properties (251). Since then, a corroborating study (252) also found improved muscle strength at ovulation, with isokinetic peak torque knee flexors improved by ~9 N m and maximum voluntary isometric contraction knee extensors by ~5 N m.

There are no clear mechanisms for these noted effects, but a recent publication reviews this area in much more detail (253). In an elegant experiment sequentially assessing limb blood flow and skin and deep tissue temperature change during the MC, isometric endurance was shown to be lower during the end of the FP, due to (1) the cyclic variation in muscle temperature, (2) direct effects of the MC on circulation, and (3) direct effects of the MC on muscle (254). More recent work has attempted to link changes in electromyography variables over the MC to increased muscle fatigue (255, 256).

Progesterone does not appear to have substantial effects on either muscle strength or function, but testosterone definitely has anabolic actions in females (257). Although not systematically studied, testosterone varies over the MC under the control of luteinizing hormone (LH), and also from peripheral conversion of androstenedione, with very little actually produced by the ovaries. Concentrations are lowest in the early FP, highest just prior to or at the time of ovulation, and then fall during LP (258). Dehydroepiandrosterone (DHEA) and its sulfoconjugate DHEA-S, which are secreted by the adrenal glands, as well

as androstenedione, secreted by adrenals and ovaries, are all of physiological importance in women (259) and peak prior to or at the time of ovulation (35). Exhaustive physical exercise (an ergocycle test at 75% of their $\text{VO}_{2\text{max}}$ until exhaustion) induces an increase in circulating DHEA-S and testosterone in young women (260, 261).

Studies looking at effects of resistance exercise on circulation of androgens in women are still contradictory: both estradiol and progesterone increase after a single bout of resistance exercise in mid-LP, but not in early FP (259). These different responses have prompted some investigators (262) to suggest periodization of training cycles during different phases of the MC to maximize anabolic effects. An early study modeled *menstrual cycle-triggered training* (MCTT) in seven healthy women—every second day in the FP and once per week in the LP—vs. *regular* training periodization (every third day over the entire MC without regard for cycle phase) for a total of 4 weeks (263). Trainability of isokinetic strength of one-leg knee extensor muscles was slightly greater during FP, compared to when the respective leg was trained for 4 weeks without regard for the cycle phase (33% increase vs. 13% increase). It is known that estrogen may govern the regulation of a number of downstream genes and molecular targets (264), improving the intrinsic quality of skeletal muscles by enabling fibers to generate force through myosin binding to actin during contraction (265). Estrogen may reduce protein catabolism; in contrast, progesterone influences amino acid oxidation and protein degradation with increased protein catabolism, greater in LP than in FP, both at rest and during exercise.

ORAL CONTRACEPTIVES AND STRENGTH

At one time it was hypothesized that the androgenic component of the OCs might be ergogenic and help to increase muscle strength. In 1987 the International Olympic Committee (IOC) actually contemplated banning compounds containing norethindrone, a more androgenic progestin. Fortunately, this situation was successfully challenged and overruled. An early study suggested that OCs might have an effect on static muscle function (240), but to date there is no consensus on this matter. Other studies did not find any significant MC phase differences or changes with OCs in various strength parameters (239, 245, 248, 266–268), even with OCs containing progestins of higher androgenicity (269). Similarly, a prospective study of strength and torque production in collegiate women softball and water polo athletes participating in a 12-week strength development program did not demonstrate any positive impact of taking combination OCs (270).

The thermogenic effect of progesterone (229), which may have a detrimental impact on forearm isometric endurance and muscle force, is minimized in athletes taking OCs (240). Some researchers believe that increased GH response to exercise in women on OCs would potentiate the effects of a training program (271). However, recent work has questioned the effect of OC administration on serum androstenedione and the bioavailability of testosterone, and suggested a potential negative impact on the synthesis and breakdown of myofibrillar proteins (272). This may also be related to the effect of MC and OCs to attenuate delayed onset muscle soreness (DOMS) 48 h post-exercise (273), through a potential protective role of estrogen in decreasing production of prostaglandins (274).

Aerobic Capacity and Reproductive Hormones

AEROBIC CAPACITY AND THE MENSTRUAL CYCLE

For the most part, aerobic performance, as measured by maximal oxygen capacity ($\text{VO}_{2\text{max}}$) and submaximal exercise responses, does not change significantly during an ovulatory MC (140, 144, 165, 168, 194, 201, 245, 275). Despite theoretical metabolic advantages, mostly to do with substrate metabolism, or enhanced glycogen stores during the LP (68), only a few studies have suggested enhanced endurance performance (68, 139).

Earlier work, with hormonal documentation of MC phase, is well summarized elsewhere (253), with predominately comparable findings. For example, a comparison of 8 eumenorrheic and 8 amenorrheic runners doing one maximal and one submaximal treadmill run (40 min at 80% $\text{VO}_{2\text{max}}$ treadmill run), during early FP and mid-LP (confirmed by urinary and serum hormone levels), did not find any phase or group differences in oxygen uptake, minute ventilation, HR, RER, RPE, time to fatigue (maximal), or plasma lactate (194). A few small studies have measured a slight decrement in aerobic capacity (245) and exercise efficiency (114) during high-intensity exercise in the LP. The latter paralleled a 5.2% increase in oxygen consumption, a 5.6% increase in metabolic rate, and a decrease in net efficiency of 5.3% (114). Testing protocols differ, generally without any standardization of other variables, such as circadian rhythms, nutritional and hydration status, glycogen stores, caffeine ingestion, and exercise during the 24 h prior to testing. In all likelihood, these confounding factors contribute more to changes or enhancement of performance than hormonal influences of either MC or OC. In addition, running economy (RE), defined as the rate of oxygen consumption (VO_2) during a given submaximal steady-state running speed, has been suggested as a better measure of performance than $\text{VO}_{2\text{max}}$ (25).

Investigations of work performance and MC have looked at physiological and psychological determinants, temperature, pain perception, and so on (237, 276, 277). There is an entire body of literature in this area (278). Most reports are based on surveys or cross-sectional data, without hormonal verification of cycle phase, but are thought-provoking, nonetheless.

In a study of MC effects on exercise in sedentary young women, 14 subjects with a peak $\text{VO}_{2\text{max}} < 45$ mL/kg/min were subjected to an incremental test to exhaustion and steady-state submaximal cycle ergometer during two phases (275). Time to exhaustion, maximum power output and total work done, absolute $\text{VO}_{2\text{peak}}$, VE, respiratory frequency and HR, and lactate and lactate threshold did not differ between phases. However, as workload increased, plasma lactate, carbon dioxide output, and RER were all lower during LP, while oxygen uptake was higher. During the steady-state tests at submaximal intensities (at workloads of 25% and 75% of menstrual cycle phase-specific $\text{VO}_{2\text{peak}}$), findings were similar. Exercise performance did not change between MC phases, but metabolic responses suggested greater dependence on fat as the energy source during LP.

ORAL CONTRACEPTIVES AND AEROBIC CAPACITY

Performance enhancement has been reported anecdotally in 8% of women on OCs (2), but conversely a detrimental impact on $\text{VO}_{2\text{max}}$ was found after 2 months on a higher-dose

OC, reversible on discontinuation (279). Women taking a low-dose monophasic OC (0.4 mg norethindrone) for 6 months had a 7% decrease in maximal oxygen uptake and 8% deterioration in exercise performance (as measured by oxygen pulse or volume of oxygen consumed per heart beat) (189). In seven women taking a lower-dose triphasic OC for 2 months, compared with a similar group on placebo, there was a smaller decrement in $\text{VO}_{2\text{max}}$ (4.7%) not evident in the placebo group, who experienced a 1.5% increase over the same time period (190). Neither anaerobic capacity nor aerobic endurance was altered in this particular study. In another investigation of ten moderately trained women, prospectively randomized to placebo ($n=3$) or an OC containing 1 mg norethindrone and 35 μg ethinyl estradiol ($n=7$) for 21 days, neither the cycle phase nor low-dose OC had any significant adverse effects on ventilatory measures or performance during a maximal treadmill test or endurance run (280). A separate research group has been studying *within OC* differences: 13 female cyclists (mean peak VO_2 53.0 ± 5.6 mL/kg/min) had alterations in several physiological variables between the tests done during pill consumption, and during early and late withdrawal phases, but had no performance differences in a 1-h cycle endurance test (281).

Others have looked at aerobic capacity during various MC phases (10 women) compared with low-dose OC (5 women) (282). Again, there were no differences between mid-FP and late LP or OC test results at two time points. However, there was altered metabolism *between groups* (plasma ammonium was higher in the non-OC group), and *within the OC group* (blood lactate and ammonium were higher within the first week compared with the second week on OCs), believed to represent differences in substrate metabolism. Another study of ten women on a monophasic OC found a somewhat lower (3–5.8%) decrease in VO_2 when participants were on early and late OC use, compared to off OC, during a 12-min treadmill run (4 min each at 7, 8, and 9 km/h) (283). This was actually associated with an improved running economy.

More contemporary work investigated peak exercise capacity in six moderately active women during the FP and LP, and after 4 months on the same triphasic OC (284). There were no significant MC changes, but all subjects experienced decrements in $\text{VO}_{2\text{peak}}$ (average: 11% L/min; 13% mL/kg/min), as well as decreased time to peak exercise (14%) and peak power output (8%). These were not different between tests during OC and the week off OC (low and high ethinyl estradiol levels), suggesting a persistence of OC effects. Finally, contrary to the above findings, one study comparing two monophasic OCs with differing dosages of synthetic progestins (single-blind, randomized, counterbalanced, crossover study) suggested a higher $\text{VO}_{2\text{peak}}$ with usage of OCs for more than 6 months, with the effect more pronounced in the higher progestin OC (285). Obviously, much more accurate research is needed.

Anaerobic Capacity and Reproductive Hormones

ANAEROBIC CAPACITY AND THE MENSTRUAL CYCLE

Anaerobic capacity refers to the maximal amount of adenosine triphosphate (ATP) resynthesized via anaerobic metabolism during a specific bout of short-duration exercise. There is a paucity of research in this area, but for the most part there is either no

difference in anaerobic power output during the different phases of the MC, or greater anaerobic capacity and peak power during the LP. Older studies suggested no MC effects on an anaerobic endurance test on a cycle ergometer (no hormonal analyses of cycle phase) (286) or a 600-yd run test, with postexercise blood tests (287). Another investigation without hormonal verification measured mean power output and peak power output during a modified Wingate test as greater in MF than in either ML or menstrual phases (288). Others have reported poorer performances during the menstrual phase in a 50-m swim exercise (289) and standing broad jump (1).

It appears that the activity and fitness level of the women may be a factor. Previous studies (using cycle history only) have shown some decrement in performance during menstruation, but *more active women* (collegiate athletes) (290) were less influenced by MC phase than women defined as *fairly active* (11% less anaerobic capacity and 6% less anaerobic power in FP than in LP) (291) or healthy *active women* (242). Fifteen sedentary females (ages 19–23 years) performed a Wingate test on a Monark 818E ergometer with 75 g/kg load on the 7th, 14th, and 21st cycle days randomly. There were no differences in peak power, mean power, and fatigue index, nor any correlations with estradiol or progesterone levels (292). Studies of fit athletes ($\text{VO}_{2\text{max}} > 50 \text{ mL/kg/min}$) with more precise hormonal documentation showed no changes in the anaerobic treadmill speed test or AST (subjects run at 3.52 m/s at a 20% grade until unable to maintain the set pace—average times approx. 28–29 s) between FP and LP (245).

A small study of six women doing ten 6-s sprints on a cycle ergometer during the FP and LP revealed no differences in peak power, oxygen intake, or capillary blood lactate. However, average work was greater in LP, as was the recovery VO_2 between sprints (293). A different protocol tested eight females doing repeated 30-s sprints with 2-min rest periods at three phases—FP, just prior to ovulation (midcycle), and LP (high endogenous hormones). There were no measured changes in peak power output, recovery, lactate, blood pH and ammonia, or estimated plasma volume (294). Maximal accumulated oxygen deficit (MAOD) was assessed in 12 women with an average $\text{VO}_{2\text{max}}$ of 34.9 mL/kg/min, during repeated submaximal cycling exercise at 50%, 60%, 70%, and 80% of $\text{VO}_{2\text{max}}$ for 10 min, followed by repeated sprint cycling for three times at 120% of $\text{VO}_{2\text{max}}$, with 20-min rest between sprints. Again, no significant differences were found in any physiological parameters (295).

ORAL CONTRACEPTIVES AND ANAEROBIC CAPACITY

In a model of performance using OCs to simulate the MC, five female rowers were tested for anaerobic power (10-s all-out effort) and anaerobic capacity (1,000-m row) at two time points in each of three OC cycles: on OC days 16–18 (high exogenous estrogen and progesterone levels) and OC days 26–28 (i.e., during the week off OCs) (296). Peak power output was higher, and rowing performance better, at the low (exogenous) hormone levels. Pre- and postexercise glucose concentrations, plasma resting and postexercise TG were also lower at this time. Endogenous hormones were not significantly different between phases.

A study using a multijump test, squatting jump test, and force velocity test on a cycle ergometer saw no differences between MC phases, consistent throughout three different time phases in a normal cycle—menstruation (days 1–4), mid-FP (days 7–9), and mid-

LP (days 19–21) (confirmed by serum progesterone)—or three testing times in a subsequent OC cycle; however, these particular tests might not have adequately stressed the lactic acid system (297). One of the largest studies examining the effects of reproductive hormones on anaerobic performance tested seven women with normal menstrual cycles during the menses and LP (documented by urinary LH levels), and 17 women on OCs (OCs) (268). No significant cycle phase differences were found in the women with regular cycles, or between active-pill (high hormone) and withdrawal phases in the OC users on the Wingate cycle test and associated parameters (such as peak power, anaerobic capacity, and power decline), or in power for the Margaria-Kalamen staircase test. Another group compared nine OC users and eight normally menstruating subjects (NM) in an all-out 30-s sprint on a treadmill, and did not find any difference in peak and mean power output (298). However, the integrated GH was greater in the OC group, suggesting that the high-androgenicity OCs in this study caused a higher GH response to sprinting. Again, this begs the question of some type of anabolic effect of OCs through the effects on GH secretion during exercise.

Of note in many of these studies, however, is that the women are on different OCs, both multiphasic and monophasic. One group of investigators (282) studied five recreationally active women doing an intermittent running protocol (20-s sprints of increasing speeds with 100-s passive recovery until fatigue). Performance was the same after 2 weeks of OC, but higher lactate levels were found during the first week of testing, suggesting a possible alteration in metabolism with OC. Yet in a subsequent study of nine untrained women doing intermittent treadmill running followed by a run to exhaustion, there were no differences in performance or in energy metabolism between tests conducted during menstruation and after OC for 19–21 days (299). In conjunction with anaerobic performance, the anaerobic threshold (300) appears to be affected by usage of low-dose OCs.

Female Reproductive Hormones and Overall Sport Performance

MENSTRUAL CYCLE AND OVERALL SPORT PERFORMANCE

Optimal sport-specific performance is the primary goal of every athlete. Success in events such as long-distance running and cycling is more dependent on aerobic capacity, whereas sprinting activities require anaerobic capacity. Weightlifting obviously necessitates great strength, while fluid retention might be critical for weight-dependent sports as well as for those where the body has to be lifted off the ground (high jumping, gymnastics, etc.). Early studies were based on subjective feelings, and relied on subject recall of menstrual status (a substantial source of error and bias), without measurement of hormone levels (301, 302). Many athletes described a decrement in performance during the premenstrual and menstrual phases, while others reported improved performance (303, 304). Perimenstrual symptoms, such as abdominal or low back pain, fatigue, or nervousness during menstruation, may also come into play. Survey questionnaires of NCAA athletes (305) and 241 Turkish athletes (306) did not yield any clarifications. Nevertheless, gold medals have been won and world records set at all different phases of the menstrual cycle.

Some previous studies have addressed MC and performance in specific sports (9). For example, swimming speed was found to be highest during menstruation and lowest during the premenstrual period (289, 307); cross-country skiers performed better in the early LP and in the late FP (308); and in runners, no effect of MC phase on aerobic parameters or perceived exertion was identified (194). A more recent study of performance in a cycling time trial found it to be enhanced during the late FP, with the preovulatory surge in estradiol and suppressed progesterone (309).

Several contemporary studies on rowers should be reassuring for elite female athletes in this sport: 11 rowers were tested on a 1-h ergometer exercise at 70% $\text{VO}_{2\text{max}}$ in the FP and LP, finding no effects on energy expenditure, heart rate, blood lactate, or substrate oxidation (RER) (310). Other work by this same group compared 24 rowers—8 competitive cyclic athletes, 7 recreationally trained cyclic athletes, and 9 recreationally trained rowers taking OC during 2 incremental tests to voluntary exhaustion. Higher values of VE/VCO_2 were documented during the LP at both intensities in the cyclic athletes, compared with FP in the group on OCs (not really phases then), but no other significant differences were found, particularly not in any performance variable (311).

ORAL CONTRACEPTIVES AND OVERALL SPORT PERFORMANCE

The question of whether OCs help or hinder overall athletic performance still remains largely unanswered (21). Studies have documented fewer musculoskeletal injuries in women taking OCs, likely secondary to amelioration of PMS symptoms and dysmenorrhea (312–314). Presently, extended- and continuous-use oral contraceptive regimens may give even better cycle control and reduce performance-disrupting dysmenorrhea and PMS symptoms, as well as the more severe premenstrual dysphoric disorder (PMDD). Current formulations include Seasonale, with 91 consecutive days of active OCs (315), and Lybrel (levonorgestrel/ethinyl estradiol), with continuous active OCs for 1 year. Both regimens are safe and efficacious (316, 317), but current usage by athletes and any effects on performance are unknown.

As mentioned above, endurance capacity (1-h rowing ergometer test at an intensity of 70% $\text{VO}_{2\text{max}}$) was not significantly different in eight rowers on a monophasic OC, during the active-pill and non-active-pill phases (318). Another study did not demonstrate any effects on an endurance test in women taking a triphasic OC, during similar testing phases (296). Several different performance tests were examined in ten female team sport players using a monophasic OC: during pill consumption and during early and late withdrawal phases (319). Only reactive strength during a drop jump landing from the 45-cm height varied; it was higher during the OC consumption phase. The authors postulated a possible implication of hormones on neuromuscular timing and the stretch-shortening cycle, which are both important for sprinting and jumping performance.

The most recent research in this area raises some other interesting questions. Swimmers performed a 200-m time trial in three phases of a monophasic OC: during consumption and both early and late withdrawal phases. Swim times were not different, but there was decreased blood lactate and increased pH during the withdrawal phase, postulated to occur because of increase in fluid retention, plasma volume, and cellular

alkalosis (320). There are potential implications for coaches and elite-level athletes when basing training programs on lactate levels.

FEMALE REPRODUCTIVE HORMONES AND SPORTS INJURIES

ACL Injuries

The incidence of sports injuries in females is nearly tenfold that of males. This has been found in ball games, running, biking, military training, and other physical activities. Noncontact injuries to the anterior cruciate ligament (ACL) occur at a rate 4–6 times that of males participating in the same jumping and cutting sports, such as soccer and basketball. Postulated mechanisms that may be impacted by the female hormones (estrogen, progesterone, and relaxin) include anatomical and biomechanical factors, neuromuscular control, ligament laxity, knee instability, and others; these have been reviewed in detail by ACL research groups (321–323) and even by the IOC Medical Commission (324). Most studies focus on knee injuries and the changes in ACL laxity throughout the MC. Estrogen and progesterone are believed to affect tensile properties of ligaments (325) as well as neuromuscular function. Estrogen has potential mechanical effects on the collagen-rich structures that contribute to joint stability. For example, increases in knee joint laxity at the time of ovulation have a detrimental effect on mediolateral knee joint loading during cutting maneuvers, which is subject specific (326). This is thought to lead to increased joint loads (327).

Both estrogen and progesterone receptors have been found in synoviocytes in the lining of the knee, fibroblasts in the stroma of the ACL (328), and cells in the blood vessel walls of the ligaments in both men and women (328–330). There are also relaxin receptors in the ACL (331, 332). The importance of these findings and any causal relationship to an increased incidence of ACL injury is still under debate, although a recent prospective study monitored 143 NCAA Division I athletes over their 4-year career. Those elite female athletes sustaining ACL injury had elevated serum relaxin levels, and therefore may have been at increased risk (333).

Much of the early information came from animal studies (334), later replicated in humans (335). The results of in vivo assessment of knee joint laxity in humans are somewhat mixed (336–338), documenting either no changes with MC or, similar to animal model studies, increased compliance at times of high estrogen (i.e., ovulation) (338) and/or during the LP (339, 340). No changes in ACL laxity were found among three phases of the MC in high school-aged girls (341), including at the time of ovulation (determined with the OvuQuick™ One-Step Ovulation Predictor (Quidel Corp., San Diego, CA)). However, because ovulation lasts 24–36 h in a normally cycling female, this phase in the menstrual cycle is more correctly referred to as *near ovulation* to reflect this.

Various investigators have attempted to characterize high- and low-risk MC phases (Table 1). An early study, using only interviewer-administered questionnaires to 28 women, reported a significant statistical association between the MC and likelihood of ACL injuries (greater during the ovulatory phase and less during the FP in 28 women) (342). Interestingly, a group of researchers reanalyzed the data and found a different chi-square value, which was not significant (343). The authors responded appropriately with

Table 1
Hormonal risk factors for ACL injury during the menstrual cycle

<i>Study design</i>	<i>Injuries/subject</i>	<i>High-risk phase</i>	<i>Hormones</i>	<i>References</i>
Questionnaire	28	Preovulatory ^a	No	(342)
Case-series	65	Preovulatory	Urine	(344)
Prospective cohort	46/69	Menstrual	No	(345)
Prospective cohort	17/23	Late LP	No	(346)
Unmatched case–control	37/38	FP	Salivary	(335)
Case-series	83	Preovulatory	No	(348)
Matched case–control	46/91	Preovulatory	Serum	(350)
Descriptive	18/37	Ovulatory	No	(349)
Matched case–control	93/186	Preovulatory	No	(351)

FP follicular phase; *LP* luteal phase

^aLater found not to be significantly different (see ref. (343))

a retraction, but maintained that there were fewer injuries than expected in the FP and more in the ovulatory phase. These original investigators published a later study using urinary hormone levels for cycle phase verifications, and validated their hypothesis of greater than expected incidence of ACL injuries during midcycle (ovulatory phase), but less than expected during the LP (344). Other researchers have also shown the risk to be greater in the premenstrual period or late LP (345, 346), but there are little objective data using large numbers of subjects. There appears to be some periodicity to these serious injuries, regardless of OC status, but this is dependent on the method of analysis (347, 348). Teenaged female athletes have been shown to be more prone to ACL injuries during the FP (341, 349). More than half of the respondents in the first study also reported feelings of diminished athletic performance during menstruation, which is in agreement with the findings of greater variability during a cyclical athletic movement. Several studies in skiers found an increased incidence during the preovulatory phase: a matched case–control study of alpine skiers (with verification of cycle phase) (350) and a self-reported questionnaire (351).

Tissue qualities are also affected by estradiol concentrations (352). Using hormonal measurements, investigators found lower musculotendinous stiffness (MTS) during cyclical hopping tests at the ovulatory phase, in contrast to the menstrual phase and FP (353). They postulated that the resultant increase in compliance leads to greater reliance on reflexive responses from the contractile component of the muscle, due to decreased contribution from passive elastic structures. This would increase electromechanical delay and therefore risk of injury. A reduction in knee stiffness of approximately 17% at ovulation was associated with increased knee laxity in another study (354). Such MC changes have not been found in other tissues, such as the gastrocnemius (355), patellar tendon (356), or Achilles tendon (357). Some researchers have found no changes in muscle and tendon properties in knee extensors and plantar flexors (358). More recent work has examined variations in varus/valgus and internal/external rotational knee lax-

ity (359). Muscle stiffness of, and neuromechanics of, the hamstrings have also been studied at different MC phases and with OCs (360–362).

Various neuromuscular performance characteristics, such as gender differences in muscle strength, recruitment order, and peak torque production, also likely contribute to the higher ACL injury rate in women (363). Some have found no variation across MC (337, 364), while others have documented alterations in neuromuscular control patterns during landing or postural control (365). Knee joint kinesthesia, as measured by performance in a square-hop task, improved at ovulation when compared to the pre- and menstruation phases of the cycle (366). Sympathetic neural responses to upright tilt have also been noted (367). Given that muscle forces influence knee joint dynamic restraint, neuromuscular control of the lower limb musculature will dictate the propensity for ACL injury (368). A previous study reported modified co-contraction patterns of the gluteus maximus and semitendinosus at different stages of the MC, with increased synchronicity of the contraction between the two muscle groups around ovulation (369). Knee joint position sense accuracy decreased during menses (370), but others have found no differences in knee or hip loading, using a variety of measures, during across MC phases or with OCs (371). Currently there is much ongoing sophisticated research on landing biomechanics (372).

Additionally, PMS, with its effects on balance and motion perception, may be important. Motor skill performance may decrease during the premenstrual phase (121). A connection between the MC cycle and soccer injuries (313), with fewer injuries in women on OCs (314), has been reported, but these authors used a faulty calendar system for calculating MC phase, which may have underestimated the incidence of injuries.

Other mechanisms of increased susceptibility to injury during specific MC phases (and/or while taking OCs) include variations in muscle strength (266), neuroendocrine activation (373), motoneuron excitability and anterior tibial displacement (374), and, most recently, tibial acceleration variability (359, 375). Risks can be ameliorated with various neuromuscular training programs, including plyometrics, proprioceptive exercises, and correction of dynamic valgus (drop jumps). Improved knowledge of predisposing factors will also facilitate screening of athletes at increased jeopardy of sustaining ACL injuries (376).

Keeping in mind the above mechanisms, OCs use may stabilize the hormonal milieu, and thus may function to either passively or actively stabilize the knee joint (377, 378). To this end, OC use was found to influence injury risk in some (348), but not all, studies (379), in addition to neuromuscular properties (378, 380) and sporting performance (319). During steady-state running, female athletes who do not take OCs exhibit greater variability in gross mediolateral acceleration at the proximal tibia during menstruation compared to during ovulation (378). Given that mediolateral tibial acceleration can be used to represent varus/valgus movement of the knee, and that valgus collapse is a commonly cited gender-specific risk factor for ACL injury, further research is needed to examine the ideal level of movement variability in this population around the time of menstruation. While OC ingestion may diminish the somewhat significant association between ACL injury and the ovulatory phase, there is no consensus on any proven protective effects (351).

Much work remains to be done in the investigation of sex hormones and anterior knee laxity. Variations in the MC, with different hormonal profiles, will also have an

impact (381). There is great difficulty with validated outcome measures using serum urine or salivary progesterone in combination with MC data (382). In addition, there may be a phase delay of hormonal effects, or they may be more cumulative and chronic, rather than acute. There are many excellent reviews on this important topic (323, 338, 383–385).

SUMMARY

Although understanding of the unique physiology of the female athlete has increased, there are still many unanswered questions. Both endogenous and exogenous female sex steroids can influence numerous cardiovascular, respiratory, and metabolic variables, but most likely have minimal impact on the athletic ability of most recreational athletes. In elite athletes, however, even a statistically nonsignificant change can mean the critical difference between first and second place. There appears to be individual variability in the response of different performance parameters to MC phase and/or OC administration. In particular, there may be subtle alterations in substrate metabolism, and increased susceptibility to heat stress under conditions of high heat and humidity. The latter is most important for female athletes competing in endurance events. Similarly, MC phase and OC use may have some implications in terms of management of MLA, prevention of ACL tears, and the response to periodized strength and endurance training. These areas warrant further scientific investigation.

Although the majority of research to date suggests that regularly menstruating female athletes do not need to adjust their MC to maximize performance, it is difficult to extrapolate controlled laboratory findings from a study population to an individual competitor on the playing field. It is critical for each woman to monitor her own physiological responses and to *listen to her body*. For women with menstrual dysfunction and/or the need for contraception, OCs may provide a stable hormonal milieu for training and competition, and predictable onset of menstrual bleeding. Potential side effects (and any concomitant impact on performance) can be minimized with the lower-dose triphasic pills and the newer progestins. Continuous pills are another promising new option. Further large-scale, prospective, randomized clinical trials are needed on trained athletes, using accurate hormonal measurements for verification of MC phase, to further elucidate the short- and long-term effects of cycle phase and OCs in exercising women.

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17

Exercise and Pregnancy: Hormonal Considerations

R. Carlton Bessinger, PHD

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INTRODUCTION

This chapter summarizes information related to exercise during pregnancy and endocrine responses to that exercise. The hormones addressed in this review are a combination of those commonly investigated in exercise science and sports medicine research (1) and those identified from a Medline search using the terms exercise, physical activity, pregnancy, gestation, hormones, and individual hormone names. Hormones are typically listed by the major site of origin and include hormones released from the hypothalamus, pituitary gland, adrenals, pancreas, adipose tissue, thyroid, parathyroid, liver, and placenta. The hypothalamus secretes hormones that stimulate or suppress the release of hormones in the pituitary gland. For example, corticotropin-releasing hormone (CRH) stimulates adrenocorticotrophic hormone (ACTH) release from the anterior pituitary gland. In turn, ACTH controls the secretion of hormones from the adrenal cortex, including cortisol. In addition to ACTH, other pituitary hormones

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include growth hormone, thyroid-stimulating hormone (TSH) which stimulates the thyroid gland to release triiodothyronine and thyroxine, prolactin, vasopressin or antidiuretic hormone (ADH), and B-endorphin. Adrenal hormones, other than cortisol previously mentioned, include epinephrine and norepinephrine. The pancreas secretes insulin and glucagon. Leptin, a hormone associated with body weight/body fat regulation, is mainly produced in adipose tissue. The parathyroid gland produces parathyroid hormone (PTH). The liver produces 25-hydroxyvitamin D and it is converted to 1,25-dihydroxyvitamin D, primarily in the kidneys. However, 25-hydroxyvitamin D is considered the best measure of vitamin D status. Placental hormones include estrogens, progesterone, insulin-like growth factors 1 (IGF-1) and 2 (IGF-2), human chorionic gonadotropin (hCG), placental growth hormone (PGH), human placental lactogen (HPL), and relaxin.

With a number of hormones, exercise studies during pregnancy are limited or are nonexistent. Therefore, there may be an occasional reference to studies involving nonpregnant women or men. Finally, there are several factors that may affect the measurement and assessment of hormones and therefore the interpretation of results when trying to evaluate a particular study or compare different studies. These factors include the exercise intensity and duration, mode of activity, and environmental conditions; the timing, method, site of specimen collection, subject posture, and the analytical assays used for measuring the hormones; age, nutritional status, training status, and life stress; and genetics. Consideration of these factors should lead to more consistent research findings which in turn should improve interpretation of those findings. For discussion of these and other factors that may influence the hormonal responses to exercise, the reader is referred to the following references (1–3).

HYPOTHALAMUS

Corticotropin-Releasing Hormone

CRH-stimulated ACTH is released from the anterior pituitary gland (4). While the hypothalamus is a source of CRH, CRH is also synthesized by the placenta during pregnancy and the placental CRH appears functionally the same as CRH produced in the hypothalamus (5). CRH increases as pregnancy progresses (6) and placental synthesis of CRH appears to account for the increased blood levels during pregnancy (7). Elevated levels are associated with increased risk for preterm delivery (7). Exercise in men consisting of a 1 h ride on a cycle ergometer at 70% maximal oxygen consumption increased CRH (8). Since exercise increases CRH in males during moderate prolonged exercise, the combination of pregnancy and exercise may have an additive effect on levels of CRH. Studies examining the effect of exercise during pregnancy on CRH concentrations are needed.

PITUITARY

Growth Hormone (GH)

GH stimulates lipolysis (9) and gluconeogenesis (10). GH levels appear to increase during pregnancy (11, 12) and GH, especially during late gestation, appears to be a

combination of pituitary GH and a placental derivative (13, 14). Functionally, the placental GH should act as a GH agonist sharing most of its biological properties, since the placental derivative has been found to bind to hepatic GH receptors with an affinity similar to pituitary GH (14, 15). The overall role of GH, along with other hormones, may be to regulate metabolism to ensure that the fetus is provided with adequate glucose and amino acids (16).

As with a number of hormones, little information exists as to how exercise during pregnancy affects GH. One study (17) during pregnancy, using a graded exercise protocol and lasting an average of approximately 13 min with a peak intensity of about 79 W, found no significant changes in GH. The short period of exercise and lower intensity may explain the findings since increases in GH are related to exercise duration and work load (18). In another study (11), circulating levels of GH increased as a result of exercise during pregnancy. There was a greater increase at 14 weeks postpartum compared to 22 and 33 weeks of pregnancy. The greater increase at postpartum may be explained by the difference in resting values. While there were no differences in GH values after exercise, resting values were 50% lower at 14 weeks postpartum compared to 33 weeks gestation. The limited data preclude any conclusions regarding the effect of exercise during pregnancy on GH.

Thyroid-Stimulating Hormone

TSH stimulates the thyroid gland to release triiodothyronine (T3) and thyroxine (T4) (9). One study (19) investigated TSH's relationship with hCG in 9,562 women during the first and second trimesters. The researchers concluded that centile levels of TSH were associated with TSH being inhibited by hCG at any level of hCG. These results were interpreted as hCG itself possibly being the primary substance responsible for stimulation of the thyroid gland. In another study (20), higher TSH levels were associated with increased risk of child loss. However, the risk was described as small. There does not appear to be a clear explanation for these findings. Research, in general, suggests that TSH levels rise during short-term submaximal exercise (2). However, it is unclear if TSH levels increase during pregnancy as a result of exercise, and therefore it is unclear as to any potential effects.

Adrenocorticotrophic Hormone

ACTH controls the secretion of hormones from the adrenal cortex (21). ACTH increases during pregnancy but uncertainty exists about the possible causes as to why (7). Placental production may account for some of the increase. ACTH may be associated with the high levels of cortisol seen during pregnancy (7). ACTH levels did not change in 14 male subjects exercising for 1 h on a bicycle ergometer (22). However, 11 of the subjects performed a graded exercise on the bicycle ergometer until exhaustion and ACTH levels rose along with increasing intensity. There was a significant positive relationship with beta-endorphin. Limited research (23) discussed below under the "Endorphins" section suggests an increase in beta-endorphin as a result of exercise during pregnancy. Therefore, ACTH levels might be expected to increase as a result of exercise during pregnancy. In nonpregnant subjects, short-term submaximal exercise is

associated with increases in ACTH (2). If ACTH did respond similarly to beta-endorphin, then one might expect transient changes with no meaningful effects.

Prolactin

In women, prolactin stimulates milk production (24) and prolactin concentrations increase during late pregnancy (25). In one study (25), conducted at an average of 35 weeks, ten pregnant women exercised for 10 min with the average heart rate rising from 93 to 157 beats/min by the end of exercise. While prolactin values did not change during exercise, values increased approximately 66 ng/mL (45%) during 30 min after exercise. Prolactin concentrations remained high for an hour or more. A later study (26) reported that exercise at 60% maximal oxygen uptake (VO_2 max) resulted in prolactin levels that were approximately 23% lower during water exercise compared to immersion alone in lactating postpartum women. Although the meaning of this decrease is unclear, it appears that any changes in prolactin are small and/or temporary and would not affect women during pregnancy or lactation after delivery.

Vasopressin or Antidiuretic Hormone

Vasopressin promotes water uptake by the kidneys and increases blood pressure (4). In 37 pregnant women with normal blood pressure and 15 women with gestational high blood pressure, vasopressin concentrations decreased by 36 and 59% respectively from week 12 to 36 (27). The authors concluded that the decreases in vasopressin and increases in oxytocin suggested that oxytocin worked with vasopressin to concentrate urine since there were no changes in sodium and water excretion. Cycle ergometer exercise at 100, 175, and 225 W in 15 young males resulted in increases in vasopressin, but the increases were only significant when the intensity exceeded 40% VO_2 max (28). In another study (29) of six trained males, vasopressin increased as a result of exercise at 90% VO_2 max for 10 min. Also, ACTH increased, but CRH did not. The authors suggested that, based on their results, vasopressin may be more important than CRH in stimulating ACTH during short-term intense exercise in males. Any implications for exercising pregnant women are unclear.

Endorphins (B-Endorphin)

Although not completely understood, endorphins appear to have opioid effects on the central nervous system and may be associated with the relief of pain while also producing a feeling of elation (4, 21). Beta-endorphins are also synthesized by the placenta (30). Resting levels of beta-endorphins appear lower in pregnant women compared to nonpregnant women. In one study (31) concentrations of beta-endorphin were significantly lower across gestation while in another study (32) beta-endorphin levels were lower during the first and second trimesters compared to nonpregnant subjects. Plasma levels increased toward term and during labor. The authors suggested that the increase in levels may be associated with the stress of labor.

As with a number of hormones, exercise studies during pregnancy investigating beta-endorphins are limited. However, one study (23) followed 12 women across gestation

and concluded that 20 min of cycle exercise in the water at 60% predicted maximal capacity increased beta-endorphin levels during pregnancy but not postpartum. The 15th-week increase was greater than the 25th- or 35th-week increase. Beta-endorphin concentrations had returned to resting levels by 20 min after exercise during all trials. Thus, it appears that the changes were transient with no noticeable lasting effects.

ADRENALS

Cortisol

Cortisol is involved in macronutrient metabolism including stimulating lipolysis and protein degradation and increasing blood glucose levels (9). Pregnancy elevates resting levels of cortisol (11, 25, 33). Resting levels of maternal cortisol during pregnancy increase to levels associated with Cushing's syndrome (7). An enzyme, 11-B hydroxysteroid dehydrogenase 2, which converts cortisol to an inactive metabolite, provides protection for the fetus in early pregnancy/critical development time (5, 7). Cortisol may play a role in insulin resistance since cortisol decreases glucose entry into insulin-sensitive tissues (34).

Plasma cortisol was unaffected by mild physical activity during pregnancy in earlier studies (25, 35). Both studies were of short duration, 15 and 10 min, respectively, and low intensity. Another study (33) in pregnant women found plasma cortisol concentrations to be lower than resting levels during 20 min of moderate exercise in the water at an average heart rate of 132 beats/min. A later study (36) in pregnant women found increases in circulating cortisol levels after a 40 min treadmill walk at heart rates averaging 135 beats/min, which was about 60% of maximal aerobic power. Increases in cortisol were found in pregnant women walking on a treadmill for 30 min at 65% of predicted heart rate (11). The differences between studies may be related to both the intensity and duration of exercise. There appears to be a threshold intensity of approximately 60% of an individual's maximum aerobic output that must be exceeded in order to elicit a rise in cortisol (37).

Elevated levels of cortisol as a result of exercise may depress immune function, but this appears to be a transient effect (38). However, maternal stress may decrease the activity of the enzyme mentioned previously, 11-B hydroxysteroid dehydrogenase 2, which provides protection for the fetus by converting cortisol to an inactive metabolite (39, 40). Thus, the placenta and fetus may be exposed to greater concentrations of cortisol (40). While the authors (40) mention undernutrition as a potential stressor, exercise may be a potential stressor in some pregnant women. Exercise as a stressor has been recently reviewed (2, 3). However, while most exercise bouts might be expected to result in a stress response that was temporary, certain conditions, if present, could affect the stress response to exercise. Those conditions include, but are not limited to, nutrition status, training status, type of activity, environmental conditions, more anaerobic exercise, and age (2, 3).

Epinephrine

Epinephrine stimulates muscle glycogenolysis and lipolysis and also stimulates liver glycogenolysis (4). Fifteen minutes of moderate-intensity treadmill exercise resulted in

a significant increase in epinephrine in pregnant women during the third trimester (35). In another study (25), 10 min of cycle ergometer exercise during the third trimester of pregnancy resulted in a 51% increase in epinephrine concentrations. Based on previous studies, as might be expected, epinephrine concentrations increased after 30 min of moderate-intensity cycle ergometer exercise (41). By the third trimester, the increases were considerably smaller. In a follow-up study (42), exercising for 25 min at a heart rate of approximately 150 beats/min during a prenatal exercise class caused an increase in epinephrine during the first and second trimesters, but not in the third trimester or in a control group. While 35–40 min of exercise at 70–75% VO_2 max increased epinephrine, the response was the same during pregnancy and postpartum (43). Epinephrine levels during pregnancy increased at heart rates of 135–155 in as little as 20 min of treadmill walking or aerobic dance with epinephrine levels tending to increase as exercise intensity increased (44). So, epinephrine appears to increase as a result of moderate-intensity exercise although the increase may be blunted in later gestation. The implications of this are unknown.

Norepinephrine

Among other effects, norepinephrine stimulates lipolysis in adipose tissue (4). Light treadmill exercise during the third trimester at an oxygen consumption rate of 0.5 L/min produced an increase in norepinephrine levels (35). This appeared to be a transient effect and baseline values were reestablished approximately 30 min after exercise. Low-level exercise during pregnancy in 13 insulin-requiring diabetic patients and 42 control subjects led to increased norepinephrine concentrations (45). In a study (41) designed to investigate how exercise and pregnancy affected different hormones and substrates, seven nonpregnant and six pregnant women in the second trimester and eight women in the third trimester participated in 30 min of bicycle ergometer exercise at heart rates of 130–140 beats/min. Norepinephrine increased with exercise.

In one study (46), 36 pregnant women performed leg exercise on a bicycle ergometer. Near-maximal exercise caused a significant rise in norepinephrine, both in pregnant and in nonpregnant women. In addition to norepinephrine, renin levels were measured and exercise renin values were twice resting values. The authors concluded that the circulatory system during pregnancy is well adapted to changes in the renin angiotensin system.

Norepinephrine values increased by 139% after 10 min of submaximal cycle ergometer exercise during the 35th week, on average, of pregnancy (25). The authors reported finding what they described as mild irregular uterine activity in four of their ten subjects, but none experienced any regular contractions. Although the increase in norepinephrine as a result of exercise may stimulate uterine contraction, it appears that the increase in epinephrine counters the norepinephrine effect (25). Norepinephrine was measured with subjects performing treadmill exercise at 2, 3, and 4 METS for three continuous 10 min sessions during pregnancy and after delivery (47). Norepinephrine concentrations at peak exercise increased 64, 42, and 29% (not significant), respectively, when exercise was compared to rest. Of the 14 women participating in the study, ten experienced increased uterine activity during the exercise

or recovery periods. Thus, research suggests that while uterine activity may be affected by norepinephrine in some cases, low to moderate exercise would not be expected to lead to preterm delivery.

Estrogens and Progesterone

Although the adrenal gland may be a source of estradiol and progesterone, it appears that any contribution to plasma levels is small (48) and therefore, they are reported on under the “Placenta” section.

PANCREAS

Insulin

Insulin promotes glucose uptake by tissues, glycolysis, glycogenesis, triacylglycerol synthesis, and protein synthesis (49). Resting insulin levels tend to increase as pregnancy progresses (11, 41). Moderate exercise is associated with decreases in insulin concentrations during pregnancy. In one study (41) during pregnancy, 30 min of bicycle ergometer exercise at heart rates of 130–140 beats/min led to lower insulin concentrations after exercise. Also, during the second and third trimester, glucose concentrations declined 25 and 31% respectively. A subsequent study (42) during pregnancy reported a reduction in insulin levels after 45 min of aerobic exercise at heart rates approximately 150 beats/min. Another study (36) during pregnancy found decreases in insulin after 40 min of aerobic dance and treadmill walking at heart rates averaging about 135 beats/min. Further studies (50) using bicycle ergometry found decreases in insulin concentrations during the third trimester after 60 min at 50–60% VO_2 max. Glucose levels progressively decreased through the 60 min of exercise and the decreases ranged from 16% during the first 15 min to 29% at 60 min. Finally, a study (11) involving 30 min of treadmill walking at approximately 65% of predicted maximal heart rate reported that insulin decreased at 22 and 33 weeks of gestation and 14 weeks after delivery. Blood glucose also decreased in this study by about 13% at 33 weeks. Thus, exercise studies carried out for 30–60 min at heart rates of approximately 130–150 beats/min, involving different modes of exercise, have shown decreases in insulin when exercising during pregnancy. Also, three of the studies mentioned above reported decreases in blood glucose as a result of exercise.

Glucagon

Glucagon stimulates liver glycogenolysis and gluconeogenesis (49) while also promoting lipolysis (4). Glucagon has been reported to increase after light exercise during pregnancy (35). The exercise consisted of 15 min of treadmill walking at an oxygen consumption of less than 0.5 L/min. However, in another study (45), glucagon levels did not change as a result of mild exercise during pregnancy. Moderate exercise at 65% predicted maximal heart rate caused glucagon to increase during pregnancy, although there was a blunted response at 33 weeks (11). Glucagon increased 10% at 33 weeks pregnancy compared to 36% at 14 weeks postpartum. One possible explanation

for this blunted response may be related to the catecholamine responses in exercising pregnant women. Some studies (41, 42) have reported blunted catecholamine responses to exercise by the third trimester. Since increased circulating epinephrine stimulates glucagon secretion, a blunted epinephrine response might be expected to limit glucagon stimulation.

In addition to three studies (11, 41, 50) previously cited, several other studies (33, 43, 51) reported decreases in blood glucose levels of 10, 14, and 28% respectively after exercise during pregnancy. The intensity in the studies ranged from 47% to 70–75% maximal capacity and the duration from 20 to 40 min. The greatest decrease of 28% was associated with the higher intensity and longer duration of exercise. In one study (35), there were no changes in blood glucose concentrations during a 15 min bout of low-intensity exercise. Therefore, both the intensity and duration of exercise may have affected the results in the different studies.

Blood glucose concentrations are typically the result of liver glucose release from glycogenolysis and gluconeogenesis, and peripheral glucose uptake. In studies (11, 50, 51) in which respiratory exchange ratio (RER) values were reported, while there was a reduction in blood glucose, there were no changes in RER, suggesting that there was no change in carbohydrate utilization. If there were decreases in blood glucose, but no change in substrate utilization, what may be occurring?

It has been hypothesized (11) that the drop in blood glucose does not necessarily represent increased utilization but rather an inability to maintain blood glucose homeostasis. As mentioned above, glycogenolysis and gluconeogenesis help control blood glucose levels. If either is not functioning normally, blood glucose may be affected. Gluconeogenesis appears to take on added importance during pregnancy (52). Forty years ago, research (53) suggested that gluconeogenesis was compromised during starvation in pregnancy. Alanine uptake by the placenta was suggested as an explanation for the findings. Alanine is a key gluconeogenic substrate (53) and continuous uptake by the placenta leads to reductions in blood levels and thus an impairment in gluconeogenesis. If glucagon is blunted during exercise in pregnancy (11), then that may help explain the decreases in maternal blood glucose since glucagon stimulates gluconeogenesis. Also, if gluconeogenesis is impaired, insulin by itself or possibly the ratio of insulin to glucagon may have inhibited gluconeogenesis. To what extent, if any, maternal blood glucose decreases have on fetal glucose and any consequences are unknown. Further studies are needed to clarify reasons for the decreases in blood glucose as well as to identify any impact on the fetus.

ADIPOSE TISSUE

Leptin

Leptin is involved with regulating body weight/body fat (54). While leptin is produced mainly in adipose tissue, it is also produced by the placenta during pregnancy as well as being produced by skeletal muscle, bone, stomach, brain, and arterial endothelium (55). During pregnancy, there is an increase in resting leptin concentrations mainly from adipose tissue and the placenta (56). In one study (57) of 135 pregnant women of low socioeconomic status, mean leptin concentrations were the greatest at 22–27 weeks,

measuring 29.8 ng/mL and decreasing to 25.2 ng/mL at 34–39 weeks gestation. The increased levels of leptin during pregnancy may be a reflection of leptin resistance.

One exercise study (54) reported an increase of 29% in leptin in pregnant women participating in a home-based, moderate (approximately 65% of predicted VO_2 max)-intensity stationary cycling program. The program aimed for 40 min of activity up to a maximum of five times per week. Baseline values were determined at 19 weeks and late gestation values were assessed at 35 weeks for both a control group and an exercise group. Although not significant, a trend was noted toward lower free fatty acids when compared to controls during late gestation. According to the authors, the increase in leptin may reflect a placental response to exercise resulting in a decrease in nutrient availability and may contribute to the reduction in birth weight seen in this study. However, using the coefficient of determination or value of the squared r from the authors reported r value for the relationship between late pregnancy changes in maternal leptin levels and offspring birth weight, those changes in leptin levels would only explain 6% of the birth weight. Therefore, it appears that leptin has a small effect. In contrast to this study, another study (58) using weight-bearing exercise found a blunted increase in leptin during pregnancy. The reasons for the inconsistent findings are unclear. However, different modes of exercise were used and one study group (58) had less fat mass which may have affected leptin levels. Because of few studies with inconsistent findings and the possibility of a reduction in fetal birth weight, more studies appear warranted.

THYROID

Triiodothyronine (T3) and Thyroxine (T4)

Thyroid hormones mainly affect the metabolic rate of tissues (4, 21). While thyroid hormones decline slightly during pregnancy (59), the significance of this is unclear. However, fetal development may be compromised if maternal hypothyroidism is present during the first 20 weeks of pregnancy (60). Specifically, permanent brain damage or preterm delivery and fetal death have been associated with overt maternal hypothyroidism occurring in the first trimester (20).

In a study (61) with 60 well-trained male athletes riding a bicycle ergometer at 45, 70, and 90% of predicted maximum heart rate, T3 and T4 concentrations tended to increase with the intensity of the exercise. However, all values were still within euthyroid adult values. Since hormonal responses to exercise appear similar for men and women (18), any changes that might occur during pregnancy would be expected to be minor.

PARATHYROID

Parathyroid Hormone

PTH acts to increase calcium levels by its effects on bone, kidneys, and intestines (21). Small studies have reported decreased PTH levels during pregnancy (62, 63). In one (63) of the studies, there was an initial decrease of 47% at 16 weeks compared to the baseline value. From 16 weeks, PTH levels increased through 36 weeks. However, the PTH level at 36 weeks was still 23% below baseline levels. A review (64) of exercise and its effect

on PTH levels concluded that those levels were related to both the intensity and duration of exercise. Of the studies reviewed, only those with a high intensity (15% above ventilatory threshold) and long duration (greater than 50 min) or a lower intensity of 50% VO_2 max and a duration of 5 h led to increased concentrations of PTH. Any potential effect of changes in PTH on bone mineral density during pregnancy is unknown. However, based on the results of studies with nonpregnant populations and the type of exercise typically performed during pregnancy, little change in PTH might be expected.

LIVER

Vitamin D

The biologically active form of vitamin D is 1,25 dihydroxyvitamin D (1,25 (OH)₂ D) and is formed mainly by the kidney and functions like a steroid hormone. However, 25-hydroxyvitamin D (25 (OH) D) is considered to be the best measure of vitamin D status and is formed primarily in the liver. Proper fetal skeletal formation along with tooth enamel acquisition is dependent upon adequate maternal vitamin D status (65). Also, lower birth weights may result from inadequate vitamin D intake during pregnancy (66). There is a strong correlation between fetal and maternal levels of 25 (OH) D (65). Pregnant and lactating women, thought to be protected by taking a prenatal supplement, have been found to be at risk of vitamin D deficiency with one report (67) indicating that 73% of the women and 80% of the infants were deficient at birth.

Maternal vitamin D status may be associated with long-term effects on the infant (65). These effects include inadequate maternal vitamin D status and the hypothesized development of obesity in children (68). Also, the risk of osteoporosis later in life is increased by vitamin D insufficiency in pregnant women and its effect on intrauterine development (69). The previous two studies are examples of how certain conditions and/or diseases may have fetal origins (70) as a result of the intrauterine environment.

A case control study (71) with 1,357 male and 1,264 female controls aged 55–74 was conducted in order to identify modifiable predictors of vitamin D status, serum 25 (OH) D. Vigorous physical activity equal to 3 or greater than 3 h/week was found to be strongly associated with improved vitamin D status. The authors point out that while the association between physical activity and vitamin D status has been explained in the past by physical activity simply reflecting time outside and thus exposure to sunlight, there may be something about physical activity itself affecting vitamin D status. Thus, it is possible that exercise during pregnancy may help with vitamin D concentrations. 25 (OH) D is stored in fat and possibly muscle (72, 73). If muscle stores 25 (OH) D, then exercise and muscle activity may lead to the release of 25 (OH) D into the plasma. The vitamin D status of pregnant women and possible exercise effects on 25 (OH) D warrants study.

PLACENTA

The placenta produces hormones and therefore should be viewed as an endocrine organ. Estrogens and progesterone are produced by the placenta after the first 8–10 weeks of pregnancy (74, 75) and related studies are reviewed in this section. While

IGF-1 and IGF-2, also known as somatomedins, are produced in the liver, skeletal muscle, bone, and other tissues (9), they are also produced in the placenta and fetus (76) and studies are discussed in this section. Other placental hormones include variants of hormones normally produced elsewhere such as GH and prolactin (discussed in the pituitary section) and may affect fetal growth directly or indirectly (77).

Estrogens

Metabolic effects of estrogen during pregnancy include increased lipid synthesis and storage and protein formation (74). At an average of 35 weeks, ten pregnant women exercised for 10 min with the average heart rate rising from 93 to 157 beats/min at the end of exercise (25). Estriol increased significantly during exercise but decreased to slightly below (approximately 2.5 nmol/L) beginning values in an hour. In the same study, estradiol did not increase during the 10 min bicycle ergometer exercise session, but it showed a significant decrease 60 min after exercise. A later study (41) during pregnancy, involving 30 min of cycle ergometer exercise at 130–140 beats/min, resulted in increases in estriol. However, in a subsequent study (42) of pregnant women in an exercise class exercising for 25 min at a heart rate of approximately 150 beats/min, changes in hormones, including estriol, were described as transient and not significant.

Progesterone

Along with estrogen, progesterone stimulates lipid storage (74). Progesterone did not increase during the third trimester when ten pregnant women exercised for 10 min with the average heart rate rising from 93 to 157 beats/min at the end of exercise (25). However, like estriol and estradiol, progesterone concentrations were significantly lower 60 min after exercise (25). Thirty minutes of cycle ergometer exercise at 130–140 beats/min resulted in increases in progesterone (41). However, in a subsequent study (42) of pregnant women in an exercise class exercising for 25 min at a heart rate of approximately 150 beats/min, changes in hormones, including progesterone, were described as transient and not significant.

Insulin-Like Growth Factors 1 and 2

IGFs are produced in the placenta and fetus (76) and are a family of growth-promoting proteins (9). IGF-1 may be an important regulator of fetal growth (78, 79). During early pregnancy, plasma levels of IGF-1 decrease and then increase after 24 weeks gestation (56). Increased levels of IGF-1 and insulin lead to increased fetal fat and liver glycogen concentrations, increased protein synthesis, and thus overall growth (56). In a study of non-exercising pregnant women, IGF-1 levels increased during pregnancy and were associated with bone turnover markers (63).

There is little research regarding exercise during pregnancy and the effects on IGF-1 and IGF-2. One study (80) of pregnant women found that fetal IGF-1 and IGF-2 levels were less in the children of an exercise group compared to the children of the controls. The home-based exercise program during pregnancy consisted of 40 min of stationary

cycling for a maximum of five times per week. Although the authors suggest that exercise was associated with the reductions in fetal IGF levels and lower birth weight in the exercise offspring, it does not appear that differences in birth weight were statistically significant. The difference in birth weights between the controls and exercise group was 143 g, which may or may not have clinical/practical significance.

Human Chorionic Gonadotropin

In early pregnancy, hCG stimulates the corpus luteum to produce estrogen and progesterone (74). Thirty minutes of cycle ergometer exercise at 130–140 beats/min resulted in no change in hCG concentrations in one study (41) of pregnant women. Also, in a 1995 study (42) of pregnant women, changes in hCG during exercise were described as transient. The subjects had participated in an exercise class exercising for 25 min at a heart rate approximately 150 beats/min. Thus, little information is available to suggest that exercise during pregnancy affects hCG levels.

Placental Growth Hormone

While GH has been addressed under the pituitary section, a couple of brief comments are included in this section. PGH stimulates maternal gluconeogenesis and lipolysis providing nutrients for the fetus (81). Since PGH is not found in fetal blood, PGH appears to have an indirect effect on fetal growth (81).

Human Placental Lactogen

HPL concentrations increase during pregnancy (56). The role of HPL is unclear (82). Although not known with certainty, HPL may play a role in increased food intake, therefore stimulating weight gain (56). An earlier exercise study (83) described strenuous cycling during the last month of pregnancy as having no effect on HPL. While additional studies appear to be limited, one study (41) of pregnant women, involving 30 min of cycle ergometer exercise at 130–140 beats/min, resulted in increases in HPL. Aerobic exercise at a heart rate of approximately 150 beats/min resulted in brief limited changes (42). So, the little information to date suggests that no major changes occur with HPL as a result of exercise during pregnancy.

Relaxin

Relaxin, along with estrogen, is thought to affect the laxity of ligaments or connective tissue composition (84, 85). However, while one study (86) found five of seven joints increased in laxity during gestation and postpartum, there was no association with serum relaxin concentrations. Also, pregnant diabetics may have increased relaxin levels compared to normal pregnant women (87). It is unclear what the physiological consequences are of increased relaxin levels in pregnant diabetics.

SUMMARY

Although a number of hormonal responses to exercise during pregnancy have been described, the important role each plays is not completely clear. Research on exercise during pregnancy and hormonal changes related to exercise during pregnancy appears limited and needs expansion. Much remains unanswered and more studies with more subjects are needed before confident conclusions can be reached regarding exercise during pregnancy, changes in hormonal concentrations, and interpretations of the meaning of those changes.

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18

Vitamin D and Exercise Performance

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INTRODUCTION

In 1645, Daniel Whistler first described the physical manifestation that came to be known as rickets in the equivalent of a PhD dissertation. Francis Glisson, an English physician, reported similar observations 5 years later in one of the first pediatric texts published in London (1). In this text, *A Treatise of the Rickets: Being a Disease Common to Children*, he gave a complete description of rickets and also differentiated between rickets and infantile scurvy. He did not, however, note the importance of diet or the origin of the disease in his clinical description. As early as 1822, however, Sniadecki

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published observations that children in Warsaw, Poland, had a higher incidence of rickets while children living in rural areas outside of the city had a much lower prevalence of the disease (2). He hypothesized that the development of rickets was due to a lack of adequate sun exposure. Rickets was also observed with a high prevalence in the UK and India in children of the very rich who were kept from the sunlight. It was also seen in children living in highly industrialized areas like London and New York City.

Close to the beginning of the twentieth century there were three major discoveries regarding vitamin D. The first, from both UK and US researchers, was that vitamin D was a dietary compound (2). McCollum and Davis conducted extensive investigations with development and growth in rats fed a variety of diets (3). They noted that diets consisting simply of purified proteins, carbohydrates, and fats were insufficient to promote growth in young rats. These studies led the way for the work of Sir Edward Mellanby. Mellanby induced rickets in dogs through their diet to show that McCollum and Davis were working with a compound other than vitamin A, as previously thought (4). Mellanby noted that some of the fastest growing dogs in his experiments were fed diets low in vitamin A. He was also one of the first investigators to note the antirachitic effects of cod liver oil which is rich in vitamin A. McCollum was later able to eliminate vitamin A and correctly identify a previously unidentified compound, referred to as “calcium-depositing vitamin” in earlier journal articles, which he named vitamin D (5). McCollum focused on the antirachitic properties of this vitamin and was able to show that cod liver oil, even after it had been oxidized, was still effective in preventing and curing rickets in rats. His work focused on comparing a variety of dietary fats, including cod liver oil, butterfat, and several vegetable oils including peanut, rapeseed, olive, and coconut oil.

The second major discovery was the discovery of the reported benefits of ultraviolet light by Steenbock and Black (6). Steenbock and Black irradiated rations of hog millet with a mercury arc lamp, which produces a broad spectrum of light including UVB, and fed it to rats. Rats fed with the irradiated rations had increased growth and significantly higher ash content in the femur compared to the controls. This was further supported by the work of Huldschinsky who showed that children with rickets improved after exposure to a mercury arc lamp (7). Infants with rickets in a hospital in New York City also showed complete recovery from rickets following exposure to sunlight on the roof of the hospital (7). This included complete eradication (or disappearance) of signs including beading of the ribs, bone deformity detected by X-ray, and increased deposition of inorganic salts at the epiphyses of the long bones. The infants were exposed to sunlight for 15 min to 1 h 4–5 days a week depending upon the weather (8). This important breakthrough led to the realization that irradiating certain foods and even the skin of animals and humans can be beneficial. As early as 1923, Steenbock and Black noted that the properties of the irradiated substances were related to calcium, which we now recognize as a classic function of vitamin D (6). This discovery of irradiating foods led to the irradiation of milk, the first food to be fortified with vitamin D.

The final early twentieth-century discovery concerning vitamin D resulted in a Nobel Prize for Adolf Windaus in 1938. Windaus was a steroid chemist from Germany and his contribution to the vitamin D puzzle was identification and chemical synthesis of vitamin D (9). Through a series of irradiation experiments, Windaus was able to determine that ergosterol is an intermediary compound to the active form of vitamin D.

With this knowledge and the ability to fortify foods, rickets was essentially eradicated from the US population. Vitamin D became a miracle vitamin and was added to a myriad of foods including dairy products, cereal and breads, hotdogs, soda, and peanut butter (2). Schlitz Brewery even introduced beer containing vitamin D (Fig. 1) and marketed it as “the beer containing sunny energy in both summer and winter” and advertised with the slogan “keep sunny summer health, drink Schlitz all winter.”

Europe followed suit with vitamin D fortification, but after World War II, fortification procedures weren't closely regulated and accidental over-fortification of vitamin D in milk led to vitamin D intoxication in some children and infants (2). This led to legislation that prohibited the fortification of vitamin D in foodstuffs in many European countries. Even today some European countries still ban the fortification of dairy products but allow vitamin D fortification in margarine and cereals.

PHYSIOLOGY OF VITAMIN D

Although labeled a vitamin by McCollum, vitamin D actually acts like a hormone (1). There are 4 major factors that classify vitamin D's actions as a hormone. The first is that vitamin D is metabolized into more than 41 metabolites, most importantly 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D (1,25(OH)2D). The formation of 1,25(OH)2D is also regulated in the kidney. Furthermore, the main metabolites are transported outside the cell through circulation by lipoproteins, albumin, and vitamin D-binding protein (DBP), and inside the cell through the vitamin D receptor (VDR). The final factor involves the identification of the VDR as a nuclear transcription factor which regulates transcription of a large number of genes.

VITAMIN D, CALCIUM REGULATION, AND BONE HEALTH

Vitamin D is classically recognized as playing an important role in calcium homeostasis with the target organs being bone, intestine, and kidneys. Vitamin D stimulates calcium transport from these organs to the blood. Production of the active form of vitamin D (1,25(OH)2D) is stimulated by parathyroid hormone (PTH) (10).

Vitamin D serves to increase serum calcium concentrations in three ways (11). First, it induces gene expression of the proteins involved in active intestinal calcium absorption which includes the protein calbindin. Calbindin is a calcium-binding protein in the intestine and has been localized primarily in absorptive cells of the mucosa. This supports the role of calbindin as a facilitator of calcium diffusion (12). This occurs through the cell interior towards the basolateral membrane. Vitamin D also allows mobilization of calcium from bone when it is absent or deficient in the diet. This action is through stimulation of osteoblasts to produce receptor activator nuclear factor- κ B ligand (RANKL). RANKL then subsequently stimulates osteoclastogenesis and activates resting osteoclasts. Activated osteoclasts increase bone resorption. It has been reported, *in vivo*, that both 1,25(OH)2D and PTH are required for this to occur (11). The final event is enhanced calcium absorption in the distal renal tubules which also requires both vitamin D and PTH. The distal tubule is responsible for reabsorption of the last 1% of the filtered load of calcium and can represent a significant amount of calcium retention (e.g., as high as 7 g).

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SUMMER HEALTH**



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ALL WINTER**



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WITH SUNSHINE VITAMIN-D



The Beer That Made Milwaukee Famous

Fig. 1. 1936 Schlitz advertisement for vitamin D beer.

ADDITIONAL ROLES OF VITAMIN D

In addition to the classic targets for vitamin D, more recent research has discovered other roles for this hormone (Table 1). Vitamin D is now known to play a role in immune modulation and reproductive function and may protect against multiple sclerosis, certain cancers, diabetes, high blood pressure, and cardiovascular disease. Grandi et al. reported an inverse association between circulating 25(OH)D concentration and cardiovascular disease incidence and mortality (13). Covic et al. reported an association between low VDR activation and increased risk of hypertension (14). While the exact mechanism for the protective effect of vitamin D in diabetes mellitus has yet to be elucidated, proposed mechanisms include pancreatic β -cell dysfunction, chronic inflammation, and peripheral insulin resistance due to compromised status (15). VDR has been identified in pancreatic islets, indicating a possible role for vitamin D in insulin secretion (16). VDR has also been identified in human sperm and human testis, while vitamin D has been shown to be necessary for estrogen biosynthesis in both male and female gonads (17–19). Vitamin D and VDR are both thought to play a role in skeletal muscle growth differentiation and function (20). The binding of 1,25(OH)2D to the VDR also results in enhanced transcription of several proteins. A summary of the effects of vitamin D can be seen in Table 1.

VDR has been identified in pancreatic islets, indicating a possible role for vitamin D in insulin secretion (16). VDR has also been identified in human sperm and human testis, while vitamin D has been shown to be necessary for estrogen biosynthesis in both male and female gonads (17–19). Vitamin D and VDR are both thought to play a role in skeletal muscle growth differentiation and function (20). The binding of 1,25(OH)2D to the VDR also results in enhanced transcription of several proteins. A summary of the effects of vitamin D can be seen in Table 1.

Table 1
Effects of vitamin D on various systems

<i>Effects of vitamin D</i>	
Bone (45, 112–114)	Decreases the risk of osteoporosis and osteoporotic fractures Hypothesized to be the best predictor of fracture risk Increases bone mineralization in adolescents
Intestine (12, 115, 116)	Increases calcium and phosphorus absorption in the small intestine Increases synthesis of calbindin Promotes intracellular calcium transport
Kidney (11, 117)	Increases calcium reabsorption
Skeletal muscle (28, 100, 118–120)	Decreases risk of falls Inhibits type II muscle fiber atrophy Inhibits fatty degeneration and infiltration of fat, fibrosis, and glycogen granules Deficiency promotes nonspecific muscle pain
Reproductive system (19, 121)	Increases the likelihood of in vitro fertilization success Indirectly reduces the risk of preeclampsia by regulating calcium homeostasis Positively influences sperm function

VITAMIN D SYNTHESIS AND METABOLISM

Both exogenous and endogenous cholesterol can be used as a precursor for vitamin D synthesis. In the initial step of endogenous synthesis, acetyl CoA and acetoacetyl CoA react to form hydroxymethylglutaryl CoA (HMG CoA). HMG CoA is acted upon by HMG CoA reductase, the rate-limiting enzyme in cholesterol synthesis, to create mevalonate. Mevalonate is phosphorylated to farnesyl phosphate and subsequently converted to squalene and squalene 2,3-epoxide. Squalene 2,3-epoxide is then converted to lanosterol and finally to cholesterol which is the precursor to 7-dehydrocholesterol. Cholesterol is stored in the membrane of skin cells and converted to 7-dehydrocholesterol upon exposure to UVB light (wavelength 290–315 nm) (21).

Vitamin D is synthesized cutaneously from UVB rays when 7-dehydrocholesterol is converted to cholecalciferol (previtamin D₃) (1, 2, 10). This complex conversion involves photochemical and thermal reactions, and no enzymes are involved. Cholecalciferol is transported to the liver by vitamin DBP where it is hydroxylated in the liver to 25(OH)D by cytochrome 25-hydroxylases. This conversion involves the addition of a hydroxyl group on carbon 25. 25(OH)D is hydroxylated to 1,25(OH)₂D by 1 α -hydroxylase in the cytochrome of the kidney under the direction of PTH when serum calcium and phosphorus concentrations drop. 1,25(OH)₂D is the active form of vitamin D. 1 α -hydroxylase tightly regulates the production of 1,25(OH)₂D.

At least four different isoforms of the 25-hydroxylases have been identified (1). These 4 enzymes are all microsomal cytochrome P₄₅₀ (CYP) isoforms (CYP2DIII, CYP2D25, CYP3A4, and CYP2R1). Although four enzymes have been identified, CYP2R1 is considered to be the key enzyme because a homozygous mutation was reported in a patient with low circulating concentrations of 25(OH)D and exhibiting classical symptoms of vitamin D deficiency, including rickets (22). In contrast to the 25-hydroxylases, there is only one 1 α -hydroxylase (CYP27B1). CYP27B1 is most abundant in the kidneys. Production is positively regulated by low serum calcium concentration, elevated PTH, and growth hormone and IGF-I and negatively regulated by phosphate, fibroblast growth factor 23 (FGF23), calcitonin, and 1,25(OH)₂D (1). Although the kidney seems to be the only site for production of serum 1,25(OH)₂D, CYP27B1 is expressed in the skin, monocytes, placenta, and bone cells and thought to produce paracrine and autocrine actions (23). This has been identified by immunohistochemical and Western blot analyses in both normal and diseased tissues. Although the function of CYP27B1 in several extrarenal tissues, including adrenal medulla, brain, pancreas, and colon, remains to be elucidated, the staining pattern may indicate intracrine actions in peripheral tissue related to vitamin D (23). While 4 25-hydroxylases have thus been discovered, only one gene has been identified in the catabolism of both 25(OH)D and 1,25(OH)₂D. This multifunctional gene, CYP24A1, converts both metabolites to either calcitroic acid after initial 24-hydroxylation or a side chain lactone after 23-hydroxylation (22, 24). Calcitroic acid is secreted in the bile. Figure 2 gives an overview of vitamin D synthesis and metabolism.

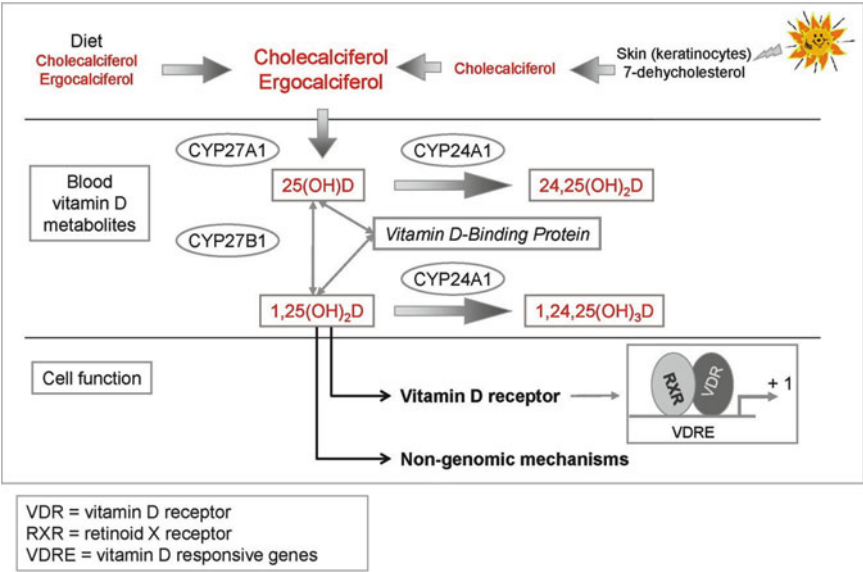


Fig. 2. An overview of vitamin D synthesis and metabolism (reproduced, with permission, from Elsevier Limited (122)).

Table 2
Adapted from Holick (2)

Factors that decrease cutaneous synthesis of vitamin D

- Latitudes >37° north or south
- Increased melanin content in the skin
- Air pollution and cloud cover
- Zenith angle of the sun (November to February)
- Proper sunscreen application
- Time of day (early morning and late afternoon)
- Increased age

CUTANEOUS SYNTHESIS

Several factors affect the synthesis of vitamin D (see Table 2). Time of day can alter synthesis of vitamin D with decreased synthesis in the early morning and late afternoon. Optimal synthesis is seen from 10:00 to 15:00 in the spring, summer, and fall (2). Air pollution can block UVB rays and decrease synthesis, as can time of year. Although the sun is closest to the earth from November to February, the zenith angle is more oblique so the UVB photons pass through a greater distance of the ozone layer, which allows the ozone to more efficiently absorb the photons (2). This decreased synthesis is especially seen in latitudes above 37° where the UVB photons may be decreased from 80 to 100% during the winter months (25). Increased melanin content serves as a natural skin

Table 3
Vitamin D values of selected foods. USDA National Nutrient Database
for Standard Reference, Release 24

<i>Vitamin D source</i>	<i>Serving size</i>	<i>IU per serving size (IU)</i>
Fortified orange juice	8 oz. (1 cup)	137
2% milk	8 oz.	100
Skim milk	8 oz.	100
Fortified soy milk	8 oz.	100
Fortified rice milk	8 oz.	100
Sardines packed in oil	1 can (3.75 oz.)	178
Salmon—pink	½ fillet (124 g)	522
Cod liver oil	1 tbsp	1,360
Atlantic herring	1 fillet (143 g)	546
Portabella mushroom		
Irradiated	1 mushroom cap (84 g)	375
Nonirradiated	1 mushroom cap (84 g)	8

protectant, but also decreases vitamin D production. Wearing one ounce of SPF 8, the recommended amount to cover the entire body surface, can decrease cutaneous vitamin D synthesis more than 95% whereas wearing SPF 15 can reduce the capacity more than 98% (2). As the skin ages, less 7-dehydrocholesterol is available and this also serves to decrease synthesis (26). Adipose tissue is thought to serve as an irreversible “sink” for vitamin D which does not appear to affect the synthesis of vitamin D, but it does affect the availability, once it has been produced.

DIETARY INTAKE OF VITAMIN D

Very few foods are natural sources of vitamin D. It is estimated that only 100–200 IU of vitamin D comes from food sources daily (Table 3) even in athletic populations (27, 28). A cohort study in Finland found that fortifying milk with vitamin D was still not sufficient in resolving hypovitaminosis D in young healthy men (29). Of the few food sources, oily fish are among the best sources. These include salmon, herring, and sardines. Cod liver oil, which has been considered critically important for bone health for hundreds of years, is also a very good source. Beef liver and irradiated mushrooms are also sources of vitamin D. Egg yolks may also contain vitamin D, but the total amount is highly variable and they are not generally considered a good source because of their high cholesterol content. Fortified foods include milk (dairy and nondairy), margarine, orange juice, and some breads and cereals. Table 3 lists several food sources of vitamin D and the IU in a serving.

VITAMIN D-BINDING PROTEIN

Vitamin D and all of its metabolites are transported in the circulation bound to vitamin DBP. DBP is highly polymorphic and belongs to the albumin superfamily that includes albumin, α -albumin, and α -fetoprotein. This family is characterized by unique cysteine

residue arrangements which have the ability to form disulfide bonds with other distally located cysteine residues (30). The functional domains differentiated by these bonds are distinct from albumin and help to define the physiologic roles of DBP. DBP is synthesized predominantly by hepatic parenchymal cells, but other cells can also produce DBP. DBP has been detected in cerebrospinal fluid, seminal fluid, saliva, and breast milk in addition to plasma (31, 32). Researchers originally thought that DBP detected in extrarenal tissue was artifact from plasma contamination during preparation, but this has been disproven. The presence of DBP in tissue is now thought to be indicative of the numerous functions of vitamin D in renal tissue (33).

Several functions for DBP have been identified. DBP depolymerizes and binds actin and tightly binds 25(OH)D (33, 34). It has been shown that 25(OH)D binds to DBP with a tenfold higher affinity than 1,25(OH)₂D (31). Additionally, DBP also binds fatty acids, controls bone development through osteoclast activation, and modulates immune and inflammatory responses, including leukocyte C5a-mediated chemotaxis and macrophage activation (30, 31, 35). The introduction of another name for DBP, macrophage-activating factor (GcMAF/DBP-MAF), highlights the importance of its macrophage-stimulating activities (36). There is also evidence to show that DBP is associated with the surface of cells which include neutrophils, fibroblasts, monocytes, B and T cells, B lymphoblastoids, placental cytotrophoblasts, human sperm, and smooth muscle cells (31).

DBP has potential therapeutic properties, separate from its actions on vitamin D. In addition to binding actin intracellularly, DBP also acts as an extracellular actin scavenger. The serum protein gelsolin (GSN), which is an actin-binding protein, acts with DBP to scavenge actin following cell lysis and is usually seen with physiologic stress. Following such tissue injury, intracellular actin is released into the circulation which can result in damage to the microvasculature through microemboli (33). Both actin-DBP and actin-GSN complexes have been detected in the circulation following tissue injury (37). DBP binds to g-actin and prevents further nucleation and polymerization (35). This binding sequesters g-actin and prevents it from polymerizing into f-actin. DBP is unable to bind with f-actin but (GSN) can both bind and sever the filaments. By removing actin from circulation, DBP is thought to attenuate clot formation and prevent the consequences of actin toxicity (32).

VITAMIN D RECEPTOR

The VDR is a nuclear protein that binds 1,25(OH)₂D. VDR expression has been identified in nearly every human tissue, which gives further evidence to the importance and myriad functions of vitamin D. The VDR is a ligand-activated transcription factor and is a member of the superfamily of nuclear receptors for steroid hormones (38). The VDR has been identified in mammals, birds, amphibians, and fish with a calcified skeleton. Genome mapping has identified 2,776 positions occupied by the VDR and 229 genes that have significant changes in expression in response to vitamin D (39). Although almost all nucleated cells express the VDR, its expression is variable. A few tissues and cells have either low or absent VDR, and these include red blood cells and some highly differentiated brain cells including Purkinje cells of the cerebellum (1). It is seen with a high degree of homology in ligand binding, functionality, and

structure. In nonchordate species such as crabs, mollusks, and octopus, the VDR is undetectable (1).

The VDR functions through heterodimerization with any of the three retinoid X receptor (RXR) isoforms (RXR alpha, beta, and gamma), much like the other members of group I nuclear receptors (NR). 1,25(OH)₂D binds to the VDR and the complex is then able to modulate the expression of target genes. Once bound with 1,25(OH)₂D, VDR is phosphorylated and the surface conformation is reconfigured. This is thought to result in the release of corepressors. Corepressors are substances that inhibit the expression of genes indirectly through interaction with repressor proteins. The VDR then recruits RXR and binds to vitamin D-responsive elements (VDREs). Many of the genes that are regulated by vitamin D will have multiple VDREs (40). Peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC1-alpha) has also been shown as a coactivator for the VDR and can augment ligand-dependent VDR transcription (41).

METHODS OF MEASUREMENT AND OPTIMAL CONCENTRATIONS

Vitamin D concentration is assessed by measuring 25(OH)D, the inactive form of vitamin D. Serum assays can be done on 1,25(OH)₂D, but this is not an accurate portrayal of an individual's vitamin D status. 1,25(OH)₂D has a shorter half-life than 25(OH)D. 25(OH)D has a half-life of ~2–3 weeks in circulation while the half-life of 1,25(OH)₂D is ~4–6 h in circulation (42). The circulating concentration of 25(OH)D is a thousandfold higher than the circulating concentration of 1,25(OH)₂D. Most importantly, in vitamin D insufficiency, elevated PTH stimulates the hydroxylation of 1,25(OH)₂D, which increases circulating 1,25(OH)₂D concentration. In fact, it is not uncommon to find normal or even elevated serum 1,25(OH)₂D in vitamin D-insufficient or vitamin D-deficient individuals (2, 42).

Common methods of assessing vitamin D status in serum include radioimmunoassay and chromatography techniques. Radioimmunoassay methods detect antibodies that are directed against 25(OH)D₃ and of 25(OH)D₂. Chromatographic methods, which include liquid chromatography–tandem mass spectrometry (LC–MS), separate and quantify 25(OH)D₂ and 25(OH)D₃ from its epimers. There are advantages and disadvantages to both. Immunoassay methods are less expensive and have a shorter turnaround time. They also can be automated, require fewer skilled personnel, and employ more user-friendly equipment. Chromatographic methods are more expensive, require more skilled personnel, and have a much longer turnaround time. On the other hand, chromatographic methods are able to differentiate between 25(OH)D₂ and 25(OH)D₃ while immunoassay methods can only assess total 25(OH)D (43). Because of the many advantages, notably cost and shorter turnaround time, immunoassays are often the method of choice for assessing vitamin D status. In the past, concerns were raised because of the reported variability between labs, often due to a difference in methods of assessment, continuing to confound the diagnosis of hypovitaminosis D (44). Recent work has shown that this variability has decreased markedly as techniques and equipment improves, but there is still a move towards international standardization (43, 44).

Although methods of assessment have improved, there continues to be much debate on the appropriate serum 25(OH)D concentration for ideal health (Table 4). Many researchers

Table 4
Concentrations of 25(OH)D

<i>Status</i>	<i>25(OH)D concentrations for health (2, 42, 45–47)</i>
	<i>Concentration</i>
Optimal	>40 ng/mL (>100 nmol/L)
Sufficient	≥30–32 ng/mL (>75 to 80 nmol/L)
Insufficient	21–29 ng/mL (51–74 nmol/L)
Deficient	≤20 ng/mL (50 nmol/L)
Toxicity	>150 ng/mL (374 nmol/L) and hypercalcemia

agree that a 25(OH)D concentration less than 20 ng/mL (50 nmol/L) is indicative of deficiency, 21–29 ng/mL (51–74 nmol/L) is suggestive of insufficiency, and greater than 30 ng/mL (75 nmol/L) identifies sufficiency (2, 42, 45). Researchers further agree that maintaining serum 25(OH)D concentration of 30–32 ng/mL (75–80 nmol/L) or greater is sufficient in the general population (46). This is because intestinal calcium absorption is maximized at concentrations at or above 32 ng/mL (80 nmol/L) (47). Bischoff-Ferrari et al. have reported evidence from clinical trials that fracture prevention efficacy is optimized when 25(OH)D was \approx 40 ng/mL (100 nmol/L) (48). They further reported that serum 25(OH)D concentration of 36–40 ng/mL (90–100 nmol/L) indicated the best results for lower-extremity strength in older adults. These and other studies in breast cancer have led some researchers to speculate that optimal concentrations are greater than 40–50 ng/mL (100–125 nmol/L) which is believed to be the serum concentration in which the human genome developed, i.e., when humans spent time outdoors without sunscreen (49).

Maintaining adequate to optimal stores of vitamin D has been found to be critical for health. Supplemental vitamin D (500–600 IU/day) has been associated with a 40% reduction in the risk of developing multiple sclerosis, and vitamin D deficiency is common in patients with autoimmune diseases (50). It has been proposed that supplementing individuals with higher doses of vitamin D (800 IU/day) can decrease the incidence of autoimmunity (51). Additionally, vitamin D intake (>500 IU/day) was seen to have an inverse relationship with the development of breast cancer in premenopausal women (52).

VITAMIN D AND IMMUNITY

Vitamin D is suspected to play a strong role in autoimmune rheumatic diseases (Table 5). This includes rheumatoid arthritis, undifferentiated connective tissue disease, and systemic lupus erythematosus (53). Low serum 25(OH)D concentrations are often correlated to the severity of the disease. 1,25(OH)₂D can exert its effect on several immune-cell types including dendritic cells, macrophages, and T and B cells (Table 6). This includes both systemic and locally produced 1,25(OH)₂D (24). Additionally, the identification of VDR in cells of the immune system and the presence of 1 α -hydroxylase in dendritic cells and macrophages suggest that vitamin D has regulatory autocrine and paracrine functions, particularly at sites of inflammation (54). There is also evidence that 1,25(OH)₂D is an immunosuppressive agent that enhances the pathogenesis

Table 5
The relationship between vitamin D and disease states

<i>Vitamin D and various diseases and disorders</i>	
Periodontal disease (48, 59)	25(OH)D concentration associated with attachment loss (reduction in connective tissue which attaches the tooth to the alveolar bone) in individuals >50 years
Autoimmune disorders (50, 53, 60–62)	Association between high 25(OH)D concentration and lower incidence of multiple sclerosis (MS) and MS-related disability Vitamin D intake may be protective in MS development 25(OH)D concentration associated with the severity of rheumatic arthritis (RA), systemic lupus erythematosus (SLE), and systemic sclerosis 25(OH)D concentration lower in patients diagnosed with mixed connective tissue disease (MCTD) Fibrosis of the connective tissue was inversely related to 25(OH)D concentration in individuals with systemic sclerosis
Cardiovascular diseases (14, 63–67)	25(OH)D concentration associated with decreased incidence of myocardial infarction 25(OH)D concentration associated with cardiovascular events even after controlling for other factors associated with coronary artery disease 25(OH)D concentration determined to be an independent inverse predictor of end-stage renal disease (ESRD)
Diabetes (15, 68, 69)	Vitamin D supplementation (2,000 IU/day) improved β -cell function in individuals at risk for type II diabetes Low 25(OH)D and calcium concentrations negatively influenced glycemia
Cancer (70–74)	Vitamin D intake and 25(OH)D concentration inversely associated with colon or rectal cancer development Increased 25(OH)D concentration may be protective against breast cancer incidence, especially in women >60 years
Respiratory infections (75)	25(OH)D concentration inversely associated with risk of developing tuberculosis Low 25(OH)D concentration associated with increased incidence of upper respiratory infections (URIs) 25(OH)D concentration may reduce the risk of developing asthma

of T helper 1-mediated autoimmune diseases including inflammatory bowel disease and experimental autoimmune encephalomyelitis (55, 56).

In the T cells of VDR knockout mice, an inflammatory phenotype is expressed in comparison to cells of control mice. Knockout T cells proliferate twice as much in a mixed lymphocyte reaction, and transfer a more severe form of inflammatory bowel disease compared to wild-type controls (57). Additionally, increased expression of IL-1 β and TNF- α in the colon of both young (5-week-old) and old (9-month-old) VDR knockout mice was seen compared to wild-type controls (58).

1,25(OH)₂D appears to significantly inhibit adaptive immune cells. This is seen in the inhibition of T-cell proliferation and the decrease in the production of IL-2, interferon- γ (IFN- γ) mRNA, and protein in T cells (24). There are also increases in IL-4 and IL-10 in T cells (24).

Table 6
Effects of vitamin D on immunity

	<i>Effects of vitamin D on immunity (24, 75)</i>
Monocytes and macrophages	<ul style="list-style-type: none"> ↑ IL-1 ↑ Proliferation ↑ Cathelicidin ↑ VDR, CYP27B1
Dendritic cells	<ul style="list-style-type: none"> ↓ Maturation ↓ MHC class II ↓ CD40, CD80, CD86 ↓ IL-12 ↑ IL-10
Effector or memory T cells	<ul style="list-style-type: none"> ↓ IL-2, IFN-γ, IL-17 ↓ Cytotoxicity ↓ Proliferation ↓ CD4⁺:CD8⁺ T-cell ratio ↑ IL-4, IL-10 ↑ T_R1-cell and T_{REG}-cell generation
B cells or antibody-secreting cells (ASCs)	<ul style="list-style-type: none"> ↓ Proliferation ↓ IgG, IgM production ↓ Plasma-cell differentiation ↑ VDR ↑ CYP24A1

HYPOVITAMINOSIS D

Vitamin D insufficiency and deficiency has been documented worldwide and has been deemed a pandemic by some researchers (2, 45, 76). In North India, 96% of neonates (77), 91% of apparently healthy school girls (78), and 84% of pregnant women (77) were found to have serum 25(OH)D concentration less than 20 ng/mL (50 nmol/L). Up to 70% of adolescent girls in Iran (79) and 80% of adolescent girls in Saudi Arabia (80) had 25(OH)D concentration less than 10 ng/mL (25 nmol/L). In the national health and nutrition examination survey (NHANES), which evaluated over 20,000 US men and women from 2002 to 2004, 29% of men and 35% of women between the ages of 20–49 were found to have serum 25(OH)D less than 20 ng/mL (50 nmol/L) (81). In a study of young Finnish girls, 9–15 years of age, 67.7% had serum 25(OH)D concentrations less than 15 ng/mL (37.5 nmol/L) during the winter months and 3 months of supplementation with 400 IU/day of vitamin D was unable to prevent hypovitaminosis D (82). In Australia, a study that investigated serum 25(OH)D concentration in recently arrived immigrants from Africa reported that 53% of the participants had concentrations less than 10 ng/mL (25 nmol/L) and 92% had concentrations less than 20 ng/mL (50 nmol/L) (83).

Vitamin D deficiency is also a growing concern for athletes. Hamilton et al. (84) investigated Middle Eastern sportsmen and found that 91% were vitamin D deficient (<20 ng/mL (<8 nmol/L)) and the entire cohort was insufficient (<30 ng/mL

(<12 nmol/L)). Constantini et al. investigated the prevalence of vitamin D insufficiency and deficiency among young Israeli athletes and dancers (85). They reported that only 27% of the cohort ($n=98$) was vitamin D sufficient (≥ 30 mg/mL). Additionally, vitamin D insufficiency was reported in 48% of athletes participating in outdoor sports (tennis, soccer, running, triathlon, and sailing) and 80% of athletes participating in indoor sports (dancing, basketball, swimming, Tae Kwon Do, judo, gymnastics, and table tennis). Our lab looked at vitamin D insufficiency and deficiency over the course of the year in collegiate athletes and found that 12.2, 63.6, and 20.0% of athletes were either vitamin D insufficient or deficient in the fall, winter, and spring, respectively (86).

Lovell (87) investigated elite Australian gymnasts and reported that 15 of the 18 gymnasts studied had vitamin D concentrations below optimal (<30 ng/mL (75 nmol/L)). The group mean was 22.4 ng/mL (56 nmol/L). Also of particular importance was that 13 of the 18 gymnasts had experienced a bony stress injury within the previous year. Garcíá and Guisado investigated serum 25(OH)D concentrations in male professional basketball players (88). They reported a mean serum 25(OH)D concentration of 47.8 ± 21.8 nmol/L immediately following the winter months. Serum 25(OH)D concentrations were associated with vitamin D intake, independent of sun exposure. They concluded that professional basketball players were at a higher risk of hypovitaminosis D after winter. Lehtonen-Veromaa et al. investigated the incidence of hypovitaminosis D and effects of supplementation in young (9–15-year-old) Finnish female athletes (82). In the cohort, 13.4% of the athletes had severe hypovitaminosis D (<20 nmol/L) and 67.7% of the athletes had moderate hypovitaminosis D (20–37.5 nmol/L) at baseline. Additionally, 2.2% of the participants had serum 25(OH)D concentrations less than 10 nmol/L. One year later, after a minimum of 3 months of supplementation of 400 IU/day, 9.1% still had severe hypovitaminosis D and 63.4% of the athletes had moderate hypovitaminosis D.

Research has shown that there is seasonality associated with vitamin D status (89, 90). Hall et al. observed a significant difference between winter and summer serum 25(OH)D concentrations in a cohort of 72 individuals of both African and European ancestry (91). They further reported a difference in serum 25(OH)D concentrations based upon skin pigmentation (reflectance). Participants of European ancestry had significantly greater serum 25(OH)D concentrations than those of Hispanic, African, and North and South Asian descent. These findings were consistent throughout the year. Snellman et al. investigated the seasonality of vitamin D in a twin study and reported a significant difference between summer and winter serum 25(OH)D concentration (89). Collectively these studies suggest that latitude and elevation (86), skin pigmentation (91), time of day during training, percent body fat (92), and time of year (86, 89) may all influence the serum 25(OH)D status of athletes. Other important factors may include indoor training and amount of skin exposed during training.

VITAMIN D RECEPTOR AND SKELETAL MUSCLE

Identification of the VDR in skeletal muscle was an important discovery. Simpson et al. (93) first identified the receptor in cultured myoblast cells from rats which was confirmed by Boland (94) shortly thereafter who identified the receptor in myoblast cells in chicks. VDR has a high specificity for 1,25(OH)₂D and this property was

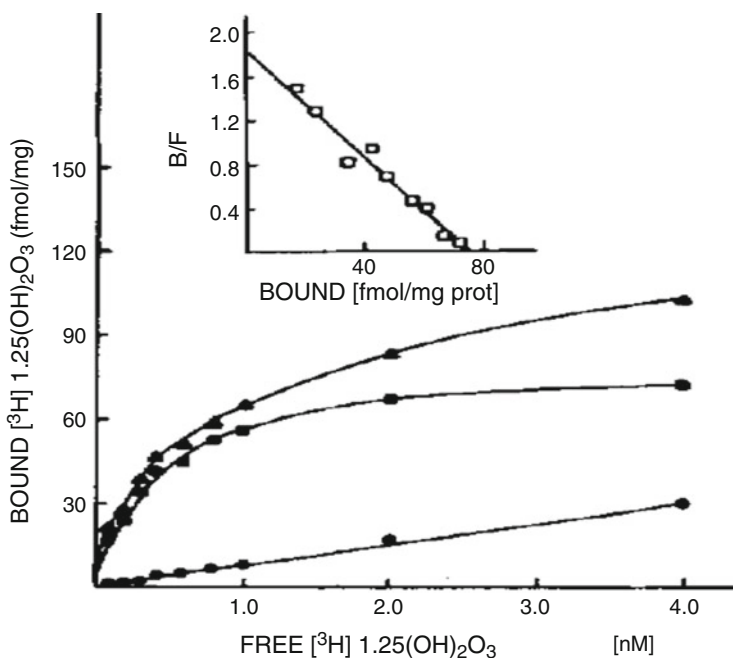


Fig. 3. Embryonic chick skeletal muscle myoblasts: saturation analysis of (3H) 1,25-dihydroxyvitamin D₃-binding cytosol from embryonic chick skeletal muscle myoblasts. Cytosol (1.0 mg protein) was incubated with increasing concentrations (0.01–4 nM) of (3H) 1,25-dihydroxyvitamin D₃ in the presence or absence of 100-fold molar excess of radioinert 1,25-dihydroxyvitamin D₃ at 4°C for 16 h. Bound and free 1,25-dihydroxyvitamin D₃ was separated with hydroxyapatite. The saturation plot is of total (*triangle*), specific (*dot*), and nonspecific binding (*filled circle*) (reproduced, with permission, from Elsevier Limited (94)).

instrumental in the initial identification. The binding shown by Boland was both specific and nonspecific, but the specific binding had a high affinity and a low capacity (Fig. 3) (94). The binding affinity refers to the strength of the interaction, and capacity refers to the actual amount of the sample that binds to the medium. This evidence was in support of the theory that skeletal muscle is a target organ with direct physiological actions for 1,25(OH) $_2$ D. Simpson et al. (93) also identified the VDR but were able to show additionally that VDR concentration decreased after myoblast cells fused and differentiated into myotubes and that DNA synthesis and cell proliferation of the myoblast line were inhibited by 1,25(OH) $_2$ D. These findings are indicative of direct physiological action of vitamin D in skeletal muscle, at least in the myoblast stage.

More recently, Endo et al. (95) worked with VDR null mice to investigate skeletal muscle development. They found that VDR null mice had significantly smaller muscle cells (approximately 20% in diameter) at 3 weeks than their wild-type littermates and more prominent changes in the muscle cells at 8 weeks. This suggests that the effects may be either additive based on systemic metabolic changes or progressive based upon the length of time with no VDR. Additionally, VDR null mice had increased expression of embryonic- and neonatal-type myosin heavy chain (MHC) at 3 weeks which was still expressed at 8 weeks only in VDR null mice. Type II MHC was expressed equally between the VDR null mice and wild-type littermates.

These findings were further supported by the increased expression in VDR null mice of Myf5, E2A, and myogenin (95). Myf5 is responsible for regulating muscle differentiation, E2A further differentiates into genes E12 and E47, and myogenin is responsible for the coordination of skeletal muscle development and repair. This increased expression of MyoD transcription factors suggests that the downregulation of myogenic differentiation factors require VDR and 1,25(OH)2D.

It is also likely that these morphological changes are due to a primary physiological rather than a secondary effect. In the aforementioned series of studies, the authors put forth three lines of evidence to support the direct physiologic role of VDR actions in skeletal muscle. First the VDR null mice developed apparent morphological abnormalities in skeletal muscle and a deregulated pattern of muscle gene expression before weaning. Secondly, the same changes were still observed in older rescued VDR null mice fed with a high-calcium diet, and thirdly, direct negative regulatory effects of 1,25(OH)2D on muscle gene expression were at least in part reproduced in cultured myoblasts *in vitro* (95). The authors postulate that this may have implications in adult models as well, particularly in cases of remodeling following injury, denervation, or immobilization. Further research, however, is necessary to confirm these earlier findings and fully elucidate the impact of VDR on differentiating myotubes.

VITAMIN D AND SARCOPENIA

Sarcopenia is the term used to describe the loss of muscle mass and strength that is often associated with aging. This is considered to be an important connection with impairment and may be a factor in disability of the aged and elderly (96). Sarcopenia is thought to exhibit a disproportionate atrophy of type IIa fibers and a decrease in the synthesis rate of myosin heavy-chain proteins (96, 97). There may also be a loss of growth factors, an increase in catabolic factors, or a combination of the two. Growth factors include neural growth factors, growth hormone, and estrogens and androgens, while catabolic factors may include inflammatory cytokines.

Vitamin D has been indicated to have a role in reducing the age-related decline in muscle function. Several well-designed clinical studies in older men and women have reported correlations between reduced 25(OH)D concentration and reduced muscle strength, gait speed, grip strength, and muscle mass (98–102). Lower 25(OH)D concentration has also been shown to correlate with increased PTH concentration and increased risk of falls (20, 100).

More recent studies have focused on the role of vitamin D in older populations, particularly regarding sarcopenia. In a prospective study of community-dwelling, older men and women (62 ± 7 years), Scott et al. (103) reported that individuals with 25(OH)D less than 20 ng/mL (50 nmol/L) had lower average leg strength, leg muscle quality, and appendicular lean mass. A higher 25(OH)D concentration was also modestly but significantly associated with greater muscle mass and was also predictive of greater muscle strength and muscular quality. In a 3-year longitudinal study, Visser et al. reported that individuals with baseline 25(OH)D concentration of less than 10 ng/mL (25 nmol/L) were more likely to develop sarcopenia compared to those with 25(OH)D concentration of at least 20 ng/mL (50 nmol/L) (98).

Rejnmark reviewed 16 randomized controlled trials that assessed muscle function after vitamin D intervention (102). Overall, supplementation between 800 and 1,600 IU/day of vitamin D was not found to improve grip strength, but several trials reported improvement of gait speed and body sway. It is important to note that of the 16 trials, only one involved patients less than 50 years of age (10–17 years old). Randomized controlled trials that have investigated various levels of vitamin D supplementation have received mixed results. Moreira-Pfrimer et al. reported a 16.4% increase in strength of hip flexors (SHF) and a 24.6% increase in strength of knee extensors (SKE) in a group of institutionalized elderly (≥ 60 years of age) receiving calcium/vitamin D treatment for 6 months (99). The participants in the treatment group were supplemented with 1,000 mg/day of calcium throughout the trial and received 150,000 IU of vitamin D once a month for the first 2 months and then 90,000 IU once a month for the next 4 months. This treatment increased serum 25(OH)D concentrations from an average of 18 ng/mL (46 nmol/L) to 34.9 ng/mL (87 nmol/L). This was compared to no improvement in a calcium/placebo group in the absence of physical training. Bischoff et al. reported a 49% decrease in falls in elderly women residing in a long-term geriatric care facility, after receiving a calcium/vitamin D treatment of 1,200 mg/day and 800 IU/day, respectively, for 12 weeks (100). Although the individual strength scores did not report a significant improvement in musculoskeletal function in the calcium/vitamin D treatment group, there was a significant improvement in overall muscle function when comparing groups over time.

Although several studies have reported a significant increase in muscular strength and function with vitamin D supplementation, there are other studies that have reported no significant improvements with supplementation. Cordless et al. reported no change in muscle strength or activities of daily living following 6 months of supplementation with 9,000 IU/day of vitamin D₂ which increased status from 7.2 ng/mL (18 nmol/L) to 48 ng/mL (120 nmol/L) (104). The subjects, who were elderly patients at an in-care facility on a geriatric ward, also had no reported improvement in mental assessment scores as compared to the placebo treatment group. Latham et al. investigated whether a single oral dose of 300,000 IU of vitamin D₂ would reduce falls and improve physical health in frail older people after hospitalization (105). After 6 months of treatment which increased average serum 25(OH)D concentrations from 15.2 ng/mL (38 nmol/L) to 24 ng/mL (60 nmol/L), there were no significant changes reported in strength, balance, and a timed walking test. There were also no reported differences in number of falls during the 6-month trial period, compared to the placebo group.

The variability of the results of these studies may be attributed to several factors. First, there was a wide range in the age of the participants (50–99 years). Research has shown a decrease in skeletal muscle VDR associated with aging. Secondly, while there was a significant improvement in mean serum 25(OH)D concentrations in all of the studies, many of the individual participants were still considered vitamin D deficient or insufficient. Finally, several of the trials did not report PTH concentrations which, if elevated, would have drastically impacted the results. Additional research investigating the role of vitamin D in sarcopenia is essential, particularly randomized clinical trials that explore the role of vitamin D supplementation on muscle strength and function.

VITAMIN D AND EXERCISE PERFORMANCE

The role of vitamin D on athletic performance was first investigated by German researchers in the 1920s (106). In the late 1920s German sports teams were using UV radiation as an artificial ergogenic aid. By the 1930s, Russian researchers followed suit. One study reported a 7.4% improvement in 100-m dash time for male students undergoing physical training and irradiation treatment (106). This was compared to a 1.7% improvement in men undergoing the same physical training but without irradiation treatment. This was followed by abundant research in Germany, albeit not of the quality of research of today's standards, on UV irradiation and physical performance which included studies on gymnasts, swimmers, untrained adults, and even schoolchildren (106).

In the USA, the first published study involving irradiation and physical performance was conducted in 1945 when Allen and Cureton irradiated 11 male college students for 10 weeks and compared them with a control group undergoing identical physical training (107). The treatment group had a 19.2% improvement in cardiovascular fitness compared with a 1.5% improvement in the control group. Rosentswieg (108, 109) investigated the effects of ultraviolet radiation on both endurance and strength in 23 college-age women. While trends towards an improvement in both strength and endurance were noted, these improvements were not significant. In both studies, however, participants were tested within 4 h of the radiation treatment, which may not have been enough time for the ultraviolet exposure to increase vitamin D availability.

Recently, Ward et al. (110) investigated the relationship of 25(OH)D and PTH with muscle power and force in 12–14-year-old postmenarcheal females from an inner city middle school ($n=99$). The low average 25(OH)D concentration (11.6 ng/mL) and elevated PTH concentration (4.87 pmol/L) indicated that the majority of girls were vitamin D deficient. Vitamin D status was strongly predictive of jump velocity, jump height, power, and body mass-adjusted force (expressed as N/kg) but not absolute force. In this study, weight was used as a quadratic term in the model because a linear regression didn't hold across the whole weight range. Although none of the girls had any symptoms of vitamin D deficiency, vertical jumping may detect subclinical effects of 25(OH)D status. Jumping mechanography was used as a marker of muscle function because previous reports had suggested that proximal muscles are most often affected in vitamin D deficiency and proximal muscles are the most important muscle groups in the jumping mechanism (102).

These results suggest that young vitamin D-deficient individuals may not be able to maximally generate force which, in turn, may not be able to maximally load and develop bones. Typically, when an individual jumps, the maximum force generated is 3–3.5 times an individual's body weight, which wasn't supported in this study. These results may be of particular importance for various populations. In younger populations this may result in abnormal bone mineralization, whereas it may decrease performance in athletic populations and impair bone health maintenance in older populations.

In a follow-up study, Ward et al. supplemented a subpopulation of the original cohort ($n=72$) over the course of 1 year with four doses of 150,000 IU of vitamin D (approximately 1,650 IU/day) and retested them for muscle jump velocity (111). After 1 year of supplementation, efficiency of movement was increased by 5% in the treated group but no other improvements were found. Improvement in efficiency of movement, however,

suggests a higher flexibility and increased muscular coordination due to treatment. It is worth noting that there was also a significant baseline 25(OH)D by group interaction for jumping velocity which was driven by greater change in jump velocity in those with the lowest baseline 25(OH)D concentrations (111). Because the vitamin concentrations in the treated group were still suboptimal (56.0 nmol/L), there may have been a significant change if the vitamin D concentration was within the optimal range.

El-Hajj Fuleihan et al. investigated the effects of 1 year of supplementation with two different doses of oral vitamin D (1,400 or 14,000 IU/week) in school-aged children (10–17 years of age) (101). They found a significant increase in lean mass in premenarcheal girls. Consistent trends for increased bone mineral density (BMD) and/or bone mineral content (BMC) were reported. There were significant increases for trochanter BMC in both treatment groups and at the lumbar spine BMD in the lower treatment group. No significant improvements, however, were found in lean mass, BMD, or BMC in boys or postmenarcheal girls.

SUMMARY

Vitamin D has a long and interesting history that began with the first description of rickets in 1645 and continues today with research investigating its role in immunity, chronic disease prevention, and even muscle function and athletic performance. As a vitamin that “acts like a hormone,” vitamin D plays a long-recognized role in calcium homeostasis targeting bone, intestine, and kidneys and a more recently recognized role in immune modulation and reproductive function. It may protect against multiple sclerosis, certain cancers, autoimmune rheumatic disorders, diabetes, high blood pressure, and cardiovascular disease.

Vitamin D can be obtained in the diet or can be synthesized in the skin from UVB light. Very few foods contain vitamin D including oily fish like salmon, mackerel, and sardines; cod liver oil; and fortified foods like milk, margarine, and juice. Several factors influence cutaneous synthesis including latitude, elevation, skin pigmentation (reflectance), age, time of day, season, and sunscreen use. Because of these factors, vitamin D insufficiency and deficiency has been observed worldwide in various populations. Future research is needed to elucidate the role of vitamin D in both muscle function and athletic performance.

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19

The Effects of Altitude on the Hormonal Response to Physical Exercise

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INTRODUCTION

One of the most important roles of the endocrine system is to allow adaptation to new environmental conditions. The neuroendocrine system “feels” and “informs” the body on such environmental conditions and then triggers biological responses to induce adaptive processes. Most of the studies performed in acute or chronic hypoxic conditions focused on hormones involved in water and electrolyte balance or on the adrenergic system,

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whereas scanty data on the other endocrine axes are available. Hormones act through membrane or intracellular receptors (nuclear or cytoplasmic). Hypoxia has been reported to modulate the expression of membrane and nuclear receptors in terms of both down- and upregulation. The study of altitude effects on endocrine variations induced by physical exercise is an extremely interesting but complicated field. Most studies show limitations due to the low reproducibility of experimental protocols, to the number of subjects enrolled, and to the fact that the identification of changes of a single hormone is impaired by the concomitant adaptation of the whole endocrine system in hypoxic conditions. Moreover, several environmental factors affect the endocrine and metabolic response at the same time. For example, high altitude means both hypoxia and low temperature and is a sufficient trigger for appetite suppression and reduced caloric consumption leading to weight loss. Based on the foregoing, in this chapter we summarize literature data and our personal experience.

GROWTH HORMONE/INSULIN-LIKE GROWTH FACTOR-I AXIS

Physical exercise is an important environmental regulator of the growth hormone (GH)/insulin-like growth factor (IGF)-I axis activity (1, 2). The GH response to exercise is dependent on the duration and intensity of the exercise bout, the fitness level of the exercising subject, the refractoriness of pituitary somatotroph cells to the exercise stimuli and other environmental factors (3, 4). The neuroendocrine pathways that regulate GH secretion during exercise include the cholinergic, serotonergic, α -adrenergic, dopaminergic and opioidergic systems (5–7). The exercise-induced GH release is influenced by fluid intake, environmental and nutritional factors as well as some pathological states (8–11). Gender regulates the relationship between exercise intensity and GH release, since GH secretion is greater in women than in men (12). The acute GH response to aerobic or resistance exercise is reduced with age (7).

Exercise leads to increases in IGF-I levels, which are likely to occur via GH-independent mechanisms (13). Haemodynamic or metabolic effects of exercise per se might play a role, although long periods of exercise training are able to stimulate IGF-I gene expression (14).

The influence of altitude-induced hypoxia on the somatotroph response to physical exercise has been clearly described in different experimental conditions. Similarly to other hormonal axes, many variables have been shown to be involved in altitude-induced hypoxia, such as the actual altitude, the duration of exposure, the training degree of the subjects investigated and the type of exercise performed.

There is scanty knowledge about the response of the GH/IGF-I axis to extremely high altitude (15, 16), while more is known about the metabolic adaptation (17–21). The metabolic adaptation occurring during high-altitude exposure is mainly characterized by an increased dependence on blood glucose as a fuel with a concomitant increase in insulin sensitivity and lipolysis coupled with a decreased reliance on lipid substrates (17, 18, 21). We have recently suggested that this metabolic profile could be determined by remarkable changes in GH/IGF-I axis function (16). We found that well-trained acclimatized climbers show clear-cut increases in mean GH concentration right after strenuous physical activity and this agrees with evidence that physical exercise represents a neuroendocrine-mediated stimulus of somatotrophic secretion (1, 2, 5) as well as

with the enhancement of the GH response to GH-releasing hormone recorded in subjects chronically living at high altitude (22). Accordingly, it had been also reported that low-altitude natives adapted to high altitude show a more marked GH increase than non-acclimatized subjects (23).

Interestingly, in our study the change in GH status was coupled with a concomitant increase in mean total IGF-I and IGFBP-3 levels (16). Indeed, IGF-I is the best marker of GH status although IGFBP-3, a GH-dependent IGF-I-binding protein, also reflects chronic variations in the status of somatotrophic function (24). The clear increase in IGF-I and IGFBP-3 together with the enhancement of mean GH levels therefore clearly points towards increased activity of an anabolic axis like the GH/IGF-I at high altitude. In fact, increased activity of the GH/IGF-I axis is likely to trigger protein anabolism and might also play a role in the adaptations occurring in glucose and lipid metabolism at high altitude (18, 25).

Though performed in a simulated setting of hypoxia, a very interesting study by Engfred and colleagues underlined the importance of training in the modulation of hormonal responses to exercise in hypoxic conditions. Quite surprisingly, a 5-week training-induced changes in GH response to exercise in a group of previously untrained subjects, studied in a hypobaric chamber at a simulated altitude of 2,500 m, were not influenced by hypoxia per se (26).

Although elevated GH levels (27–29), with maintained circadian rhythm (27), have been widely described, and no changes in resting GH concentrations of sea level residents were observed during acute exposure to high altitude (28, 29), the somatotroph response to exercise has been shown to be influenced also by acute exposure to hypoxia, again suggesting a relationship with metabolic aspects.

In fact, GH concentrations during hypoxic exercise (20 min at 750 kpm/min on a cycle ergometer) performed in a hypobaric chamber (decompressed to a simulated altitude of 4,550 m) have been shown to be higher than under normoxic conditions (30). Together with other hormonal and metabolic responses, involving glucose, free fatty acids, cortisol and insulin, these changes in GH/IGF-I axis seem to be aimed to increase fat mobilization and gluconeogenesis, in order to optimize energy substrates availability (30).

Interestingly, more than 30 years ago Raynaud and colleagues hypothesized a modification of GH secretory pattern during submaximal exercise in hypoxic conditions, in presence of normal absolute hormone concentrations (28). In fact, compared with lowlanders, in highlander natives (3,800 m) the rate of GH increase at the beginning of a submaximal exercise session was faster and earlier, but the mean maximal value reached at the end of the exercise bout was similar. Moreover, the GH response pattern in lowlanders during the early stages of exposure to hypoxia resembles that of highlanders (28). The potential underlying explanations of these observations include an alteration of the hormonal clearance through a more pronounced reduction of hepatic blood flow or a difference in the state of the pituitary gland prior to the exercise bout. However, the subsequent studies did not allow to reach definitive conclusions.

In another study by Van Helder and colleagues the balance between oxygen demand and availability was suggested to be an important regulator of GH secretion during exercise (31). In five normal men, performing seven sets of seven squats at a load equal to 80% of their seven repetition maximum, these authors found a plasma GH increase during and after the completion of the exercise, coupled with a significant linear correla-

tion between GH changes and the corresponding oxygen demand/availability ratio (31). Interestingly, the existence of a significant correlation between changes in plasma GH levels and the demand/availability ratios over a wide variety of exercise (aerobic and anaerobic, continuous and intermittent, weight lifting and cycling), in both fit and unfit subjects under normoxic and hypoxic conditions, has been demonstrated.

Accordingly, almost the entirety of the studies investigating somatotroph function at high altitude, where the oxygen demand/availability ratio is increased, particularly when individuals perform physical activity, observed an increase in GH secretion. Therefore, this increase could play a role in modifying the endocrine–metabolic response to exercise to satisfy the increased needs at high altitude.

The additive stimulatory effect of hypoxia per se on the GH response to exercise has been also described in simulated conditions (32, 33), though not in terms of hepatic IGF-I production.

Differently from chronic conditions, the acute exposure to hypoxia has been shown to blunt the GH response to submaximal physical exercise in untrained individuals, but not in trained subjects (34). In line with this observation, a previous study indicated that the hormonal response, including GH, to exercise is influenced by hypoxia and physical training, mainly via changes in the relative workload (35).

These data once again emphasized the central role of physical fitness in the modulation of hormonal and metabolic adaptive responses to exercise also in altitude-induced hypoxic conditions.

PROLACTIN

Prolactin is most of all considered fundamental for lactation though it has been recognized to have important metabolic activities. In fact, it is well known that prolactin stimulates insulin synthesis and release and that pathological hyperprolactinaemia is characterized by hyperinsulinism and insulin resistance both reverted by normalization of prolactin levels (36, 37). Moreover, prolactin acts as an important connection between the endocrine and the immune system, being also produced by extrapituitary sites including immune system and being involved in lymphocyte survival, activation and proliferation (38). The endocrine/paracrine prolactin has been shown to stimulate the immune cells by binding to prolactin receptors, which are expressed on many cells of the immune system, including haematopoietic stem cells, T cells, B cells, monocytes, macrophages, NK cells, neutrophils and thymic epithelial cells (39). Prolactin is implicated in lymphoproliferation, cytokine production and antibody secretion, but it not seems to be essential, since both prolactin-deficient and prolactin receptor-deficient mice have normal haematopoiesis (40–42).

Data on prolactin and hypoxia as well as physical exercise are scanty and not concordant. Hypoxia per se, independently of concomitant physical exercise, has been shown to apparently influence prolactin secretion (43, 44), but prolactin resting levels at high altitude are reported to be elevated (43) or decreased (44, 45).

Some studies reported that prolactin levels transiently increase with exercise and this response is proportional to the exercise intensity (5, 46, 47) and others that prolactin increments occur when the anaerobic threshold is reached and appear to be correlated with pro-opiomelanocortin derivatives, ACTH and beta-endorphins (48, 49). Moreover, the prolactin increase may be related to changes in body temperature and dehydration,

is exaggerated by stress, is reduced with habituation and hypoxia and is unresponsive to metabolic events (5, 16).

Other studies suggested that dopamine, the main factor involved in prolactin regulation, and possibly noradrenaline, inhibit prolactin secretion at high altitude (50). However, while in hypoxia conditions noradrenaline consistently increases, dopamine changes are inconsistent, being either reported unchanged or increased (51–53). Another potential prolactin modulator at high altitude is erythropoietin that could promote dopamine release and, therefore, inhibition of prolactin secretion (54).

Currently very few studies specifically investigate the role of altitude on the prolactin response to exercise.

In agreement with the observation that prolactin levels are influenced by oxygen availability, an inhibition of exercise-induced blood prolactin response has been described after acute exposure to hypoxia (55). Moreover, high-altitude cold exposure has been suggested to diminish the exercise-induced prolactin response (56), though it does not seem to influence baseline levels (43).

In our model of maximal exposure to altitude over a period of 2 months, in association with a vigorous physical exercise, prolactin levels increased, but persisted within the normal range (16). An adaptive metabolic purpose could explain this significant increase in lactotropic secretion that followed the exposure to high altitude, in agreement with a previous study (43). In fact, prolactin has been shown to markedly affect glucose metabolism (36), but, on the other hand, chronic stressful conditions are known to increase prolactin secretion most likely via neuroendocrine mechanisms (57).

In a study of chronic exposure (3–12 months) to high altitude, prolactin levels have been shown not to be different from sea level values in acclimatized men, similarly to high-altitude native residents (58). On the other hand, another study in women reported a decrease in basal prolactin levels, related to the degree of hypoxaemia (44), in agreement with the low serum prolactin concentration, described in native high-altitude women (45).

However, considering the scanty data available, definitive conclusions about the actual prolactin response to exercise after chronic exposure to high altitude cannot be drawn. Nevertheless, prolactin secretion in response to exercise during acute or short-term exposure to high altitude seems to be preserved, though attenuated, accordingly to the reduced baseline prolactin levels shown at altitude. The underlying mechanism is likely represented by an enhanced dopaminergic or noradrenergic tone. In fact, an alteration at the hypothalamic level appears to be less likely since prolactin response to thyrotropin-releasing hormone stimulation was not altered by exposure to high altitude (59).

THYROID FUNCTION

The thyroid function changes secondary to physical exercise represent a complex physiological response, which is influenced by several individual and environmental factors. Levels of thyroid-stimulating hormone (TSH), thyroxine (T4), free T4 (fT4), triiodothyronine (T3) and free T3 (fT3) have been reported to be unaffected, increased or decreased varying with the type and duration of exercise, ambient temperature and energy intake (60–63). Although these divergent findings are difficult to interpret due to the highly variable exercise sessions and to procedural limitations (64), one of the more consistent findings is reverse T3 increase, particularly when a caloric energy deficiency is associated with exercise (65).

It is plausible to hypothesize a role of thyroid function in the adaptive process to altitude hypoxia, considering the well-known ability of thyroid hormones to increase oxygen availability by increasing ventilation and cardiac output as well as red blood cell mass. Moreover, thyroid hormones are known to increase levels of 2,3-diphosphoglycerate in erythrocytes, facilitating the unloading of oxygen to tissues through a rightward shift in the oxyhaemoglobin dissociation curve (66).

Accordingly, an increase in thyroid hormone release at high altitude has been described by many authors (16, 22, 67–77). In particular, most authors agree that high altitude induces an elevation in plasma concentration of both free and total T4 levels (16, 22, 67–77). T3 has been also shown to be increased (70, 73, 74, 76), although to a lesser extent than T4. On the other hand, some authors reported an increase in reverse T3 only (72, 75, 78), suggesting the possibility of a hypoxia-induced inhibition of T4 to T3 conversion, with a concomitant rise in rT3 concentration and in T4:T3 ratio (72, 78). An increase in corticosteroids secretion could explain these changes, similarly to other stressful conditions. In fact, marked physical exhaustion due to high-altitude-related hard context could negatively influence thyroid hormones levels (78). Moreover, cold per se may also contribute to inhibit thyroid hormones secretion. In fact, T3 that plays a pivotal role in cold habituation decreases with cold exposure, whereas T4 and TSH remain unchanged (79).

No clear difference in thyroid function was observed between subjects resistant or susceptible to acute mountain sickness (AMS) (71, 80), suggesting that thyroid hormones may play a different role in the different time course of acclimatization process.

The interrelationship between high altitude, hypoxia, physical exercise and thyroid axis has been extensively studied (16, 72, 74, 76, 77, 81). Although the effects of physical exercise per se are not unambiguous (60–63, 81), environmental conditions have been reported to play a relevant role (82). A study in subjects who had a short-term stay at extreme high altitude during Mt. Everest climbing reported an increase in total T4 and T3 concentrations associated with an increase in TSH levels (72). On the other hand, a significant elevation of free T4 levels after 3 weeks at 4,300 m without any change in TSH levels have been reported (77). In our study, after a 2-month stay at high altitude, we confirm the lack of change in TSH levels as well as an increase in fT4 and a significant reduction of fT3 levels that were below the lowest limit of the normal range (16). This picture suggests a high altitude-induced low T3 syndrome that would reflect an impairment of peripheral fT4 to fT3 conversion under chronic exposure to high-altitude hypoxia. Indeed, it is reasonable that prolonged exposure to hypobaric hypoxia at extreme high altitude induces a low T3 syndrome that would also be explained by the status of negative energy balance caused by strenuous physical exercise (81) and characteristic of high-altitude exposure (16, 83, 84).

As anticipated, a dissociation at high altitude between TSH (unchanged) and thyroid hormone (increased) levels has been reported by several authors (16, 22, 59, 69, 70, 73–75, 77, 85, 86).

Several explanations for the TSH-independent T4 rise have been proposed. Pituitary dysfunction has been likely excluded, since the TSH response to TRH administration has been shown to be preserved at altitude (22, 70, 72, 74, 75), although at extreme altitude an increased TSH response to TRH was found, suggesting that the severe hypoxic stress or the association with other stressors (such as cold) could influence the

pituitary response (72). A change in hormone levels can be caused by either a modified secretion rate, a disturbed clearance or haemoconcentration and vascular shift. The T4 rise at high altitude cannot be simply explained by dehydration and haemoconcentration evaluated by the concentration of total plasma proteins (87). Actually, in contrast with a potential decreased T4 clearance, the T4 degradation rate has been shown to be increased during acute exposure to altitude (20). A potential role of increased thyroxine-binding globulin (71, 73, 87–90) or enhanced β -adrenergic stimulation (68) has been hypothesized but not definitely demonstrated.

The impact of caloric restriction on the endocrine response to physical exercise at high altitude has been elegantly investigated by Barnholt and colleagues (77). During 3 weeks at 4,300 m they found no difference in fT4 (increased) and TSH (unchanged) secretory patterns between a group of active subjects adequately fed to maintain body weight and another under caloric restriction. The authors hypothesized that the hypoxic stimulus at altitude is capable of overriding the fall in T4 induced by caloric restriction (77).

On the whole, thyroid response to physical exercise at high altitude seems to be increased with respect to sea level in different experimental models and during different types of exercise, likely contributing to the adaptative process. However, some environmental factors associated with altitude exposure, such as cold, could negatively modulate thyroid function. The apparent dissociation between TSH and thyroid hormones is not fully understood, but does not likely reflect an alteration at the pituitary level.

GONADAL FUNCTION

The effects of physical activity on the reproductive axis in males vary with the intensity and duration of the activity, the fitness level of the individual and his nutritional metabolic status. Short, intense exercise usually increases while prolonged exercise usually decreases serum testosterone levels (91–93). The exercise-associated increment in circulating testosterone does not seem to be mediated by luteinizing hormone (LH). Possible mechanisms such as haemoconcentration, reduced clearance and/or increased testosterone synthesis may be involved (94, 95). Both central and peripheral mechanisms may explain the testosterone decrease during and subsequent to more prolonged exercise, including decreased gonadotropins, decreased or increased prolactin levels, alterations in testosterone production and/or secretion (91, 96–98).

Few studies have investigated the impact of altitude on the relationship between gonadal hormones secretion and physical exercise. In fact, most of the studies describing gonadal function at altitude do not analyse the concomitant specific effect of physical exercise. Moreover, there are no studies specifically focusing on this topic in females.

Testosterone resting levels have been reported to be increased after acute (few days) exposure to moderate altitudes (99, 100) by some authors, but not by others (43, 101). An activation of adrenal function could contribute to the increase in testosterone levels, whereas increased prolactin and estradiol concentrations could account for a decrease in testosterone secretion (16, 43, 101).

On the other hand, chronic exposure to hypoxia apparently does not influence testosterone levels in adult high-altitude natives (102), although an earlier increase in

testosterone and subsequent onset of puberty have been described in young high-altitude males (103).

Some authors have reported high altitude-induced increase in progesterone levels but no change in pituitary, gonadal and adrenal hormones in subjects who had a prolonged stay at high altitude but were not performing any physical activity (58). Accordingly, progesterone has been suggested to positively modulate the hypoxic ventilatory response in polycythaemic high-altitude residents (104). Other data conversely reported that a prolonged exposure to high altitude was coupled with an increase in prolactin but decrease in LH and testosterone levels (43).

We have recently reported a significant testosterone decrease, associated with a concomitant increase in progesterone, in climbers exerting strenuous physical exercise at high altitude (16). Accordingly, a reversible spermatogenic and Leydig cell dysfunction has been suggested in members of another alpine high-altitude expedition (105).

Testosterone decrease would simply reflect a stress-induced depression in the function of the gonadal axis, mainly due to the combined negative influence of hypoxia and strenuous physical exercise. In fact, reduced testosterone levels have been recorded in men performing physical exercise in hypoxic conditions (16, 43, 77, 106, 107) as well as in subjects undergoing endurance training (91). Moreover, this high-altitude gonadal profile would be negatively affected by prolactin increase (16, 108) and the fact that the GH/IGF-I axis is concomitantly activated while testosterone is decreased (16) may explain the lack of anabolism and the increased dependence on glucose utilization.

The increase in progesterone levels in hypoxic conditions at high altitude could be viewed as a stimulus for the respiratory drive. In fact, progesterone has been suggested to be a potent respiratory stimulant in the physiological regulation of breathing by increasing the sensitivity of the respiratory centre to carbon dioxide (CO₂) (109, 110).

Moreover, this positive progesterone effect would be favoured by the concomitant decline in testosterone levels that are known to exert a downregulation of progesterone receptors (109).

It has been clearly suggested that the chronic testosterone response to physical exercise at high altitude may also be modulated by caloric consumption (77). Acutely, testosterone levels increased regardless of energy balance, but when food intake was controlled in order to maintain body weight at 4,300 m, a gradual rise in serum testosterone concentration persisted (77).

However, in line with the hypothesis that effects of altitude and caloric restriction would oppose each other, the influence of chronic altitude exposure on testosterone levels seems to be mitigated when the energy balance is negative. In fact, some studies taken after periods of intense trekking or diminished caloric intake (58, 105) often show a decrease in pituitary–testicular hormone release that may be the result of the confounding influences of a negative energy state rather than altitude. Therefore, the steady decline of testosterone levels over time in negative conditions of energy balance may represent an adaptive response of the reproductive system to a low-energy, catabolic state (111).

Interestingly, during intense anaerobic exercise the relationship between LH and testosterone is modified, since testosterone increases without any significant elevation of LH (112). On the other hand, resting high-altitude levels of LH and follicle-stimulating hormone (FSH) tend to decrease (43, 99, 101, 113), but without concomitant

variations in testosterone secretion, likely reflecting modulations of other mediators (43), as occurred during intense physical exercise.

When lowlanders acclimatized to 3,542 m trekked to an extreme altitude of 5,080 m, plasma testosterone decreased but then progressively normalized after 6 months of stay at 6,300 m (58). Concomitantly, LH levels after trekking to 5,080 m was higher than at an altitude of 3,542 m, but decreased thereafter during prolonged residence at extreme altitude. Plasma progesterone was increased after a 6-month stay at extreme altitude (58).

The potential impairment of reproductive function has been recently investigated in male mountaineers involved in an expedition at high altitude (5,900 m) (114). The authors concluded that exercise at high altitude might be associated with a direct transitory testicular dysfunction, resulting in a reduced number of ejaculated sperm, mostly due to a defective spermiation (114).

On the whole, considering the testosterone decrease in the short term and the subsequent normalization of gonadal hormone profiles observed at high altitude, as well as the unlikely presence of an insufficient pituitary function (59), a transitory Leydig cells dysfunction could be hypothesized, mainly due to high-altitude hypoxia per se.

SYMPATHOADRENAL SYSTEM

The sympathoadrenal system plays a critical role in regulating a number of physiological functions necessary to control the stress imposed by physical exercise and by altitude exposure, such as heart rate, vascular resistance, stroke volume and blood pressure (115).

An increase in the plasma concentration of catecholamines during dynamic as well as during static exercise has been reported in humans (60). Work intensity, relative workload and the duration of exercise are the major determinants of the sympathoadrenergic response to exercise (35, 116–118). Similarly the acute exposure to reduced partial pressure of oxygen at high altitude stimulates the sympathoadrenal system (119), likely stimulating arterial chemoreceptor, although some studies did not show an acute hypoxia-induced modification of resting plasma or urinary norepinephrine (119).

A differential adaptative response between sympathetic neural activity and adrenal medulla activity has been shown during exposure to high altitude, as described by Mazzeo and co-workers (120). On arrival to 4,300 m, epinephrine arterial concentration was significantly increased both at rest and during prolonged low-intensity exercise (120). On the contrary, arterial norepinephrine concentrations were lower than those observed at sea level in resting conditions, while were increased to similar values after only 45 min of submaximal exercise (120).

Plasma epinephrine and norepinephrine levels during mild exercise are not affected by the inhalation of a gas mixtures containing a concentration of oxygen (19–13%) equivalent to an altitude of 700–3,700 m above sea level. However, the higher the intensity of exercise (>50% of VO_2max), the greater the increment of plasma epinephrine and norepinephrine levels during hypoxia (121). In addition, the response to short duration exercise under hypoxia is closely linked to the relative workload (35).

A strong correlation between catecholamines and glucose/lactate turnovers has been reported (17, 120, 122, 123). Thus, circulating norepinephrine concentration correlates with the glucose rate of appearance (17), allowing an increased use of blood glucose

during hypoxic exercise. It is important to underline that exposure to cold environments could also stimulate the release of catecholamines, which, in turn, stimulate thermogenesis (82).

The most important modification of the adrenergic system in response to prolonged hypoxia is the desensitization of β -adrenergic receptors. This leads to a lower heart rate response to adrenergic activation which is similar to that observed in response to physical training (89, 124).

Therefore, a chronic exposure to high altitude could cause an abolition of the adrenergic system acute modifications. In fact, residents at high altitude show plasma catecholamine concentrations similar to those in lowlanders (125).

In contrast, most studies on subjects staying more than 1 week at high altitudes reported an increased sympathoadrenergic activity, suggesting that the duration of hypoxic exposure is one of the major determinants of the sympathoadrenergic response to exercise (126).

Once again, the study from Barnholt and colleagues pointed towards the importance of energy balance in modulating the hormonal response to exercise even at high altitude. In fact, they showed that the expected acute altitude-induced epinephrine increase in subjects under caloric restriction was significantly lower than that experienced by subjects in adequate energy balance (77). On the other hand, these authors found that norepinephrine levels rose gradually over the first days of exposure in both groups (77), suggesting that the sympathetic nervous activity increased independent of energy intake status.

The adrenergic pattern secretion described by Barnholt is in line with the “dissociation” theory between the adrenal medulla and sympathetic response to high-altitude exposure (127, 128). Therefore, it could be hypothesized that hypoxia per se can act directly on the adrenal system to secrete epinephrine based on the severity of hypoxaemia, even before sympathetic activity is elevated (129).

The dampened epinephrine rise in physically active subjects under negative energy balance (77) supports the concept of a negative interactive effect between hypoxia and caloric restriction on the adrenal medulla.

Importantly, this adrenergic pattern in response to exercise at high altitude may diminish the body compensatory response to reduced arterial oxygen, placing calorie-restricted sojourners at a greater risk for altitude sickness and decrements in physical performance. Furthermore, the negative influence of caloric restriction on epinephrine availability could also reduce muscle glycogenolysis directly and hepatic glucose production indirectly, thus decreasing carbohydrate availability and use upon acute altitude exposure.

HYPOTHALAMUS–PITUITARY–ADRENAL AXIS

The hypothalamus–pituitary–adrenal (HPA) response to hypoxia has long been investigated due to its possible involvement in high-altitude acclimatization (130). Whereas acute exposure to moderate altitude does not appear to increase plasma glucocorticoid levels, more severe hypoxia does result in an increase in adrenocorticotrophin hormone (ACTH) and corticosteroids in various species (126).

Accordingly, a more marked rise in plasma and urinary cortisol has been shown. This may precede the onset of symptoms in subjects developing AMS (131). The diurnal rhythm of cortisol is maintained at high altitude and is accompanied by a parallel

variation in AMS score (132). After 1 week at 6,542 m, plasma cortisol has been found elevated at first and then decreased with subsequent acclimatization, further supporting an association with AMS (132, 133).

Exercise represents a potent physiological stimulus on the HPA axis (134). Glucocorticoids exert many beneficial effects in exercising humans, increasing the availability of metabolic substrates for the need of energy of muscles, maintaining normal vascular integrity and responsiveness and protecting the organism from an overreaction of the immune system in the face of exercise-induced muscle damage (135).

Exercise intensity and duration are the major factors affecting the activation of the HPA axis (112, 136, 137). The response of the HPA axis to physical activity is independent of age and gender, and is affected by hypohydration, meals and time of day (135, 138–141).

When the HPA axis is repeatedly challenged by exercise, adaptation processes are activated in order to protect the body from the severe metabolic and immune consequences of increased cortisol levels (142).

Under moderate hypoxia the cortisol response to physical exercise was found to be unchanged (143), augmented (131) or decreased (144). Moreover, the cortisol response to exercise is correlated with ACTH in normoxic, but not in hypoxic conditions (144), suggesting a decreased adrenal sensitivity to ACTH when exercising in hypoxia.

However, the cortisol release from adrenal cells *in vitro* is not affected by changes in oxygenation nor in adrenal blood flow (126). Since chronic carotid body chemodeneration attenuates the ACTH and cortisol response to acute hypoxia (126), it can be hypothesized that peripheral arterial chemoreceptors are essential to the HPA response to exercise under acute hypoxia.

ACTH is not the sole controller of cortisol secretion during hypoxic stress. In fact, other factors can also interfere, such as adrenal blood flow (higher in spontaneous ventilation), stimulation of pulmonary stretch receptors (which can inhibit ACTH response) and low adrenal tissue pO_2 (145). A possible direct relation between hypoxic ventilatory response and plasma cortisol has been suggested (145) but not subsequently demonstrated.

In addition to the effects of intrinsic characteristics of the exercise stimulus, many other stress factors could modulate the hormonal response to exercise (i.e. intensity and/or oxygen availability, hydration, nutrition, low temperature). For example, as frequently observed at high altitude, corticosteroids also become elevated during hypothermia (82). An inverse relationship between 11-hydroxy-corticosteroid plasma concentrations and the degree of hypothermia exists. In one study, the highest corticosteroid concentrations were measured in hypothermic individuals who died, with respect to those who survived (82). However, another study did not find any correlation between plasma cortisol concentration and core temperature with respect to survivability (82).

One very recent study hypothesize that the cortisol response to exercise, also in altitude conditions, could be influenced by the degree of stress in athletes, secondary to a potential state of anxiety and low self-confidence (146).

All these data suggest that acute high-altitude exposure does not significantly modify the physiological HPA response to physical exercise. There is some evidence for an increase in pituitary ACTH content and number of corticotropic cells in the anterior pituitary in rats chronically exposed to hypoxia, suggesting a chronic activation of the HPA axis (126).

Indeed, pituitary and adrenal hypertrophy have been demonstrated in rats exposed to a simulated altitude of 5,500 m (147). In humans, plasma and urinary cortisol both increase with the time and level of exposure to altitude (126). Little information is available about the effect of lifelong exposure to hypoxia on cortisol concentration. Maresh and co-workers reported a greater cortisol response to a simulated altitude of 4,760 m in lowlanders as compared to moderate-altitude natives, suggesting some adaptation of the system to chronic hypoxia (148). However, the cortisol response to maximal exercise was similar in both groups whether tested at their respective residence altitude or in hypobaric chamber, suggesting that the stimulus provided by exercise alone, and not the hypoxic environment, is responsible for increased cortisol levels after exercise (149).

Altitude per se has been shown to exert a strong influence over the HPA axis (15, 99). Recently, Barnholt and colleagues confirmed an acute and persistent increase in cortisol levels in active subjects over a short-term stay at high altitude (77). This pattern of cortisol secretion has been shown to be accentuated when altitude exposure is associated with another stress, such as caloric restriction (77).

On the other hand, other studies showed no significant changes in cortisol secretion with respect to sea level (16, 58, 150). This lack of significant changes in cortisol and ACTH levels can be explained by the low reliability of a single basal evaluation of cortisol, as performed in many studies, to adequately investigate the HPA axis function and therefore to exclude some stress-induced derangement.

Such discrepancies in different studies (15, 16, 58, 99, 150) and the difference in acute cortisol response depending on energy balance (77) may reflect a varying response based on substrate availability. In fact, cortisol has a catabolic effect on fat and proteins and is known to increase circulating free fatty acids, glycerol and amino acids (151, 152). Therefore, since energy deficiency at altitude has been shown to increase dependence on lipid metabolism (153), elevated cortisol concentrations may help to compensate for the early changed glucose response in calorie-restricted subjects by providing free fatty acids as an alternate fuel and/or stimulating gluconeogenesis via elevated precursor availability (glycerol and amino acids).

ANTIDIURETIC HORMONE

The relationship between antidiuretic hormone (ADH) system and altitude exposure, during which relevant changes in body water distribution and electrolytes occur, is intuitive. Hypoxia has been suggested to alter ADH regulation by raising the osmotic threshold and increasing ADH responsiveness above that threshold (148).

It is important to note that plasma ADH assay is quite variable (154). Thus, the potential reproducibility of results from studies on the interrelationship between ADH, physical exercise and altitude exposure should be interpreted very cautiously.

It has been shown that ADH increases during exercise, its response being modulated by the intensity and duration of exercise and, at the same time, by the degree of hypoxia (155). In fact, a short-term exercise fails to increase plasma ADH, which increases after 2 h of a similar workload bout (155).

Similarly, a very short-term exposure (20 min) to mild hypoxia (about 3,400 m) was found to result in a significant reduction in plasma ADH, which returned to sea level values with more severe hypoxia (5,000 m). On the contrary, as soon as hypoxia begins

to be intolerable (presence of nausea, headache, respiratory distress), ADH increases, oedema worsens and antidiuresis is present. That is because diuresis, allowed by a decrease or lack of increase in ADH, is believed to be a normal response to hypoxia as long as altitude is tolerated (126).

The most important action of ADH is to facilitate the reabsorption of free water from the glomerular filtrate. It is obvious that a water deprivation stimulates and a salt-free diet decreases ADH secretion. These could be confounding factors in the regulation of ADH response in a high-altitude context.

Plasma ADH does not appear to increase significantly during exposure to chronic hypoxia under normal conditions (126). In fact, sustained exposure (17 days) to 5,400 and 6,300 m resulted in serum osmolality increase with no change in ADH levels, suggesting a decreased ADH sensitivity to the osmotic stimulus and thus a possible hypothalamic/posterior pituitary dysfunction (156). On the other hand, exercise-induced ADH elevation was found in those subjects that were susceptible to AMS, prior to or while experiencing it (157, 158). In particular, Bärtsch and co-workers examined ADH plasma levels in subjects before and during a sojourn of 3–4 days at 4,559 m both at rest and during 30 min of exercise on a cycle ergometer (158). No significant changes in ADH occurred at rest under exposure to altitude for 4 days, both in subjects susceptible or not to AMS (158). Instead, during the exercise test, a significantly greater increase in ADH levels was observed in subjects developing AMS, compared to those who stayed without symptoms during the 4 days at 4,500 m (158). A possible reason of this association could be that the release of ADH in the central nervous system by increasing brain capillary permeability could cause AMS (158, 159).

RENIN–ANGIOTENSIN–ALDOSTERONE SYSTEM

Considering the central role of renin–angiotensin–aldosterone system (RAAS) in the physiological regulation of volume and blood pressure, it is not surprising that this was one of the first endocrine systems to be investigated in high-altitude adaptive processes. In fact, the first indirect information suggesting a decrease in aldosterone at altitude derived from 4 subjects during an expedition to the Himalayas in 1956 (160). A subsequent study in 7 subjects exposed for 24 days at 4,350 m confirmed this previous observation (161).

Nevertheless, also due to the several variables involved in the fine-tuned regulation of RAAS, the subsequent studies did not permit to draw definitive and concordant conclusions about the actual functional modifications of RAAS at altitude, in particular during concomitant physical exercise. In fact, although almost all studies describe a low plasma aldosterone concentration (PAC), they report variable responses in terms of plasma renin activity (PRA) in hypoxic conditions: resting PRA was found increased (162, 163), unchanged (15, 131, 164, 165) or decreased (99, 133, 166–172). On the other hand, PAC or aldosterone urinary excretion was more consistently decreased (131, 161, 164, 166, 167, 169–171, 173, 174), although some authors report an unchanged (165) or even increased (15, 99, 162) aldosterone response, possibly resulting from reduced sodium intake.

Exercise has been shown to represent an activator of RAAS (175–178), in particular in terms of absolute rather than relative work load (179). Hypoxia blunts the exercise-

induced increase in PRA and aldosterone secretion (170, 174, 180), although when adequate hydration is maintained, RAAS seems to be inhibited by mild and prolonged exercise and is not influenced by hypoxia (181).

Moreover, the RAAS response to altitude hypoxia can be also modulated by other associated stressors, such as the stress of a rapid ascent at altitude (162, 182), exposure to cold, danger and strenuous exercise (15).

It has been shown that, after a prolonged exposure to hypoxia, RAAS response to exercise was exacerbated under re-exposure to normoxia (183, 184). Concomitantly, a decrease in resting PRA and an abolished response to exercise have been suggested after 3 weeks at high altitude (180), likely due to a blunted response of renal β -receptors (125). This phenomenon has not been observed in high-altitude natives, in whom PRA concentration at rest was increased, though the aldosterone response to renin during exercise was attenuated (125).

Altogether, it could be concluded that normal acclimatization to high altitude is associated with a clear suppression of RAAS, likely via multiple mechanisms, such as modified renal perfusion pressure and blunted adrenergic responsiveness. Moreover, once again, the importance of diet and hydration status can deeply modulate the exercise-induced modifications in RAAS function during hypoxic conditions.

HORMONAL MODULATION OF ENERGY BALANCE AT HIGH ALTITUDE

Subjects exposed to high altitude lose significant amounts of body mass from fat mass as well as fat-free mass, particularly if involved in increased physical activity, such as climbing (83, 84). As a consequence, an energy imbalance occurs, likely reflecting increased energy expenditure and decreased, or at least inadequate, food intake probably due to hypoxia-related satiety (83, 84).

In this context, significant variations in the secretion of leptin and ghrelin, as two of the major hormones involved in the regulation of energy balance, appetite and food intake as well as in peripheral metabolism (185), are expected. In fact, a decrease in body weight is generally associated with leptin reduction and ghrelin increase, while the opposite picture is associated with body weight excess (185). Actually, the data available so far are discrepant, since leptin levels have been reported as either increased (186, 187), decreased (16, 188) or unchanged (77), while a trend towards decreased (187) or unchanged (16) ghrelin levels at high altitude has been reported. In particular, an increase in leptin coupled with ghrelin decrease has been described after acute exposure to high altitude (186, 187), but other authors reported that prolonged high-altitude exposure is associated with a reduction of leptin concentrations, likely due to the loss of body mass and the strong hypoxia-related sympathetic activation (188). The only study available that evaluated a short-term hormonal profile (i.e. mean leptin and ghrelin concentrations over 2 h) showed a nonsignificant trend towards decrease in leptin levels, but completely unchanged ghrelin concentrations, despite a significant body weight loss (16). Thus, extreme high-altitude exposure in association with strenuous physical exercise does not allow the normal physiological response of leptin and ghrelin to significantly decrease body weight and cause negative energy

balance. The mechanism(s) underlying this lack of leptin and ghrelin responses is, at present, unknown. Nevertheless, it is again important to note that the large variations in the study methodologies employed, the role of potential confounders alone or in combination (e.g. cold exposure, weight loss, diet) as well as the intrinsic characteristics of the exercise bout could explain, at least in part, the clear-cut differences observed in leptin secretion that has been shown to be in a complex dynamic flux even without hypoxic exposure (189).

CONCLUSIONS

The adaptation to a new environment needs an information system which firstly informs the body on the environment characteristics and secondly triggers biological responses that may be more or less “adaptive” to new external conditions. The neuroendocrine system represents one of the most important body networks for adaptation.

High altitude means both hypoxia and low temperature and is a sufficient trigger per se for endocrine secretions. A further stimulus is represented by physical exercise. From a finalistic point of view, activation of the neuroendocrine system has a central role. Indeed, the strong endocrine response to high altitude can improve oxygen delivery via cardiorespiratory and haemopoietic adaptations and induce an adaptive response in favour of enhanced energy preservation and activation of the immune system. Data available on endocrine responses to high altitude are currently scanty and nonhomogeneous. Thus, clear conclusions cannot be drawn although high-altitude exposure could represent a physiological model of hypoxia.

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An Introduction to Circadian Endocrine Physiology: Implications for Exercise and Sports Performance

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INTRODUCTION

This chapter presents a summary of the current understanding of human circadian rhythms, their relationship to exercise and sports performance, with a particular emphasis on endocrine physiological regulation and dysregulation. The principal anatomical basis of circadian rhythms will be described, and the diurnal and circadian variation of

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certain hormones will also be discussed. The chapter then continues with a description of measurement methods in circadian physiology followed by a brief section on sleep. Circadian rhythms and general and athletic performance will subsequently be reviewed. The chapter ends with a discussion of exercise effects on circadian rhythms, shift work and exercise, and circadian effect of light exposure. At the end there is a glossary of terms frequently used in chronobiology, the science of biological rhythms.

BIOLOGICAL RHYTHMS

Biological rhythms are physiological and behavioral phenomena which recur regularly in a living organism. Though intrinsically determined (endogenous) and persisting independent of external influences, ultimately, the timing and amplitude of biological rhythms are influenced by their interaction with the environment. Biological rhythms are involved in the maintenance of homeostasis and the various components of the mammalian biologic signaling and communication system such as the endocrine system, central nervous system, autonomic nervous system, intestinal tract, and immune system all function in a rhythmic and integrated manner (1).

The time interval for completion of one cycle, i.e., the period, is the basis of classification of biological rhythms into one of three classes—circadian, ultradian, and infradian. Biological rhythms that occur approximately every 24 h are circadian, those with periods considerably shorter than 24 h are ultradian, and those with periods considerably longer than 24 h are infradian (2).

NEUROANATOMY OF THE BODY CLOCK

It is now known that mammalian circadian rhythms are orchestrated by nerve cells in the suprachiasmatic nuclei (SCN), small paired nuclei located in the hypothalamus. The neurons of the SCN (also referred to as the circadian pacemaker or “biologic clock”) generate circadian rhythms via a negative feedback loop of clock gene expression (3–5).

The circadian pacemaker has an intrinsic rhythm slightly different from 24 h; therefore, its precise synchronization to a 24-h day (i.e., entrainment) requires environmental time cues (known as Zeitgebers, “time givers”), light being the most important (6).

Light entrainment of the SCN is mediated primarily by the retinohypothalamic tract (RHT) which projects from the retinal ganglion cells to the SCN; interruption of the RHT eliminates light (photic) entrainment (7), as well as phase shifting induced by exposure to bright light. The classical photopigments, namely, rhodopsin and cone opsins, are not primarily required for entrainment to the light/dark cycle (8). Apart from giving rise to the RHT, the retinal ganglion cells project to the intergeniculate leaflet within the lateral geniculate body of the thalamus from where projections are sent back to the SCN via the geniculohypothalamic tract (9) which is likely involved in the integration of photic and non-photoc zeitgeber influence on the SCN (10, 11). Afferent serotonergic cells also project from the midbrain raphe nucleus to the SCN and contribute to the modulation of SCN function (12). Output tracts from the SCN are distributed primarily to several nuclei in the hypothalamus, most importantly to the paraventricular nucleus, and to a lesser extent to the midline thalamus and basal forebrain (13). The projection to the paraventricular nucleus is part of the multisynaptic pathway

(involved in the modulation of melatonin secretion by the SCN) which passes from the SCN through the paraventricular nucleus, the intermediolateral cell column of the spinal cord (containing sympathetic outflow fibers), the superior cervical ganglion, to the pineal gland (14).

DIURNAL AND CIRCADIAN VARIATION IN COMMON HORMONES

Melatonin

Melatonin, first discovered in the 1950s, is predominantly synthesized and released by the pineal gland. However, cells in other organs and systems—for example, gastrointestinal tract, bone marrow, skin, leucocytes, membranous cochlea—have also been reported to release melatonin, but in a less robust manner (15). Part of melatonin's role in circadian physiology is to function as a chemical marker of the internal, i.e., biological, night (16). Increased firing of the suprachiasmatic nucleus (SCN) during the “internal” (biological) day and via the complex polysynaptic pathway described previously (17) inhibits the production of melatonin. Figure 1 (upper diagram) depicts the rise in melatonin secretion during the biological night and the suppression of melatonin secretion during the day.

Apart from being an SCN-dependent output signal, melatonin can function as a potentially resetting feedback signal to the SCN through melatonin receptors (MT1 and MT2) located in the SCN. For example, the administration of melatonin or melatonin receptor agonists to the SCN during late day to early night has been associated with reduced neuronal firing. Melatonin can phase shift circadian sleep–wake rhythms and melatonin can be used to entrain the circadian sleep–wake cycle in blind individuals (18, 19).

Cortisol

Circulating cortisol exhibits a circadian rhythm which is thought to be a result of the modulatory action of the biological clock on the hypothalamic–pituitary–adrenal (HPA) axis. In “diurnal” species (those regularly awake during the day and asleep during the night) cortisol level begins to rise during the night (Fig. 1, lower diagram) and the highest levels are usually attained in the early morning after which the level begins to decline during the day (20), with a trough during the early part of the night. Interrupted sleep during the night and nocturnal sleep deprivation are both associated with elevated cortisol in the circulation.

Prolactin

The plasma concentration of prolactin is higher during the internal night and reflective of three different types of oscillations namely two ultradian rhythms and a circadian oscillation (1). One of the ultradian rhythms has a high amplitude pulsatile quality clustered during the night and early morning hours. In both men and nonpregnant women, a significant percentage of daily secretion of prolactin occurs during REM sleep, and REM sleep activity on the EEG is in turn promoted by prolactin (21). One study (22) also indicated that exercise (90-min cycling at 70% of maximal oxygen consumption) performed between 4:30 and 6:00 p.m. resulted in increased nighttime blood levels of prolactin when compared to a day without exercise.

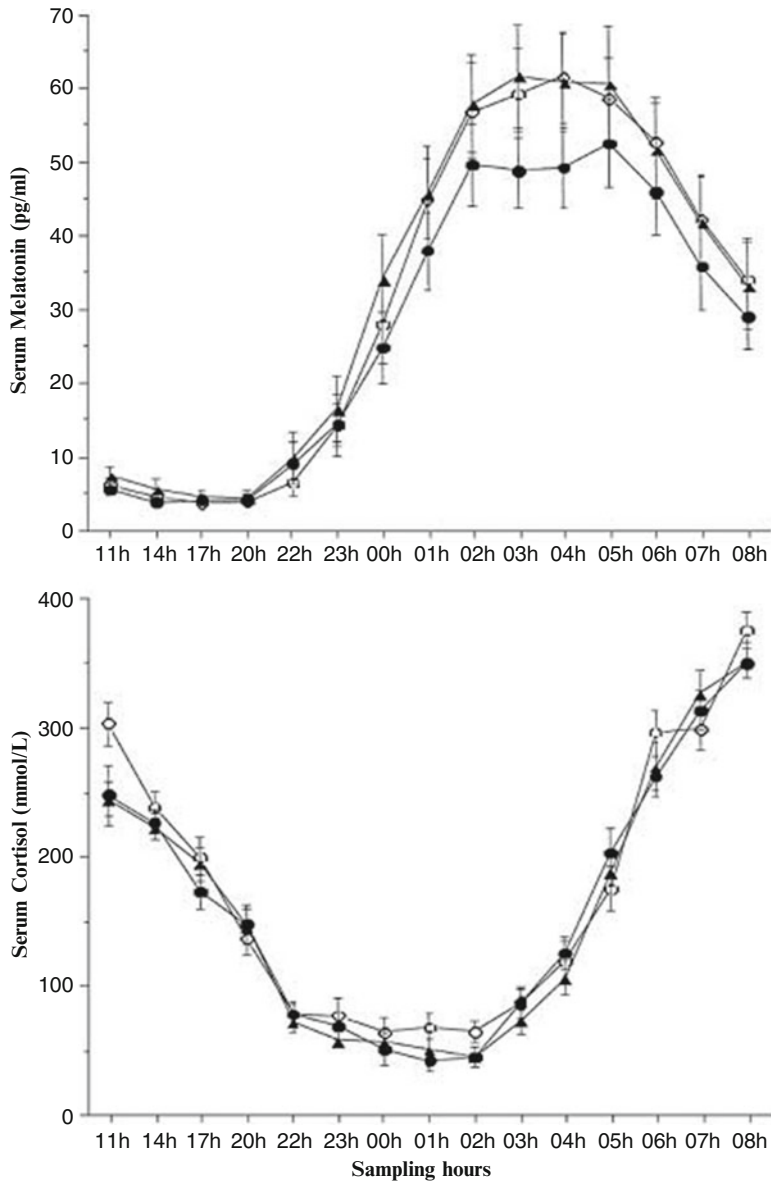


Fig. 1. Circadian rhythms of melatonin and cortisol in 31 healthy young men. The rhythms were evaluated over three different 24-h sessions with a space of 2 weeks between the first and second sessions and 4 weeks between the second and third (20). Reproduced, with permission, from Elsevier Limited.

Testosterone

In healthy men, levels of circulating testosterone vary with the time of day such that peak concentrations are observed between 6 and 8 A.M. while trough levels are measured between 6 and 8 P.M., but this variation is blunted in older men in whom overall testosterone in circulation is also reduced (23). Testosterone levels begin to rise at night

(from 21:00 h) and levels fall during the day; this is responsible for the early morning peaks and evening troughs. The findings from the study by McMurray et al. (24) suggest that the early morning peak of testosterone might be augmented by engaging in heavy resistance exercise (three sets of six exercises to exhaustion) between 7 and 8 P.M. the previous day. In addition, fragmented sleep can disrupt the rhythm of testosterone secretion, and the nocturnal rise in testosterone may be attenuated (25).

MEASUREMENTS IN CIRCADIAN PHYSIOLOGY

Some of the common methods used in circadian rhythm research will now be described briefly.

Constant Routine

It is difficult to separate the contributions of (a) external (environmental) factors, (b) sleep vs. wake, and (c) internal circadian rhythms to any observed diurnal rhythm. The circadian pacemaker (or “biological clock”) in the suprachiasmatic nucleus of the brain “drives” rhythms in physiology and behavior, but light, temperature, food, posture (external factors), as well as stress level and motivation (internal factors) all affect the pacemaker. Specifically, if a physiological variable (e.g., body temperature or melatonin) is measured in the presence of an intact sleep–wake cycle, then any data derived from such measurements may not be attributable to the circadian pacemaker alone because the underlying endogenous rhythm (i.e., the one attributable to the circadian pacemaker) may have been “masked” by the periodic behaviors involved in the sleep–wake cycle (26). Additionally, the overlap between sleep–wakefulness alternations with circadian rhythms makes it difficult to disentangle their relative contributions to observed oscillating phenomena. Thus, special procedures had to be developed in an animal or human subject and *constant routine* protocols were designed to address this problem (27).

With the use of a *constant routine* protocol the aim is to control for external factors through design and for sleep–wake-related factors statistically (26). In a *constant routine*, ambient light and temperature are kept constant, and the patient is required to maintain constant wakefulness while food and water are administered at fixed, short, and regular intervals. *Constant routines* lasting more than 24 h are usually needed to evaluate an entire circadian cycle, for example, a study of the circadian variation in temperature might require a *constant routine* of about 28 h (28). Maintaining wakefulness for that duration is challenging. Apart from the *constant routine* described above, many other less demanding protocols have also been used in studies of circadian physiology. Examples include shorter protocols, multiple nap protocols (29), and protocols that allow periodic changes in posture (e.g., bathroom breaks (30)).

Forced Desynchrony

As described above, the interpretation of circadian contribution to diurnal variation in physiology can be confounded by sleep and other “masking factors,” and the *constant routine* protocol is used to circumvent this problem. However the sleep deprivation resulting from the *constant routine* procedure (even if results are adjusted statistically

for it) can impact on the interpretation of results. For this reason the *forced desynchrony* protocol may be more suitable because it avoids sleep deprivation effects and at the same time controls for the effects of the relevant masking factors (31).

In a *forced desynchrony* protocol, the sleep–wake cycle is “forced” into a state of desynchrony with the circadian pacemaker by subjecting individuals to a period of sleep–wake schedule that is drastically different from the “normal” 24 h in entrained conditions. Common durations of “artificial days” in *forced desynchrony* protocols are either short (20 h) or long (28 h). The circadian pacemaker cannot adapt to these long or short periods of alternation between rest and activity and begins to oscillate at its own intrinsic period (free running (32)). An important feature of a *forced desynchrony* protocol is that the proportion between sleep and wake (1:2) remains constant, activities are allowed, and usually sleep deprivation is minimized. Usually the participants in such a protocol stay in a sleep-monitoring laboratory for the duration of the protocol; lighting levels are dimmed during periods of wakefulness and almost extinguished during periods of rest and typically do not have access to clock-bearing devices or cues that might convey information regarding the time of day. Since the circadian pacemaker is “free running” and desynchronized from the sleep–wake cycle, data (e.g., core body temperature (CBT), plasma melatonin, or cortisol) can be collected over successive circadian cycles to compute separately the circadian (process C) and sleep–wake (process S) contribution to the variable under investigation (see Fig. 5).

Dim Light Melatonin Onset

Measurement of circulating melatonin is a preferred marker of circadian phase, and this is because melatonin is relatively less influenced by biochemical and physiological factors. For instance CBT can be significantly influenced by caloric intake and physical activity, but these factors exert a negligible effect on melatonin (33). However one factor that has been known to significantly affect the level of melatonin is bright light, which effectively suppresses its secretion (34). Thus, measuring melatonin onset requires exposure to dim light. In an entrained healthy human subject in stable environments (e.g., no transmeridian travel, no shift work) in dim light, levels of melatonin in the blood begin to rise abruptly couple of hours before the onset of sleepiness preceding nocturnal sleep and reach the highest level, the first part of the night; the beginning of the rise is called dim light melatonin onset (DLMO). On the basis that plasma and saliva melatonin are highly correlated (35), salivary onset of melatonin is now commonly used to measure DLMO. Normal DLMO usually occurs between 19:30 and 22:00 h for adults and 19:00 and 21:00 h for children aged 6–12 years (36).

During the saliva DLMO test, the subject must remain in dim light 1 h prior to the commencement of saliva collection, and physical activities are avoided. The subject should also avoid eating bananas, drinking alcoholic beverages or coffee, and should rinse his mouth with water 15 min prior to collection of saliva—but teeth brushing is not permitted (36). Depending on the indication, the timing of saliva collection can vary from hourly collections beginning from 7 to 11 P.M., 9 P.M. to 1 A.M., 4 to 9 P.M., and 8 P.M. to midnight. The graph plotted from saliva melatonin collected for less than a 24-h period is called a partial melatonin curve. However hourly collections for 24 h may be indicated when the partial melatonin curve is not conclusive or when a free running rhythm is suspected, such as in blind persons (36).

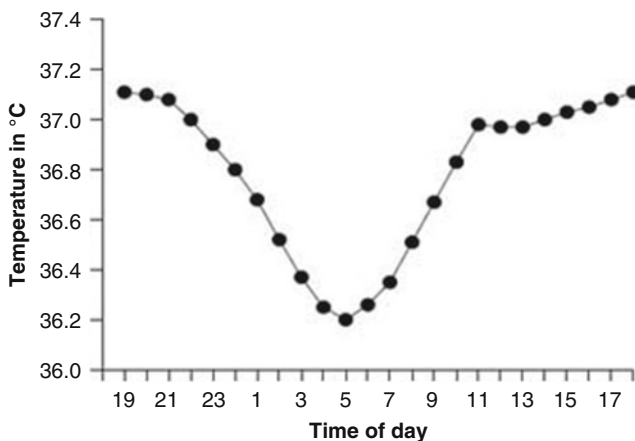


Fig. 2. Circadian rhythm of core body temperature (85). Reprinted, with permission, from Elsevier Limited.

Core Body Temperature

CBT has been used as a marker of circadian rhythms in conditions when sleep–wake, activity, and diet can be maintained constant. The circadian rhythm of CBT is such that the maximum level is reached towards the end of the biological day and the minimum level towards the end of the biological night, at approximately 4–5 A.M. for the majority of individuals (37) (Fig. 2). Though CBT can be easily measured, the relative ease with which it is influenced by “masking factors” means that *constant routine* or *forced desynchrony* protocols are necessary when CBT is used as a marker of central circadian rhythm.

Morningness–Eveningness Questionnaire

The first self-administered questionnaire to evaluate whether an individual was a “morning” or “evening” person (this is known as chronotype) was created by Horne and Ostberg (38). Morningness could be conceptualized as a natural tendency to go to sleep and wake up early (so-called larks), and be most alert in the early morning, while eveningness is a natural tendency to stay up late (so-called owls), wake up later, and feel most alert later in the evening. Since the introduction of Horne and Ostberg’s morningness–eveningness questionnaire, several other questionnaires have now been introduced. The athlete’s morningness–eveningness questionnaire (39) is shown in Fig. 3. Morningness–eveningness status of an individual is also related to polymorphisms in certain circadian genes, such as *CLOCK* (40) and *PER* genes (41).

SLEEP

Although discussing sleep and sleep impairment in relationship to hormones and athletic activity is beyond the scope of this chapter, we cannot completely avoid mentioning it considering interactions between circadian rhythms and sleep on physiology and performance. Prevalence of poor sleep quality among athletes is substantial (42).

Name: _____ Date: _____ Score: _____

Athlete's Morningness-Eveningness Scale (AMES)

Directions: This Scale is designed to help you identify your chronotype, that is, your tendency toward a morning ("lark"), mid-range or evening ("owl") performance pattern. To complete this Scale, first print out the document. Then, read each question and consider all of the responses carefully. Then, complete each of the six items on this Scale as accurately as you can; circle only one response per item.

1. At what time in the evening do you usually start feeling tired and in need of sleep?

- (7) A. 8:00 PM- 9:30 PM
- (6) B. 9:31 PM- 10:45 PM
- (5) C. 10:46 PM- 12:30 AM
- (4) D. 12:31 AM-1:45 AM
- (3) E. 1:46 AM- 3:00 AM

2. Suppose that you were able to choose your own competition hours. For some athletes, it might be useful to think about the 3-hour block when there would be a greater chance of feeling "in the zone," or performing "at peak." Which one of the following 3-hour blocks would be your most preferred time?

- (8) A. 6:00 AM- 9:00 a.m.
- (7) B. 9:00 AM- Noon
- (6) C. Noon- 3:00 PM
- (5) D. 3:00 PM- 6:00 PM
- (4) E. 6:00 PM- 9:00 PM.
- (3) F. 9:00 PM- Midnight

3. One sometimes hears about "feeling best in the morning" or "feeling best in the evening" types of people. Which type do you consider yourself?

- (8) A. Definitely a "morning" type
- (6) B. More a "morning" than an "evening" type
- (3) C. More an "evening" than a "morning" type
- (1) D. Definitely an "evening" type

4. Suppose that you were able to choose your own training (practice) hours, and organize all other daily routines to protect those hours. Which one of the following 3-hour blocks would be your most preferred time?

- (8) A. 6:00 AM- 9:00 AM
- (7) B. 9:00 AM- Noon
- (6) C. Noon- 3:00 PM
- (5) D. 3:00 PM- 6:00 PM
- (4) E. 6:00 PM- 9:00 PM
- (3) F. 9:00 PM- Midnight

Calculate your sleep score by adding the values in parentheses beside your circled answers.

Total Score:

10 to 12	=	Extreme Evening Type
13 to 17	=	Moderate Evening Type
18 to 23	=	Mid range
24 to 28	=	Moderate Morning Type
29 to 31	=	Extreme Morning Type

Fig. 3. The athlete's morningness–eveningness scale questionnaire. Reprinted, with permission, from Elsevier Limited (39); adapted, with permission, from Elsevier Limited (38).

INTERACTION BETWEEN CIRCADIAN RHYTHMS AND THE SLEEP HOMEOSTAT

Sleep is regulated via an interaction between the circadian process and the sleep homeostat, and this is the so-called two-process model (43). The circadian process is sometimes referred to as "process C" and the homeostatic process as "process S." The homeostatic and circadian processes act antagonistically to consolidate wakefulness during daytime and sleep during nighttime. The homeostatic process can be conceptualized, similar to hunger and thirst, as a buildup of pressure for sleep when a person is awake and the dissipation of the pressure when asleep. Towards the morning after a period of sleep at night, there is very little homeostatic pressure for sleep, but the body clock reduces its firing to elevate the threshold for waking, and thus consolidates the

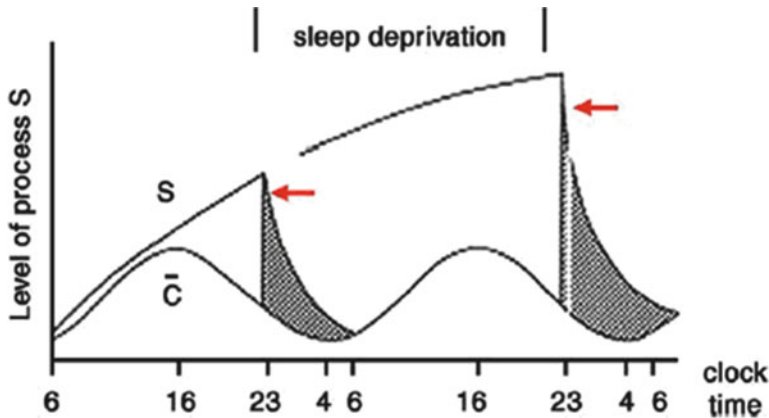


Fig. 4. Two-process model of sleep showing the saturation of appetite for sleep with time and the exponential decrease in process S during sleep. This explains why sleep debt accumulated over several hours can be paid with a short nap. Reprinted, with permission, from Elsevier Ltd. (39); adapted, with permission, from Elsevier Limited (86).

sleep state. The homeostatic pressure for sleep (similar to appetite for food or thirst) gradually builds up as the day progresses. The circadian process elevates the threshold for falling asleep (44) and thus consolidates wakefulness.

Because homeostatic pressure for sleep decreases exponentially during sleep, a significant amount of accumulated sleep debt (from sleep deprivation) can be paid by a short nap (this is shown in Fig. 4).

CIRCADIAN RHYTHMS AND GENERAL HUMAN PERFORMANCE

Circadian rhythm effects have been observed in relation to both cognitive and physical performance in human subjects. For example in a study that employed a 20-h *forced desynchrony* protocol, participants demonstrated a circadian pattern of performance in tests of psychomotor vigilance, short-term memory, addition/calculation, digit symbol substitution, and alertness (45). After controlling for homeostatic effects, peak performance was recorded near the maximum of the CBT shortly before the onset of melatonin secretion while significant dip in performance occurred around the time of CBT minimum shortly after melatonin maximum secretion (Fig. 5) (process C). After controlling for circadian effects, cognitive performance scores also decreased with increasing hours of wakefulness (process S).

The study by Freivalds et al. (46) evaluated the circadian variation in performance-related capabilities over a 25-h period. Specifically, they measured variation in elbow flexion strength (practically relevant to manual handling of materials), simple reaction time, maximum information processing rate, physiological tremor, and critical eye-hand tracking capacity. Though the amplitudes were small, circadian variation was recorded for all the above measures of job performance-related variables. Performance scores were generally better during the day and evening times when compared to night or morning times. In the study by Teo et al. (47) the circadian rhythm of cortisol and testosterone was evaluated in relation to strength and power performances at four

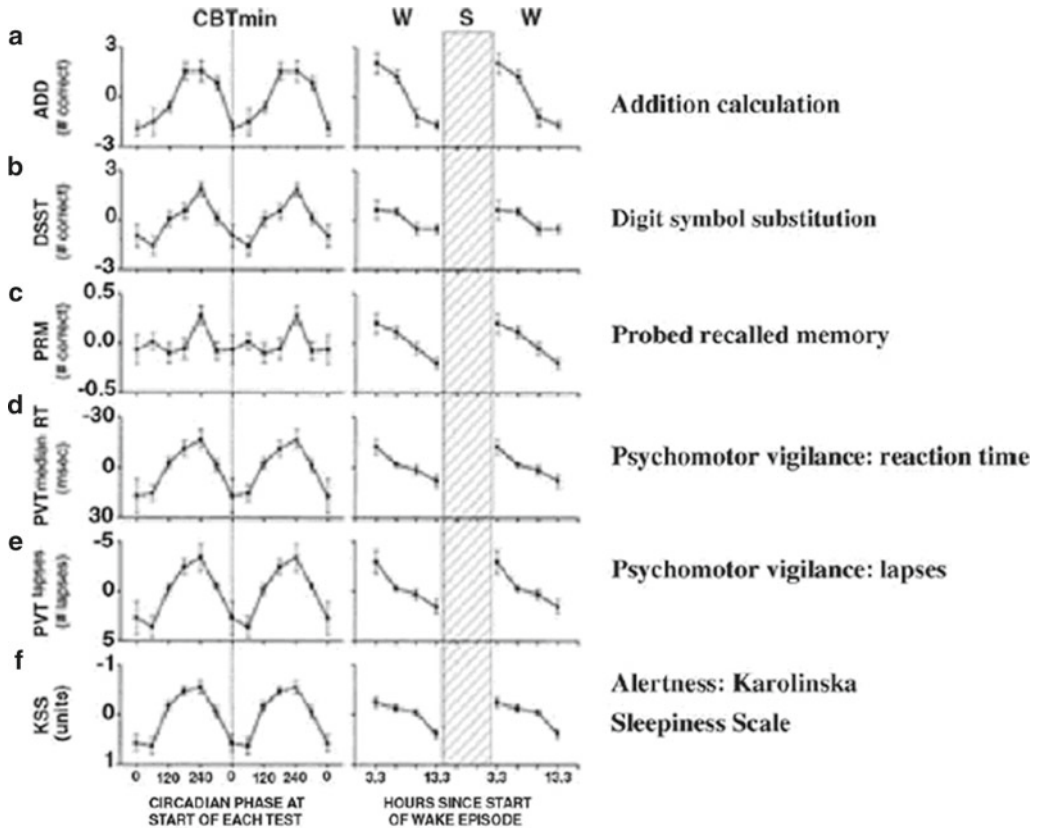


Fig. 5. Circadian (plotted on the *left*) and homeostatic (plotted on the *right*) variation in cognitive function. For each graph, lower lying data points indicate poorer performance on that neurocognitive measure. Addition/calculation test (ADD), digit symbol substitution test (DSST), probed recall memory test (PRM), psychomotor vigilance task ((PVT) which consists of the median reaction time and total number of lapses), and Karolinska sleepiness scale (KSS) scores all exhibit a pattern of maximal scores near the core body temperature maximum and minimal scores near the core body temperature minimum (CBTmin). Minimal scores are also recorded with increasing duration of wakefulness (W in the figure) as homeostatic pressure builds up from the point of awakening from sleep (S in the figure). Note that even though the trend of circadian variation was observed in the probed recall memory test, statistical significance was not reached for this measure. Reprinted, with permission, from Elsevier Ltd. (39); adapted, with permission, from BMJ Publishing (53).

different time points during the day (8:00 h, 12:00 h, 16:00 h, and 20:00 h). Power performance (maximal force production and peak power output in counter movement jump and isometric mid-thigh pulls) showed a variation with peak performance at 16:00 h when compared to the other time points. Unexpectedly, no relationship was found between cortisol or testosterone blood levels and power performance.

The studies by Freivalds et al. (46) and Teo et al. (47) are limited because they did not employ a *constant routine* or *forced desynchrony* protocol, and the number of measurements was also relatively few. Nevertheless the available evidence is in support of better cognitive and physical performance during the late afternoon than during the earlier hours of the day, probably driven by the circadian pacemaker.

CIRCADIAN RHYTHMS AND ATHLETIC PERFORMANCE

Evidence from several studies indicates that athletic performance in many types of sports exhibit circadian rhythms with peak performance usually noticed during late afternoon and worse performance in the morning (48). For example cyclists, runners, shot putters, swimmers, and badminton players have been shown to perform better in the late afternoon compared to the morning (49–51). In addition, during major sports competitions, it appears that more world records are broken by athletes competing in the early evening compared to those competing in the morning (52). However, it is important to note that many of the studies that have reported a diurnal rhythm in athletic performance have an important limitation—they did not control for confounding by “masking factors.” Therefore, the observed rhythm in athletic performance could have arisen due to environmental and behavioral characteristics that are unrelated to circadian regulation. For example, worse performance in the morning could be due to the effect of ambient temperature, sleepiness and lethargy, as well as relative stiffness of joints after an overnight bed rest.

Three studies have been conducted (48) in which “chronobiological protocols” were employed to mitigate against the impact of “masking factors” in the evaluation of circadian rhythms of athletic performance. In the first study (53), subjects participated in a 4-day 5-a-side soccer match in which 5-min breaks were allowed at the end of every hour, but sleep was not allowed for the entire 4-day period. Activity of the subjects (measured by a modified motion analysis method (54)) and heart rate exhibited a circadian rhythm with peaks in the afternoons (average 17:00 h) and troughs in the early morning (average 5:00 h).

In another study, Callard et al. (55) measured torque developed by voluntary isometric contractions of the knee extensors during cycling and at rest. The measurements at rest and during cycling were done on 2 different days a month apart. The variation in torque was circadian with peaks at around 19:00 h when measured every 4 h both at rest and during cycling. A constant environment, elimination of sleep, and constant amount of activity were part of the protocol employed in the two studies described, and they can therefore be viewed as close approximations to the “*constant routine*” protocol described earlier in this chapter.

The study by Kline et al. (51) was conducted by subjecting experienced swimmers to an “ultrashort” sleep–wake cycle in which they were allowed to sleep in the dark for an hour and mandated to stay awake for 2 h in dim light. Measurements of the time required to complete a 200 m swim was done over a 50–55-h period such that a total of 6 measurements were obtained for each swimmer. The swim performance had a circadian peak in the internal late evening/early night, around 23 h. It is interesting to note that the period of peak performance in all these studies tends to overlap with the timing of maximal CBT (see [core body temperature](#) above).

In summary, based on the review of the literature, one can conclude that the limited data suggest that several sports activities may be under the influence of circadian rhythms. Performance generally peaks (except in those with an extreme morning chronotype) in the early evening at a time when the circadian pacemaker is firing frequently to counteract the effect of accumulated wakefulness. This period is called “wake maintenance zone,” as it is the period when it is difficult, if not impossible, to

fall asleep for majority of non-sleep-deprived individuals, despite an accumulated duration of wakefulness and thus increased “appetite” for sleep. The characterization of the individual circadian rhythms of athletic performance could be important when making recommendations regarding the optimal time, internal and external, for competing, training, and practicing for elite athletes and “working out” for the majority of us for maintaining health and productivity.

EFFECT OF EXERCISE ON CIRCADIAN RHYTHMS

In addition to light and other zeitgebers, scheduled physical activity has been reported to have an effect on circadian rhythms. Hackney and Viru (56) carried out a study of the cortisol profile in physically active individuals randomly exposed to daytime high- or moderate-intensity exercise and no exercise conditions, respectively, using a crossover design with 1-week washout, and measuring daytime and nighttime cortisol levels. The nocturnal levels of cortisol were lower in the daytime exercise conditions and lower after anaerobic (i.e., high intensity) daytime exercise conditions as compared to aerobic (i.e., moderate intensity) daytime exercise conditions. Thus, daytime exercise appears to suppress nocturnal cortisol secretion. The main limitation of the study was the confounding by sleep and wakefulness, factors that were not controlled by design and not adjusted for statistically.

Furthermore, scheduled voluntary wheel-running and forced treadmill running induce entrainment and phase shifting in rodents (57–59). In humans also, exercise has been shown to have phase-shifting effects. For instance, Van Reeth et al. (60) reported a phase delay in the circadian rhythms of thyrotropin and melatonin with a single bout of exercise (alternating arm and leg ergometry at 40 and 60% of maximal O_2 consumption) centered from 5 h before to 4 h after the body temperature minimum, in 300-lux constant light conditions. The findings by Van Reeth et al. (60) were later replicated by Baehr et al. (61) in both younger and older adults (without a significant age-group effect) and this time using DLMO as a circadian phase marker. Higher intensity (stair climbing at 75% VO_2 max) but shorter (1 h) exercise had a similar phase-delaying effect on the thyrotropin rhythm (62). In that study moderate-intensity exercise appeared to delay the circadian rhythm of melatonin more potently than the higher-intensity exercise.

As light could have been a confounding factor in these studies, a developed protocol replicated the phase-delaying capacity of nocturnal exercise in very dim light conditions (three 45-min bouts of cycle ergometry with 0.65 lux in the line of sight) on melatonin circadian rhythms (63) each night. When different timings of scheduled exercise were added to the previous protocol (morning, afternoon, and night exercise sessions), nocturnal exercise significantly phase delayed, while early evening exercise phase advanced melatonin onset rhythms (64). However, it appeared that only the first bout of evening exercise significantly advanced the melatonin circadian rhythm, as the effects were diminished below significance on subsequent nights. In another study conducted in a temporal isolation facility, in which participants were subjected to an imposed external cycle of 23 h and 40 min duration (participants were subjected to 12 of these cycles), 2 h of exercise twice per day (cycling and rowing to a heart rate of 140 beats/min), a phase advance of the melatonin rhythm of 1.6 h was observed, and the phase difference

(i.e., between exercise and “no-exercise” states) was significant 6 days after the start of the exercise (65).

As exercise has phase-shifting effects, does exercise enhance or inhibit circadian phase shifting with light? It appears that the answer is “no,” as illustrated by two studies with concordant results (61, 66). It seems that the effects of light as a zeitgeber are so potent as not to be modulated by exercise.

In regard to re-entrainment (of considerable importance for prevention and treatment of jet lag), it appears that timed exercise has a significant accelerating effect on sleep–wake cycles, but not on melatonin circadian rhythms (67).

SHIFT WORK AND EXERCISE

Individuals involved in shift work might find it challenging fitting exercise schedules into their daily routines, but for those able to exercise regularly, it is important to consider whether physiological and psychological responses to exercise are altered by shift work. This is relevant since the decision to continue or quit exercise is likely going to depend on whether exercise relieves or exacerbates the extant challenges and difficulties of shift work (68).

In diurnal individuals, exercise could potentially improve sleep quality and quantity, and current hypotheses regarding the sleep-promoting effects of physical exercise include anxiety reduction, antidepressant effect, thermogenic effect, and circadian phase-shifting effect (69). Unfortunately there is a dearth of research specifically designed to evaluate physical exercise, sleep, and shift work. This fact notwithstanding, Atkinson et al. (68) speculated that exercise during certain targeted times of the day could potentially help adjust the circadian rhythm in such a manner that a circadian phase shift occurs, and this in turn can result in an alignment of the internal clock with the external time, i.e., the shift being worked. This could potentially lead to a reduction in the feelings of tiredness, cognitive dulling, sleepiness, and other negative effects of working unusual hours. This gain could also act as further incentive for a shift worker to engage in exercise. Atkinson’s speculation is plausible since exercise has been reported to act as a zeitgeber (please see above under “Effect of exercise on Circadian Rhythms”).

Härmä et al. (70, 71) evaluated the effects of exercise (jogging, running, swimming, skiing, walking, and gymnastics) sessions administered 2–6 times a week for 4 months, on a number of circadian-related variables in female shift workers (nurses and nursing aides). The variables measured were physical fitness, fatigue, sleep, psychosomatic symptoms, circadian rhythms of alertness, short-term memory performance, and body temperature. The exercise regimen was individualized based on the submaximal ergometer test, age, and the subject’s sports habits such that the heart rates during exercise were 60–70% of the maximal values for each individual. Participants in this study were involved in a shift cycle that consisted of day, evening, and night shifts, and each participant worked all three shifts during a 3-week period. Participants were randomly assigned to an intervention (exercise) group or control (no exercise) group.

For the exercise group only, alertness and short-term memory increased and there was a significant decrease in general fatigue, but these findings were observed only

during the night shift. In addition, there was an increase in the duration of sleep for the exercise group during the evening shift. The authors suggested that physical training maybe of practical use in reducing fatigue in nurses working night shifts. Despite the seemingly beneficial effects of exercise in this study, “nervous symptoms” did not decrease in the exercise group though the authors attempted to explain this finding by suggesting that the time spent exercising could have encroached on leisure and family time, and this could have increased stress in some subjects more than exercise was able to decrease it. Commenting on this finding and in relation to recommendations in shift workers, Atkinson et al. (68) wrote that “...any lifestyle change must be considered also in the context of its implications for the family, close friends and social contacts.”

CIRCADIAN EFFECT OF LIGHT

Light is the main entrainment agent in most species and exposure to single pulses of light at certain times of the day can result in phase shifting of circadian rhythms (72, 73). Light pulses administered 2–6 h before the CBT minimum usually results in phase delay of the circadian rhythm while administration of light 2–6 h after the temperature minimum phase advances the circadian rhythm. In terms of the effect of wavelength on phase-shifting potential of light, blue light appears to have a more potent effect on circadian rhythms than red light (74). The circadian phase-shifting potential of light can be useful in the treatment of cases of circadian rhythm sleep disorders such as shift work, delayed or advanced sleep phase, and jet lag (important for athletes engaged in transmeridian travel to competition sites (see section on jet lag below)).

More than 30 million Americans have been estimated to embark on air travel across five or more time zones each year (75). Jet lag has been documented to have a negative impact on the performance of athletes. For example in Major League Baseball, home teams playing against teams that had traveled eastward hit 1.24 more home runs (76). The decreased athletic performance associated with jet lag may be due to a number of processes including (1) peak performance time for an athlete and time of performance demands (training and competition) may become misaligned due to the effects of transmeridian travel on the circadian system; (2) sleep disturbances associated with transmeridian travel could potentially affect athletic performance; (3) the general malaise associated with jet lag (39, 77).

In order to minimize the potential negative effects of jet lag on athletic performance, it is advisable to make attempts to realign (or to minimize the misalignment) of the internal clock with the local time at the competition site, and the recommendations regarding the use of light for this purpose would depend on the direction of travel, i.e., eastward or westward. For athletes traveling eastward, the recommendation would be to avoid light if the time of arrival at the competition region falls anywhere between 2 and 6 h before the CBT minimum based on the time from the departure zone. To illustrate this concept, let us assume that an athlete travels from Washington DC to London and arrives at 7 A.M. (London time). If after alighting from the plane, the athlete is immediately exposed to sunlight, this would imply light exposure at a time probably 2–3 h before the temperature minimum, and this would likely result in a

further phase delay of the biological clock. The effect of this is likely going to be additional difficulty adjusting to the local time in terms of the sleep–wake cycle, and this could impact negatively on athletic performance. Therefore for this hypothetical athlete, it is better to advise using sunglasses at the time of arrival in London, until approximately 10 A.M. (the most likely time of the 4–5 A.M. temperature minimum based on time differences between London and DC). After 10 A.M., the exposure to natural or artificial light should be plentiful, as it will fall in the phase advance portion of the phase response curve and will likely reduce the desynchrony from 5 to 3 h. On the subsequent day, the prohibition of exposure to bright light would be moved 2 h earlier, i.e., at 8 A.M., with exposure recommended after 8 A.M. In addition to light exposure and avoidance, well-timed melatonin administration has also been used in combination or by itself. It is important to keep in mind that melatonin effects on circadian timing are the opposite of the effect of light exposure, i.e., the two phase response curves are mirror images of each other (78, 79). Strategic scheduling of sleep has also been recommended to reduce the adverse effects of jet lag on performance. For example, where at all possible, maintenance of the sleep–wake schedule from home after arrival at the destination location is beneficial for short-duration trips (80). Moving the sleep schedule (with or without the addition of artificial light) 1 h per day before embarking on air travel in order to bring it in alignment with the destination time zone has also been found to be beneficial (81). Caffeine, modafinil, and armodafinil, which are alertness-promoting agents, have been shown to demonstrate potential utility in the treatment of the drowsiness symptoms of jet lag (82, 83), but side effects such as insomnia, headache, nausea, and vomiting may be significant. However, it is important to note that alerting substances such as modafinil and armodafinil are performance-enhancing agents, which constitute doping and thus are prohibited for competitive athletes.

For a more detailed review of light exposure and avoidance in relationship to jet lag, please see the reviews by Postolache and Oren (84) and Postolache et al. (39).

CONCLUSIONS

1. Predictable hormonal (melatonin, cortisol, etc.) and electrophysiological changes occur with a period of approximately 24 h.
2. These changes are associated with changes in performance, cognitive and physical, simple and complex.
3. Best performance occurs during the end of the internal (biological) day (i.e., evening), except in individuals who score high on morningness.
4. Measuring circadian rhythms is challenging, because of masking effects of sleep and wake and environmental effects.
5. The most important signal for shifting circadian rhythms is exposure to bright light.
6. Knowledge of circadian factors is likely to (a) improve our understanding and ability to predict fluctuations in performance, (b) minimize effects of jet lag and sleep loss on athletic performance in competitive athletes, (c) use simple nonpharmacological techniques to shift circadian rhythms to avoid competing during individual circadian “dips” in performance, and (d) choose wisely the timing of “working out” for everyone, for maximizing health benefits and adherence.

GLOSSARY

Circadian phase This is the phase of the circadian cycle, representing the timing of the internal biological clock and is usually estimated by measuring onset–offsets, troughs or peaks, or changes in slopes (i.e., increasing vs. decreasing) in circadian markers such as hormones (melatonin, cortisol, prolactin) or CBT. Circadian phase measurement should be performed in controlled dim light conditions. In animal research, rest–activity in dim light conditions is used to estimate circadian phase.

Diurnal (in relation to circadian) Refers to variations in a physiological parameter with time of day. In contrast with circadian variations which are usually endogenous (i.e., intrinsically generated by the body clock, see below) but influenced by external light–dark cycle, diurnal variations can be either driven by the biological clock, external light–dark cycle, the sleep–wake cycle, or their interaction.

Entrainment The process by which biological rhythms are synchronized (by timing signals) to a 24-h environmental cycle (usually the day/night cycle). Under entrainment or entrained conditions, circadian rhythms usually oscillate with a period of 24 h, induced by exposure to time cues (i.e., zeitgebers).

Free-running rhythm A non-24-h rhythm seen in the absence of timing signals, most importantly in dark (or very dim light) conditions, which expresses the intrinsic circadian period of the circadian pacemaker (the suprachiasmatic nuclei, see below). Almost all animals have the internal circadian period slightly different than 24 h, either slightly shorter (as in a majority of rodents) or slightly longer (as in humans, among other animals). Animals with a longer than 24-h circadian rhythms have a tendency to phase delay from one day to another in the absence of exposure to light during morning hours.

Jet lag A circadian rhythm sleep disorder consisting of sleep difficulty at night, daytime sleepiness, impairment of daytime function, gastrointestinal disturbance, and general malaise associated with transmeridian air travel, resulting from a desynchrony between external time cues, sleep–wake, and timing of endogenous rhythms.

Masking Obscuring of circadian rhythms (driven by the circadian pacemaker) by external or internal factors. For example physiological processes and behaviors (such as opening and closure of eyelids, activity, food intake) associated with the sleep–wake cycle, exposure to bright light, eating, drinking, and standing could all be related to the rhythm of a physiological variable, though the rhythm is primarily generated endogenously by the circadian pacemaker. Therefore, measuring the rhythm of the variable (e.g., CBT) in the presence of an intact sleep–wake cycle and all the other aforementioned external factors could “mask” the true contribution of the circadian pacemaker to the rhythm and lead to inaccurate measurements.

Morningness–eveningness (ME) The natural tendency for an individual to either go to sleep and wake up early and perform best in the morning (a morning person) or to go to sleep and wake up late and perform best in the evening (evening person). The ME has been related to clock gene polymorphisms and to circadian sleep disorders.

Nadir This is the lowest point of a biological rhythm, e.g., the nadir of CBT is the lowest point on the CBT rhythm and is usually around 2 h before habitual waking time in most individuals with a stable circadian rhythm.

Phase advance Positioning of a particular circadian rhythm earlier relative to clock time or other circadian markers.

Phase delay Positioning of a particular circadian rhythm later relative to clock time or other circadian markers.

Phase response curve (PRC) A graphical illustration of the relationship between the timing of exposure to a zeitgeber or other intervention (on the x axis) and the shifting induced by the exposure to the zeitgeber or other intervention. Conventionally, on the y axis positive values represent phase advances and negative values represent phase delays. For example the circadian phase-shifting effects (advance or delay, depending on the time of exposure) of bright light or melatonin administration on any marker of circadian rhythms (such as CBT or the nocturnal melatonin secretion measured in blood—or the onset of nocturnal melatonin secretion in saliva) have been presented as phase response curves. On the x axis, timing is usually measured in relationship to a circadian marker—such as core temperature trough or the onset of melatonin secretion (i.e., internal timing) rather than external timing. Determination of circadian phase response curves is very demanding in terms of funds and time—requiring highly controlled conditions and minimizing exposure to zeitgebers.

Postprandial dip (or early afternoon dip) The dip in performance observed during the mid-afternoon hours (incorrectly called postprandial), because it occurs in anticipation and not as a physiological reaction to the main meal of the day. For most individuals, performance measures (physical and cognitive) exhibit an increase from a low at the morning wake time to peak levels in the early evening time, but in some individuals a dip in performance is observed during the mid-afternoon hours.

Suprachiasmatic nuclei A group of brain cells located bilaterally above the optic chiasm in the anterior basal hypothalamus and demonstrated to be the site of the master circadian oscillator (“body clock”) that synchronizes, as a conductor does with the orchestra, circadian rhythms of peripheral tissues, organs, and cells.

Wake maintenance zone This is the time of the day (usually in the late evening) when the propensity for sleep is lowest and wakefulness or arousal is increased. The wake maintenance zone (also referred to as the forbidden zone for sleep) is mediated by the circadian pacemaker.

Zeitgeber The name given to any external time-signaling stimuli that help maintain periodic regularity in circadian rhythms. It is a German word which literally means “time giver.” Light is considered the most potent zeitgeber. Other zeitgebers are exercise, food, temperature, and social interactions.

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21

Physical Activity and Mood. The Endocrine Connection

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SUMMARY

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INTRODUCTION

Physical Activity Causes Mood Changes

Various researchers have pointed out the affective changes brought about by physical activity (PA) (1–5). The mood changes documented are generally an increase in “positive” mood states, such as calmness and pleasantness, and a decrease in “negative” mood states, such as depression and anger. The improvement in overall mood scores is seen after most types of exercise, both aerobic and anaerobic, that last for a minimal period of time or intensity.

It has been demonstrated that the regular participation in PA is more important in enhancing mood than the overall fitness (3). This study shows that even in subjects with high fitness levels, inactivity is associated with decreased mood scores, suggesting an advantage of regular activity on fitness in influencing mood. In the group of regularly active subjects, a higher fitness level did not lead to a better mood, and affective scores remained independent of it. Fitness level would obviously be important in the physical benefits of PA, whereas the mental effects might be related to other chronic and repeated mechanisms. Further, PA deprivation in habitual exercisers increased mood disturbance scores, which improved when exercise was later resumed (6). This suggests that repeated exercising might cause some degree of “addiction.”

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PA affects many different systems in the body and in various ways. Following PA, there is a rise in core temperature, a temporary lack of oxygen to some organ systems, and the secretion of different hormones and neurotransmitters is changed. These effects of PA are difficult to isolate, since they all occur together. Additionally, they may all lead to mood changes.

Several theories exist regarding the mechanism by which PA might cause mood changes, as reviewed by Yeung (5). The “thermogenic hypothesis” regards the elevation in the body’s temperature as the cause for mood effects following PA. The “distraction hypothesis” posits that the distraction of one’s mind from everyday stressors by PA is a major cause of mood elevation. The “mastery hypothesis” associates the mood changes with the achievement sensation in sport. The “endorphin hypothesis,” which relates the affective changes to β -endorphin (β E), is the major hypothesis discussed here. Thus, we see that enjoyment from sport can be owing to a combination of changes brought on by PA, where are not all fully understood.

In a study comparing groups of volunteers before, and after, a PA class or a hobby class, several differences in mood changes were observed (1). Although the hobby class group showed less tension and depression as compared to preclass measurements, the PA group also exhibited less tension and depression, but also less anger, less fatigue, and a higher measure of pleasantness after the exercise. For several mood indices, PA had a greater effect than a hobby class. However, seeing that the part of the mood enhancement in the PA group was also seen in the hobby class, these results provide some support for the distraction hypothesis. The difference between PA and hobby groups can be related to additional mechanisms besides distraction. Other differences between groups were seen in some preclass measurements. The PA group was less sad, angry, and depressed than the contrasting hobby group, and this could be related to the chronic effects of PA on mood.

Another connection between two hypotheses—the thermogenic and the endorphin—was seen in a further study (7). It suggested that the increase in plasma β E, together with a breakdown of the blood–brain barrier following PA-induced heat load, led to higher opioid levels in the cerebrospinal fluid (CSF). This enabled the thermogenic hypothesis to “enhance” the endorphin response and to increase the cerebral action of plasma β E.

Mood Assessment: The Profile of Mood Scores

The most common test used to quantify mood changes following PA is the profile of mood states (POMS) (8). Other tests for specific measures of cognition, anxiety, frustration, fatigue, and others may also be used, but provide narrower spectra of mood indices. When taken together with the POMS, these tests generally give similar results (1, 4, 6). The POMS is a list of 65 adjectives describing various affective states, which the subject grades on a five-point scale. The test is then used to calculate six measures of mood: tension–anxiety, depression–dejection, confusion–bewilderment, vigor–activity, anger–hostility, and fatigue–inertia. The six scores may be combined to yield a “total mood disturbance” score.

One major methodological drawback of this kind of questionnaire is its subjectiveness, since it relies on self-rated, rather than observer-related mood scores. Perhaps in the

future, biomarkers that assess mood levels, such as neurotransmitter metabolites, will allow for a more objective measurement of “mood” to enable comparison.

PHYSICAL ACTIVITY, HORMONES, AND MOOD

Hormones Can Cause Mood Changes

Many hormones are found to respond to PA (9). Hormones that could participate in affective changes are thyroxine (T4), cortisol, reproductive hormones, growth hormone (GH), β E, leptin, and more. Endocrine effects may modulate neurotransmitter synthesis, metabolism, and release, or they may alter the amount of receptors present on target neurons. Several hormones, which may influence mood states following PA, will be discussed in this section.

THYROID HORMONE

It is well documented that thyroid diseases may be associated with mood disturbances. The mechanism is not clear, but could be owing to the influence of thyroid hormone on synthesis of adrenergic receptors (10). β -adrenergic receptors are also found as postsynaptic receptors for noradrenaline in the brain, so rises in thyroid hormone levels might elevate the brain adrenergic “tone.”

Free T4 levels in plasma may rise following PA (9, 11). The reason for this increase is probably owing to an elevated level of free fatty acids in plasma, which displaces T4 from its binding protein. Since free T4 is the active hormone, this displacement has a functional effect. However, there is no correlation between the rise in free T4 levels and the rise in thyroid-stimulating hormone (TSH), which is the releasing hormone for T4 (11). TSH levels have been reported previously to remain unchanged following exercise (9), so total T4 levels should also remain constant. The increase in free hormone levels does not appear to have a physiological function. Otherwise, we would expect an activation of the releasing axis and higher TSH levels. The hormonal changes might be related to the increased energy requirements of the exercising body. The effect of repeatedly higher free T4 levels in the exercising person could add to the arousal sensation originating from noradrenergic brain activity.

CORTISOL

Cortisol is secreted from the adrenal cortex following stimulation by the adrenocorticotropic hormone (ACTH). ACTH, like β E, is derived from the large precursor protein proopiomelanocortin (POMC). These two hormones are secreted together in response to stress situations. Although ACTH activates the hormonal stress system, β E counter-regulates it through its relaxing effect, discussed below. Receptors for cortisol are found in various brain areas and are associated with emotions and mood (12).

Circulating plasma cortisol levels rise following aerobic and strenuous anaerobic types of PA (13, 14). Even though ACTH levels rise during short bouts of PA (13, 15), it takes about 20 min for cortisol to reach a higher and statistically significant level as compared to pre-exercise levels. This could be owing to the fact that steroid hormones are not stored, but require synthesis following the appropriate stimulus.

When cortisol and peripheral catecholamines were measured in relation to self-expressed mood, urinary cortisol levels correlated positively with the reported

level of “alertness” by the subjects (16). However, does cortisol cause arousal, or does the aroused state activate the stress pathway? An infusion of pharmacological levels of cortisol did not cause any significant changes in the measured affective states of healthy subjects (17). Therefore, perhaps acute changes in cortisol levels, such as after PA, do not affect mood. Nevertheless, it should be noted that the cortisol effects on mood are probably more chronic and dose-dependent, as sometimes seen in patients on prolonged treatment with systemic steroids; short-term infusions may not be able to mimic the exact action of endogenous cortisol in the brain.

THE REPRODUCTIVE HORMONES

PA has been shown to affect levels of reproductive hormones in men and in women (9). In women, moderate exercise during the midluteal phase can raise estrogen and progesterone levels. However, exercising during the follicular phase does not seem to influence estrogen levels (18). Therefore, exercise-induced mood changes, which could be caused by estrogen, are expected to be dependent upon basal estrogen levels. Different conclusions may be reached with varying fertility status (prepubertal, premenopausal (amenorrheic/eumenorrheic), postmenopausal) and in different stages of the menstrual cycle (9, 18). The interaction between the reproductive hormones and the stress system (19) may also contribute to the response of estrogen levels to PA. Frequent activation of the stress hormone cascade might cause menstrual difficulties. Additionally, estrogen itself can elevate ACTH and cortisol levels (19). Another interaction of estrogen and progesterone with cortisol may be through the competition between the different steroid hormones and free fatty acids on plasma binding proteins during PA.

The mood effects of the female sex hormones can be inferred from conditions in which estrogen levels decline, such as the late-luteal phase (“premenstrual syndrome”), following child delivery and entering menopause (19, 20). In these estrogen withdrawal states, mood can be enhanced by estrogen therapy, so estrogen is regarded as a mood-elevating hormone. The mechanism by which estrogen influences mood is probably through enhancement of monoamine neurotransmitter action by adding noradrenaline (NA) and serotonin (5-hydroxytryptamine (5-HT)) receptors to neural cell membranes (21).

The counterregulatory hormone progesterone can be considered as a mood-destabilizing hormone (20) by reducing estrogen effects. Additionally, progesterone can act directly on GABA receptors as a neuroactive steroid, producing sedative or hypnotic actions (12).

In trained men, acute aerobic or anaerobic exercise may induce a rise in plasma testosterone concentrations (14). However, when testing for mood changes following infusion of supraphysiologic doses of testosterone, no such changes were found (22).

INSULIN

Insulin concentrations are also affected by PA. After a single bout of exercise, plasma insulin levels fall for a day or more. The major effect of this decline on mood would be through the contribution to the higher level of plasma free fatty acids. A lower level of insulin releases the inhibition of the hormone-sensitive lipase in adipose tissue and allows for a higher rate of lipolysis. The rise in free fatty acids in plasma would increase the free fractions of T4 and steroid hormones, as discussed previously. Another metabolite affected by this mechanism is the amino acid tryptophan, precursor of 5-HT, which might elevate 5-HT levels in the brain.

As for the effects of glucose on mood, it has been shown that lower levels of glucose are associated with more negative affective states (23). In this regard, a possible lower glucose availability to the brain during PA might be expected to depress mood. In a study in which a carbohydrate supplementation drink was given to participants in a training camp, the increased availability of carbohydrates had a limited effect on mood (24). The only variable that changed was the measure of central fatigue, which according to the POMS was only slightly lower on some occasions in the supplemented group.

The effect of glucose on mood during or following PA is probably a minor one, since glucose levels in the brain could remain sufficient for its activity during PA. The mechanism of reduced fatigue might also be via the effect of slightly higher insulin levels on free fatty acids in plasma and the consequent lower tryptophan availability to the brain.

LEPTIN

Another hormone affected by PA is the adipose tissue-derived leptin. Leptin levels may decline in physically active people owing to a decline in fat mass (25–27). Acute bouts of moderate intensity PA were not shown to influence its mean plasma levels (25, 28, 29), although sample sizes were small and changes varied among individual subjects. Only an exercise as strenuous as a marathon run could alter its levels by reducing them only a little more than 10% (27). Additionally, as with other hormones, there may be a diurnal variation in leptin levels, which may confound exercise-induced results (30).

Leptin inhibits the action of neuropeptide Y (NPY), a ubiquitous peptide neurotransmitter, thus regulating food intake. Antagonism of the NPY-Y1 receptor caused anxiety in the rat as assessed by behavior in a specific maze (31). Although the food-regulatory receptor seems quite different from the NPY-Y1 receptor, a connection between leptin and anxiety may be suggested.

β E and Mood: From POMC to POMS

BIOLOGICAL ROLES OF β E

β E is a 30 amino acid peptide that is derived from the large precursor protein POMC. This protein is synthesized mainly in the anterior pituitary gland on activation of the stress system and also produces ACTH. β E binds to opioid receptors in various tissues, especially relevant for this discussion, to the brain and skeletal muscle. In the brain, activation of μ opioid receptors in the locus ceruleus inhibits noradrenergic activity (32), which might be part of the anxiolytic effect of β E. The diffuse and para-synaptic distribution of the μ opioid receptors on these cells implies that β E reaches the neurons mostly as a hormone, and not as a neurotransmitter (33).

In the nucleus accumbens, opioids cause the release of dopamine (DA), which may explain the mood elevation and exercise dependence brought about by β E (34). This dopaminergic excitatory effect is indirect, since the action of opioids is actually on GABA containing neurons synapsing with the dopaminergic cells (35). Activation of μ receptors hyperpolarizes GABA neurons, thus releasing their inhibition from the dopaminergic neurons. The DA-containing cells, which originate from the ventral tegmental area to end in the nucleus accumbens, are believed to play a major role in opioid rewarding effects, along with additional nondopaminergic pathways (35).

A metabolic role of β E is suggested to be owing to its effect on glucose levels during PA (36). β E enhances glucose uptake by skeletal muscles. This effect could be in the muscle tissue by activation of local opioid receptors, as can be seen by a β E-induced glucose uptake by isolated muscle (37), or by increasing the levels of the glucose counterregulatory hormones (38).

Therefore, β E may have two distinct roles in exercise. The first is the cessation of the stress response, since β E is excreted together with ACTH perhaps as an intended counterregulator. β E has a longer half-life and, therefore, will terminate the stress response after ACTH is degraded. This mechanism will allow PA to be calming and relaxing. The stress response is not fully shut down, so the levels of alertness and vigor may remain elevated. The second role of β E may be the delivery of glucose fuel into the working muscle.

β E LEVELS AND MOOD CHANGES

Several clues regarding the involvement of opioid receptors in PA-induced mood changes have led to the discovery of the β E connection. The increased feeling of “pleasantness” after running (2) resembles that of the opioid drugs and is frequently termed the “runner’s high.” Cessation of habitual exercise results in withdrawal symptoms in mice (39), and in humans—the POMS reveals increased tension, anxiety, depression, confusion, and total mood disturbance (6). After observing that β E rises following acute exercise and correlates with mood improvement, a direct blocking of opioid receptors showed that PA-associated mood elevation is indeed opioid-mediated (4). In this study, subjects were given naltrexone (an opioid receptor antagonist) or placebo prior to a high-intensity aerobics class. As expected, subjects that ingested the placebo exhibited mood improvements after class for most indices, whereas subjects given naltrexone did not. The total mood disturbance score decreased in the placebo group, but not in the naltrexone group.

Various stimuli elevate β E levels in plasma. These range from mental stressors, such as public speaking (40) or listening to techno-music (41), to acute pain signals, such as 90 s of coronary balloon angioplasty (42). Different types of PA can elevate plasma β E levels, as elaborated elsewhere in this volume. In brief—regarding aerobic exercise, a minimal duration of activity, lasting for about 60 min below the anaerobic threshold (the level of exercise in which the muscles require an anaerobic metabolism), has been suggested (43). Regarding incremental exercise, the rise in β E appears after the anaerobic threshold is breached (13). In this study, Schwartz and Kindermann (13) showed that the rise in β E parallels the rise in lactate levels. β E levels differed significantly from baseline at lactate concentrations of about 10 mmol/L. During resistance exercise, a very specific and intense workout is needed. In a study that employed six different combinations of intensity and rest, only one raised β E levels above baseline (44). This protocol was the most demanding, since it raised lactate levels to peak around 10 mmol/L and also reached the highest levels of plasma creatine kinase (an index of muscle breakdown). Other protocols utilizing lower resistance volumes could not demonstrate β E elevations (44, 45).

Taken together, intensities that raise lactate levels as high as 10 mmol/L are not common among leisure time activities. The mechanism causing β E changes in a moderately exercising population would generally be the first one mentioned—a minimal period of

aerobic PA, lasting about 60 min. Naturally, large deviations from this prediction might be expected among various exercisers.

The correlation between lactate and β E levels led Taylor et al. (46) to examine the importance of acidosis in β E release. Subjects infused with a bicarbonate buffer exhibited a suppressed β E response to exercise, as compared with placebo-infused controls. Therefore, the lowering of blood pH may be a major signal for β E release. β E correlated best with the measure of base excess, implying that the buffer capacity of the individual may determine the threshold for β E release by activity intensity. Various types and intensities of exercise that can lower pH levels below a specific individual threshold will cause β E secretion and its mood elevating effects. Interestingly, other exercise-released pituitary hormones, such as GH, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), do not show this regulation (47). In this study, prolactin exhibited a higher level following exercise combined with base administration as compared to exercise plus saline, suggesting an inhibitory role of acidosis on its release.

We conclude that an exercise load sufficient to lower pH beyond a certain individual level may elevate β E levels and cause mood changes. A possible bias in this cascade could be the choice of subjects. It could be that certain individuals who do not benefit from this mechanism are the ones who dislike PA, since most studies employ trained volunteers, who willingly engage in sports. Another issue is that variations in mood states before exercise could possibly alter the positive affective response, such as if the subject did not want to perform the exercise or vice versa: athletes who want to participate in the trial could be prone to easier mood enhancements.

Monoamine Neurotransmitters

Eventually, all the hormones previously discussed as modulating mood have been shown to alter neurotransmitter action. This is trivial, since “mood” itself is a product of interaction among neurons, although complex and spanning several brain regions. We shall briefly review the exercise-induced affective roles of the classic transmitters of the monoamine family—noradrenaline (NA), dopamine (DA), and serotonin (5-HT).

NORADRENALINE (NA) AND DOPAMINE (DA)

The production of NA and DA is derived from the amino acid tyrosine. The key enzyme in their synthesis is tyrosine hydroxylase, whose activity increases following stress, including PA (48). The regulation of this enzyme is very complex, since it affects the levels of these very important neurotransmitters (49). Cortisol may elevate enzyme levels by increasing transcription rate, although much more in NA-containing cells than in dopaminergic ones. An increase in the rate of synthesis and metabolism of NA and DA could influence mood. The higher levels of arousal and alertness following PA may be affiliated with higher NA levels. On the contrary, activation of opioid receptors on NA-containing cells of the locus ceruleus would inhibit their activity, suppress the arousal level and stress, and produce calmness (32). The rewarding effect of β E in the nucleus accumbens is mainly through DA action (34, 35) via a common pathway for most addicting agents.

SEROTONIN (5-HT)

5-HT is a neurotransmitter involved in appetite regulation, pain perception, mood, and sleep; it is possible that 5-HT plays a role in central fatigue during PA (50). The synthesis of 5-HT is also based on an amino acid precursor—tryptophan. Once again, cortisol may play an important part in the regulation of the key synthetic enzyme, tryptophan hydroxylase (51). Additionally, and in contrast to NA and DA synthesis, which is not enhanced by elevated tyrosine levels, 5-HT synthesis is affected by altering tryptophan levels (52). This is owing to the fact that the enzyme is not saturated with its substrate tryptophan. Elevation of free tryptophan levels in plasma might therefore raise 5-HT levels in the appropriate neurons. Indeed, in brains of exercising rats, higher levels of free tryptophan raised 5-HT concentrations by 35% (53).

The carrier protein for large neutral amino acids introduces several amino acids across the blood–brain barrier, including tryptophan. During PA, branched chain amino acids are utilized by the active muscle (54), therefore allowing more tryptophan to enter the brain owing to decreased competition. In this case, would ingestion of branched chain amino acids influence central fatigue? A study in which supplementation of these amino acids was given to runners revealed only slight mood changes (55). For the measure of fatigue, one part of the study revealed a trend for slightly less fatigue in the supplemented group. Another part revealed no influence of the amino acid supplementation, and in both experimental and control groups, fatigue measures were higher posttrace. Some positive effect of the supplementation was found—a better coping with complex cognitive tasks.

Another reason for higher tryptophan levels in plasma during PA is the lipolytic effect of exercise. The higher level of free fatty acids being mobilized in plasma displaces albumin-bound tryptophan and raises the free fraction of tryptophan, which is now available for entry into the brain.

In whole, both mechanisms may contribute to central fatigue during exercise. Another reason for fatigue could be merely energetic: slightly lower glucose or oxygen levels might also affect brain metabolism during PA, although for glucose this is unlikely (24).

SUMMARY

Moderate physical activity is widely accepted as a “recipe” for maintaining good health. Its beneficial action on delaying the onset or preventing many common diseases of western culture, such as ischemic heart disease (56–58), diabetes (59), stroke (60), and cancer (61, 62), is widely accepted. However, an additional benefit emerges—one of psychological well-being and of a better ability to cope with modern-day stress. It too has its mechanisms, although not fully demystified at the present. Future research will certainly expand our perception of brain pathways involved in emotion and mood state.

The acute effects of exercise include hormonal changes, mainly involving the activation of the stress system, as summarized in Fig. 1. Among the substances released is the

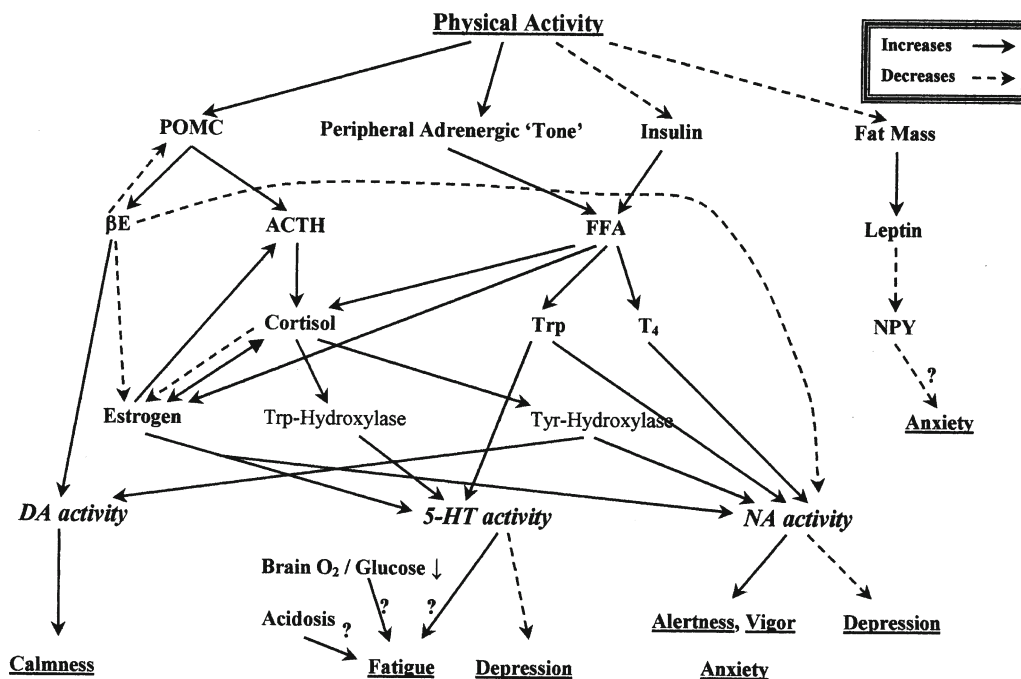


Fig. 1. Summary of the complex interrelationships among exercise, neurohumoral, and metabolic activity and their possible influences on mood states. Activation of the stress system during physical activity initiates several metabolic cascades, resulting in changes in neurotransmitter action. Both transmitter concentrations and receptor activity may be affected. Although neurotransmitters are considered, for simplicity, to be responsible for the different mood changes, the latter is probably a resultant of their combined actions. A *solid arrow* indicates stimulatory, and a *broken one*, inhibitory effects. *POMC* proopiomelanocortin; *βE* β-endorphin; *ACTH* adrenocorticotropic hormone; *FFA* free fatty acids; *T₄* free thyroid hormone; *Trp* tryptophan; *Tyr* tyrosine; *NPY* neuropeptide Y; *DA* dopamine; *5-HT* serotonin; *NA* noradrenaline.

neurohormone βE , the levels of which seem to correlate best with indicators of effort, such as blood acidosis. Its calming and anxiolytic effects are mediated mostly through modifying DA and NA neurotransmission in defined brain areas.

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Endocrine Responses to Acute and Chronic Exercise in the Developing Child

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INTRODUCTION

Physical activity plays a vital role in the developing child. Physical activity stimulates somatic growth, influences muscle development, strengthens bones, and contributes to the development of the cardiovascular, respiratory, and thermoregulatory systems. Many of these processes are regulated by the endocrine system: the release of hormones related to growth and development, as well as metabolism. For example, participating in normal physical activity during the day stimulates a pulsatile release of growth hormone (GH) from the anterior pituitary gland. GH, in turn, stimulates muscle development and bone growth. GH also stimulates the use of fat during the exercise, which in turn influences body composition. This is just one example of the study of pediatric exercise endocrinology as it relates to the understanding of the biological process of growth and development.

The study of pediatric exercise endocrinology is in its initial stages compared to what is known about adults; thus there is limited information. The research available suggests that some hormones respond to acute exercise similarly in children and adults; however, the responses of other hormones are associated with changes related to sexual maturation.

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In adults, excess adiposity modifies some of these hormonal responses to exercise, and the limited available evidence suggests that the same occurs in youth. Therefore, the purpose of this chapter is threefold: first to present hormonal responses to aerobic and anaerobic exercise in children and if these hormonal responses change as the child goes through sexual maturation, second to describe the present state of knowledge as to whether the degree of excess adiposity influences exercise-induced endocrine responses, and third to describe any known endocrine adaptations to exercise training in children.

Acute Hormonal Responses to Aerobic Exercise

CATECHOLAMINE

The catecholamine include epinephrine, norepinephrine, and dopamine. In many places outside of the USA, norepinephrine and epinephrine are commonly referred to as noradrenaline and adrenaline, respectively. There are actually two sources of these amines: the sympathetic nervous system (SNS), which releases predominantly norepinephrine; and the adrenal glands, which secrete mostly epinephrine. The catecholamine have far reaching effects during exercise influencing many systems within the body. Their response to exercise is intensity and duration dependent. During exercise, larger changes are normally seen in norepinephrine vs. epinephrine in children compared to adults (1).

During short-term high-intensity exercise, like graded exercise testing or a maximal aerobic power test (VO_2 max) usually lasting 10–15 min, studies have shown that the catecholamine respond similarly or slightly less in children compared to adults (1, 2). In particular, Lehmann et al. showed that norepinephrine and epinephrine increases with maximal exercise in boys were 25% lower than in adults, but fairly comparable during submaximal exercise if accounting for differences in absolute workload (2). The authors suggested that in children vs. adults, there is a lower maximal capacity of the SNS and the anaerobic system. The lower glycolytic capacity in children than adults has been linked to lower epinephrine release (3).

During submaximal exercise Eliakim et al. noted that 20 min of interval exercise (2:1 rest ratio) resulted in a 250% increase in norepinephrine and a doubling of epinephrine (4). Delamarche showed sex differences in catecholamine release between girls and boys during 60 min of endurance exercise (5). Boys' concentrations were higher than girls' and reached peak (specifically norepinephrine) sooner (15 min vs. 60 min into the exercise). But in general both sexes responded to acute submaximal exercise with an increase in catecholamine within 10 min (5). In summary, catecholamine release during maximal and submaximal exercise in children presents similar characteristics to adults, but the overall absolute concentrations may be lower in children than adults.

INSULIN

In healthy children, glucoregulation is not a major issue during short-term aerobic exercise as most studies find that blood glucose is unchanged or even slightly increased (2, 5). However, aerobic exercise does affect circulating insulin levels. The key functions of insulin include increasing cellular uptake of glucose, increasing glucose use for metabolism, and reducing beta-oxidation of fats, particularly in striated muscle (6).

During exercise, increases in SNS activation and the adrenal release of the catecholamine inhibit the release of insulin from the pancreas. This decrease in circulating insulin allows for better utilization of fats for energy and prevents most nutrients from being stored when they are needed to produce ATP (6). Although insulin declines, muscles can uptake glucose from the blood via the contraction-stimulated glucose transporters (7).

In general, several studies have demonstrated that the concentration of insulin decreases in response to acute exercise in children (4, 5, 8, 9) as it does in adults. Delamarche et al. studied insulin concentrations in response to 60 min of cycling exercise at 60% VO_2 max in prepubertal girls and boys (5). This study showed a 66% decrease in insulin concentrations within 15 min of the exercise session. Similarly, Viru et al. showed a decrease in basal insulin concentrations in girls who completed a 20-min cycling test also at 60% VO_2 max (9). Viru et al. followed prepubertal girls for 3 years and found that fasting insulin concentrations increased from prepuberty to mid-puberty and then decreased at the end of puberty if the children were of normal weight (9). However, the results of Viru and coworkers demonstrated that the decrease in insulin concentrations in response to acute exercise was independent of both pubertal stage and age. Interestingly, an earlier study by Wirth et al. showed different exercise-induced insulin responses: a decrease in response to acute exercise in prepuberty, no change during mid-puberty, and an increase at the end of puberty (10). To our knowledge, no other studies to date have evaluated whether exercise-induced changes in insulin are affected by pubertal stage as occurs with baseline insulin concentrations; therefore at this moment studies present conflicting results. In summary, insulin concentrations decrease within 15 min during exercise in children. Puberty clearly affects baseline insulin concentrations.

GLUCAGON

Glucagon is a pancreatic hormone and has the primary role of stimulating glycogenolysis in the liver and lipolysis at the adipocyte to help sustain metabolic homeostasis. The primary stimulus for the release of glucagon is low blood glucose levels, but increased SNS stimulation of the alpha cells also causes the release of glucagon. Unlike insulin, few studies have evaluated the changes in glucagon during acute exercise in children. In 1994, Delamarche showed a slight increase (10%) in glucagon in response to moderate intensity cycling in 8 to 11-year-old children (5). Glucagon concentrations rose within 15 min into the exercise, remained elevated at 30 min, and declined by 60 min into the exercise session. No differences between the sexes were observed in these responses. The 10% increase could have been related to hemoconcentration (known to occur with exercise) and not a result of greater production. Galassetti et al. studied the response of glucagon in adolescent girls and boys (11–15 years) in response to intermittent cycling at ~80% VO_2 max (11). Glucagon concentrations increased at the end of the 30-min bout, a change similar to those observed in adults. Two studies did not show an increase in glucagon in response to exercise. The study by Garlaschi et al. showed no change in glucagon during 30 min of aerobic exercise in children (12). Pilot data from Rubin et al. showed a lower glucagon concentration immediately postexercise compared to baseline in children ages 8–11 years who did a 30-min intermittent cycling exercise at 80% of their peak heart rate (8). The results of two of these studies suggest that in children glucagon increases during exercise, but other two studies do not support this response; more study is needed on this hormone.

CORTISOL

In response to physiological or psychological stress, the pituitary gland releases the adrenocorticotropic hormone that stimulates the release of cortisol from the adrenal cortex. Its major roles during exercise are to aid in lipid mobilization, protein breakdown, and glucose formation in the liver. In adults, cortisol increases in response to exercise bouts if exercise intensity is greater than 50% VO_2 max (moderate intensity) and may decrease during exercise if the intensity is lower than 50% VO_2 max (13).

Several studies evaluated changes in cortisol during aerobic exercise in children and presented either an increase or no change in response to the exercise bout. Del Corral showed that in 10-year-old male children exercising at 70% VO_2 max, compared to resting conditions, cortisol concentrations increased by 43 and 57% at 15 and 30 min respectively (14). After 15 min of recovery from exercise, cortisol concentrations remained elevated compared to rest. Viru et al. also showed an increase in cortisol in response to 20 min of cycling exercise at 60% VO_2 max in girls (9).

Several studies showed no change in circulating cortisol concentrations in response to high exercise intensity. Two studies used an intermittent cycling bout with ten 2-min exercise intervals separated by 1 min of rest at an intensity of approximately 80% VO_2 peak (4, 11). Both studies included children with a wide age range and showed that cortisol concentrations were lower 1 h into the recovery from exercise compared to baseline and immediately postexercise. However, the resting concentrations of cortisol ($\sim 14 \text{ mgdL}^{-1}$) were relatively high compared to other studies that evaluated this response. Data from Rubin et al. using the same intermittent protocol in children ages 8–11 years old also showed no immediate cortisol response to exercise, high cortisol concentrations at rest, and a steady decrease during the 1-h long recovery (8). Sills and Cerny who examined 30 min of continuous (50% VO_2 max) or interval (100% VO_2 max at 1:1 min work–rest ratio) exercise also found no significant effect of either exercise protocol on cortisol (15). As all studies were done in the morning, it is possible that cortisol concentrations were in their morning peak and then decreased following the natural circadian rhythm. If this was the case, the exercise-induced rise in cortisol was perhaps masked by the morning's peak levels.

A cross-sectional study showed in 235 children ages 2.2 to 18.5 years that there were no associations between baseline cortisol concentrations and either age, sex, or growth (16). In contrast, in a longitudinal exercise study, Viru et al. showed that the cortisol response to acute exercise was the highest (about a 55.5–66.2% increase) during mid-puberty, developmental stages II and III in comparison to stages IV and V (9). Hackney et al. have recently shown no difference in the magnitude of cortisol increases between adolescents in Tanner stages IV and V and adults completing a graded cycling test (17).

The response of cortisol to exercise is highly variable. Different mechanisms are involved in its secretion depending on the duration of exercise and the intensity (18). Why some studies show no response of cortisol to exercise is not understood. However, in general, the studies reviewed that showed no exercise-induced cortisol response included intermittent protocols. In summary, there are a similar number of studies showing increases or no change in cortisol in response to acute aerobic exercise. The only longitudinal study that evaluated changes with acute exercise showed a larger magnitude of

response as girls matured (9). A more recent cross-sectional study by Hackney et al., however, showed no differences in the response of cortisol in late puberty and adulthood in response to a maximal graded exercise protocol (17).

GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTOR AXIS

The growth hormone/insulin-like growth factor 1 (IGF-1) axis is a hormonal axis that involves growth hormone (GH) release from the pituitary and IGF-1 from the liver. This axis is involved in a number of physiological functions including muscle hypertrophy, body composition changes, bone mineral density, and cognitive function. The release of GH is controlled by the hypothalamus; it presents a circadian pattern, being greatest the release early after the onset of sleep (19). Once in circulation, GH stimulates hepatic release of IGF-1, which enhances bone growth and development and protein synthesis.

The GH-IGF-1 axis responds acutely to the stress of exercise. Several studies have demonstrated increases in GH in response to aerobic exercise: maximal, submaximal intermittent, and submaximal continuous (4, 8, 10, 12, 15, 20–24). The increase in GH in response to acute exercise is dependent on pubertal status (9, 10, 22–24). Children in more advanced pubertal stages respond with larger peak GH concentrations ranging from 6.9 ± 4.2 to 28.5 ± 14.3 mgL^{-1} than those children in early puberty (23). However, it is important to note that not all children in early pubertal stages respond to the stress of exercise with increased GH; this should be considered when analyzing results (21, 23). It could be expected that 5–10% of children in early puberty will not respond with increased GH in response to acute exercise.

The reason for the increase in circulating GH levels during exercise is not well understood. The increase is not related to blood glucose levels, as most studies show increased GH without hypoglycemia. Two studies have shown that the release is not related to growth-hormone-releasing hormone (4, 25). Gil-Ad has suggested that an inhibition of somatostatin may be involved (25), but this needs further study. The exercise-induced increase in catecholamine has been implicated but not proven (4). Another possibility is a downregulation of GH receptor by insulin (26), but once again, this has not been confirmed. Last, a possible metabolic regulator is the accumulation of hydrogen ions in the blood during exercise (27).

IGF-1 is the downstream hormone stimulated by GH. IGF-1 is released because of GH stimulation in the liver and also in the skeletal muscle if exercise presents a stress of sufficient magnitude. The IGF-1 response to exercise is controversial. Eliakim et al. did not find an increase in IGF-1 in children of normal weight (4). Similarly, Pomerantz et al. did not see changes in IGF-1 in boys who completed a 30-min cycling bout at 95% ventilatory threshold (24). Unpublished data by Rubin et al. also showed only a small change in IGF-1 with a similar bike protocol (8). Nemet and Eliakim (28) have suggested that during exercise, the IGF-1 increase is not entirely dependent on the release of GH, given that IGF-1 concentrations peak earlier than GH. In support of this speculation, unpublished data from Rubin et al. show an increase in IGF-1 concentration after intermittent cycling exercise in children and adolescents with Prader–Willi syndrome who present GH deficiency (8). The cause of the release of IGF-1 during exercise is not completely understood but appears independent from GH (8, 28). In summary, GH increases acutely with the stress of exercise. The increase in GH is larger with more advanced pubertal stages perhaps related to the increased testosterone.

REPRODUCTIVE HORMONES

Unfortunately, there are only a few studies evaluating longitudinal changes in the concentrations of testosterone and β -estradiol with puberty and acute responses to aerobic exercise. Viru et al. showed increased testosterone release during aerobic exercise in girls with increased pubertal development (9). In adolescent males, Hackney et al. demonstrated a higher testosterone response to a graded exercise protocol when adolescents were in Tanner stage V vs. stage IV (17). The testosterone response to exercise in Tanner stage V was similar to the one in young adults. Similarly, larger increases in response to exercise in β -estradiol were seen in developmental stage IV vs. stages II and III in adolescent females (9).

LEPTIN

Leptin is a hormone produced by the adipose tissue and is involved in energy balance (29). A decrease in leptin results from energy imbalance or low energy availability, as well as to carbohydrate availability (30). Leptin does not play a major role in the regulation of metabolism during exercise, and unless the exercise session leads to a large caloric imbalance, no apparent changes are seen in leptin (31). Leptin relevance to exercise responses is related to reproductive functions: the release of luteinizing hormone (30, 32) and its association with bone mineral density in female athletes. In children, leptin appears to be involved as a signal of energy stores in the release of sex hormones, regulating the onset of menses (33). Because of its role in the regulation of reproductive function, lower leptin concentrations have been related to bone mineral density problems in young athletes who present a caloric deficit (34). However, a recent longitudinal study by Donoso et al. showed that although dancers had low fat mass and leptin concentrations, over time, their bone mineral density was normal when compared to controls (35).

Our current understanding of the relationship between leptin and exercise in children is inadequate. It appears that there is no acute response to exercise and the acute reported increases are likely related to hemoconcentration in addition to variation of the diurnal release of leptin. Kraemer et al. were the first to evaluate acute changes in leptin to a discontinuous graded exercise protocol in adolescent females (36). This study showed an acute increase in leptin with exercise, but the increase (~15%) could have been due to hemoconcentration (36). Souza et al. showed that a maximal exercise stress test did not influence resting levels of leptin in normal weight or obese children (6–11 years) (37). No other studies to date have evaluated the acute responses of leptin to exercise in children (36, 37). Conversely, studies have shown that diminished leptin levels are evident immediately postexercise and during 48 h of recovery from exercise in adults perhaps indicating low energy availability (31).

Acute Hormonal Responses to Anaerobic Exercise

CATECHOLAMINE

Catecholamine have been proposed to be important stimulators of hormone secretion during resistance exercise (38, 39). Three studies, completed by the same group, evaluated the effects of resistance exercise on catecholamine (40–42). The first study used five sets of ten knee extensions with 40% 1RM (the maximal amount of resistance that can be moved only once) and two sets of knee extensions

until exhaustion to evaluate plasma norepinephrine levels in adolescents (Tanner stage 5), men, and women (40). No significant differences were found among the groups for norepinephrine (40). However, the increase in the peak plasma epinephrine from pre-exercise was about twice as high in boys ($5.0 \pm 2.6 \text{ nmolL}^{-1}$) as in men ($2.5 \pm 0.8 \text{ nmolL}^{-1}$) and in women ($2.1 \pm 0.6 \text{ nmolL}^{-1}$). The second study investigated plasma catecholamine responses in adolescent (15 ± 1 years) and adult male athletes at rest and after two sets of 30 half-squats at 50% 1RM with 2 min of recovery between sets (41). The researchers found lower norepinephrine concentrations in the adolescents ($15.7 \pm 7.8 \text{ nmolL}^{-1}$) compared to the adults ($32.7 \pm 13.2 \text{ nmolL}^{-1}$) while both groups exhibited an 11-fold increase (41). In contrast, epinephrine changes in response to resistance exercise were similar for both groups. The third study examined the effects of three sets of knee extensions under the influence of delayed onset muscle soreness (DOMS) also in adolescent boys and adults. During and 15 min after exercise, norepinephrine and epinephrine concentrations were significantly greater than rest in both groups (42). In this study, the increased catecholamine responses in boys and men were similar, indicating that there were no significant differences between the groups. The results of all three studies indicate that young male athletes respond with increased catecholamine concentrations to resistance exercise (40–42). However, the studies present different results in the degree of change of the hormones compared to adults.

CORTISOL

The importance of cortisol release during resistance exercise is not as well studied as in endurance exercise (43). However, the release of cortisol during resistance exercise in adolescents may be related to increasing the building blocks for protein synthesis after exercise as well as the reduction of inflammation in response to muscle damage (43). Pullinen et al. noted that following exhaustive knee extensions, cortisol concentrations increased significantly in 14-year-old boys at the end of puberty (40). Additionally, the change in cortisol concentration in boys ($0.13 \pm 0.10 \text{ mmolL}^{-1}$) was greater than in men ($-0.05 \pm 0.06 \text{ mmolL}^{-1}$). In contrast, when studying changes in cortisol under the influence of DOMS, cortisol levels were significantly lower in boys than in men during three sets of knee extensions until failure at 40% 1RM (42). These studies suggest that cortisol responses to resistance exercise in adolescents appear greater than adults but under DOMS adults release more cortisol than adolescents (18). The decreased response during DOMS could be related to (1) less total muscle mass being involved thus less damage, (2) less physical pain which would elicit less a stress response, or (3) a faster recovery rate from muscle damage. This is a fertile area for study.

GH

GH secretion relates more closely to peak exercise intensity than exercise duration or total exercise volume (6). In terms of resistance exercise, as summarized by Kraemer and Ratamess, many factors influence the magnitude of the response of GH including the muscle mass, the intensity, the rest period in between sets, the total volume, and the amount of work (27). In addition, the change in GH is closely related to the increase in lactate, which reflects the effect of all factors previously presented (27). Several studies in adults (for a complete review, see Kraemer and Ratamess) showed that during exercise, the accumulation of hydrogen ions in the blood regulated the release of GH (27).

Pullinen et al. evaluated changes in GH following exhaustive knee extensions in 14-year-old boys. GH increased tenfold from about 1.5 mgL^{-1} at rest to about 15.0 mgL^{-1} after exercise (40). However, the authors indicated that after taking the change in plasma volume into consideration, the change in GH in response to exercise was not statistically significant, possibly because of the large standard deviations. In a follow-up study in 2010, Pullinen et al. observed that adolescent boys had significantly increased GH levels during and after three sets of exhaustive knee extensions with a load of 40% 1RM compared to pre-exercise (42). Also, the boys displayed significantly greater GH levels than men during all stages of the exercise and recovery. From the scant information available, it is suggested that adolescent males respond to resistance exercise with acute increases in GH. The magnitude of the response in children is unclear at this point in terms of it being lower, higher, or similar to adults, and no data is available in females.

TESTOSTERONE

Testosterone is an anabolic hormone found in men and women, with men having much larger quantities than women. During childhood both girls and boys display similar levels of testosterone; however, during mid-puberty (about stage III) concentrations of testosterone and its free form increase significantly in boys (44). Testosterone increases muscle protein anabolism directly and also by stimulating the release of GH. Heavy resistance exercises requiring the use of large muscle groups, moderate to high volumes, and short rest periods have all been shown to increase testosterone levels after an acute bout of resistance exercise, primarily in men (45).

The response of testosterone to resistance exercise in children has been evaluated in a few studies. In general, all studies found an acute increase following resistance exercise (40, 41, 46). In an early study Kraemer et al. observed a 32% increase in testosterone in junior elite weight lifters (17.3 ± 1.4 years) after a traditional weightlifting session involving high-intensity, high-speed resistance-training exercises (46). In a subsequent study in 14-year-old boys, free testosterone levels increased by $2.1 \pm 2.6 \text{ pmolL}^{-1}$ after exhaustive knee extensions (40). Later, Pullinen et al. showed significant increases in testosterone after an exhaustive half-squatting exercise session (2 sets of 30 repetitions at 50% 1RM) in adolescent male athletes (41). The adolescent boys experienced a lesser increase in testosterone levels compared to adult males. In 2010, Pullinen et al. observed no changes in either free or total testosterone in adolescent males during and after three sets of knee extensions under the influence of DOMS (42). The adolescent boys had significantly lower levels of testosterone at rest, during, and after exercise when compared to men (42). In summary, testosterone appears to increase in response to acute resistance exercise in adolescent boys and is normalized shortly after exercise. The magnitude of the increase in testosterone is lower in adolescents than adults. Evidence is lacking on the testosterone response to resistance exercise in young children or girls.

THE ROLE OF ADIPOSITY AS A MODIFYING FACTOR OF THE HORMONAL RESPONSES TO EXERCISE

Catecholamine release during exercise contributes to lipolysis, sustaining euglycemia, and increased glycogen breakdown during exercise. These effects are important for individuals who are overweight or trying to lose weight. Given the increasing rates in

pediatric obesity, researchers in pediatrics have investigated exercise-induced hormonal differences in obese children and adolescents. Eliakim et al. demonstrated a blunted catecholamine release in response to a 30-min, high-intensity, intermittent cycling bout in obese children vs. their lean counterparts (4). In this study, obese children responded with increased catecholamine concentrations to exercise, but the degree of increase was lower than in lean children (4). The authors suggested that the blunted adrenergic response could be linked to a blunted GH release in obese children (4). In contrast, a later study involving adolescent girls did not find significant differences in the catecholamine increase after about 7 min of maximal cycling when comparing obese, overweight, and lean participants (47). However, the lean group had fairly high levels of body fat (~30%), while the overweight group had about 37%, and the obese group had 42% of body fat. In the study by Eliakim and colleagues, lean children had a mean of 20% of body fat while the ones classified as obese had a mean of 38% of body fat. Perhaps the fact that the adolescent girls in Zouhal et al. study did not have very drastic differences in body fat content among the groups masked any possible differences in catecholamine response (47). Thus, some evidence suggests that in children as in adults, high levels of body fat may blunt the catecholamine response to acute exercise (4).

It has been demonstrated by many studies now that obesity in childhood is associated with high baseline concentrations of insulin (48–50). In response to exercise lean and obese children respond with a decrease in insulin concentrations (4). Eliakim et al. found that in response to a 30-min intermittent exercise at 80% VO_2 max, the magnitude of the decrease in insulin was larger in the obese children compared to normal weight children. The larger decrease of insulin in obesity appears to be related to the higher resting insulin concentrations in the obese children compared to normal weight youth and not to a lower concentration at the end of exercise. In contrast, from the limited data comparing glucagon responses to acute cycling between normal weight and obese children and adolescents, no differences can be pointed out (8, 12).

Two studies on the cortisol responses of obese children did not show any differences in the response of cortisol to a similar exercise protocol (4, 8). Eliakim et al. noted that high-intensity interval exercise resulted in a 20% increase in cortisol in normal weight children and approximately a 5% decline in obese children exercised at the same time of day; however these differences were not statistically significant. Rubin et al. also showed that normal weight and obese children responded similarly to an intermittent aerobic protocol (8). At this point, a larger magnitude of decrease in insulin immediately after exercise has been shown in obese vs. lean children, no major differences in glucagon, and there is not enough data to demonstrate that obesity affects cortisol response to exercise. Since cortisol increases fat utilization during exercise, which could be of benefit for obese children, it would be important to have further studies evaluating changes in cortisol in children in response to exercise perhaps conducted during the afternoon.

As mentioned earlier, GH release during exercise is affected by adiposity. In 1975, Garlaschi et al. demonstrated a decreased GH secretion in obese prepubertal children compared to their lean counterparts (12). Similar findings were later presented by Eliakim et al., showing a larger GH response to a 30-min intermittent cycling bout in normal weight vs. overweight children and adolescents (4). Similarly, Oliver et al. showed that the blunting of the GH response to exercise was dependent in a dose–response manner to the degree of obesity (22); the more obese the children, the larger

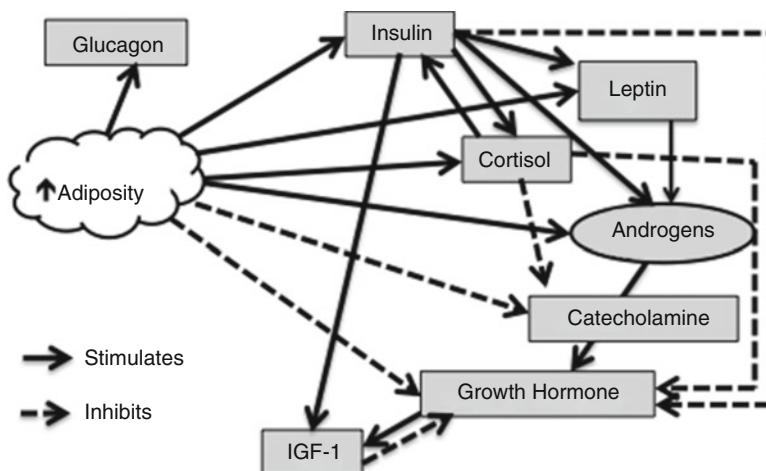


Fig. 1. Proposed influences of adiposity in the release of some key hormones during exercise.

the blunting of the GH response. Puberty also appeared to influence this response independently of adiposity (22). Last, unpublished data from Rubin and associates also shows the blunting of GH in response to exercise in obese children ages 8–11 but not in normal weight children (8). Eliakim et al. also reported a small (~15%) immediately postexercise increase in IGF-1 in obese children but not in normal weight children (4). Based on the available evidence, obesity decreases the magnitude of the GH response to exercise. However, it does not appear to alter downstream mediators such as IGF-1.

Nemet and Eliakim have speculated that blunted GH response could be responsible in obese states for a diminished response in body composition changes because of exercise training (51). It is possible that the combined reductions in catecholamine, GH, and perhaps cortisol could result in obese children utilizing less fat than normal children during exercise, increasing the difficulty of weight loss. The elevated insulin found in obesity may be one reason behind the lack of responsiveness of the GH-IGF1 axis (52, 53). In terms of the blunted catecholamine response to exercise, it may be explained perhaps by the interconnection between the adrenal cortex and medulla. It is possible that the increased insulin concentration in obesity increases cortisol and that blunts the adrenal medulla release of catecholamine (54). Figure 1 presents some of the demonstrated relationships and also some other possible interactions (13). Although we do not fully understand the mechanisms behind the alteration of some endocrine axes by excess adiposity, it is clear that adiposity in childhood and adolescence alters the functioning of the endocrine system not only at rest but also in response to exercise.

CHRONIC CHANGES IN HORMONES WITH EXERCISE TRAINING

General Responses

Little is known about the effects of exercise training on the hormonal responses of children. Most of the studies have evaluated changes in cortisol, insulin, the GH-IGF-1 axis, or reproductive hormones. To our knowledge data is not available on chronic changes in glucagon or catecholamine to exercise training.

INSULIN

Changes in insulin concentrations in children and adolescents in response to training protocols have been the focus of attention since early 1990s. Improvements in insulin concentrations have been demonstrated in laboratory-controlled, school-based, and community-based studies involving aerobic exercise. The largest changes in insulin concentrations are observed in obese children or adolescents (50, 55) or those who present the highest insulin at baseline (56). Decreases in basal insulin concentrations have been associated with increased fitness (56). Insulin responds to exercise training independently of changes in body mass (57) and in a relatively short period of time (8 weeks). Changes in insulin in response to chronic aerobic exercise have been large studied in pediatric population as in adults, and it is currently the hormone we seem to better understand in children.

CORTISOL

In an early study in two subsets of mostly prepubescent female gymnasts, the concentration of cortisol was evaluated at baseline and over 3 days of intensive training (3.5–5.5 h long) (58). No changes in cortisol were observed in this study. Kraemer also evaluated chronic changes in cortisol in adolescent female runners over a 7-week period but did not find any changes in either resting or exercise concentrations (36). Di Luigi et al. reported that the cortisol responses to soccer training decreased as subjects aged, suggesting that the intensity of the training program decreased or they were adapting to the training protocol (59). In a longitudinal study Daly et al. followed a group of prepubertal gymnasts and a control group of girls of similar age through the different phases of training in a year (60). There were no differences in cortisol concentrations because of the different training phases or between the groups. However, the testosterone/cortisol ratio was elevated in the phase of strength/conditioning only in the gymnast indicating a possible catabolic state (60). In obese boys who completed a 12-week exercise, intervention cortisol concentrations decreased but not in the control group (61). More studies are needed to clarify the chronic effect of exercise training on the baseline concentration of cortisol and its significance.

GH-IGF-1 AXIS

With the increased participation in competitive sports at a young age, a major concern was that exercise training during childhood could stunt growth, because of negative influences on the GH-IGF-1 axis. This concern was partially based on the stress generated with the training and also because of the possible negative caloric balance induced by exercise training (62).

An early study in prepubescent female gymnasts measured IGF-1 at baseline and over 3 days of intensive training 3.5–5.5 h long (58). Concentrations of IGF-1 decreased by 25% after the 3 days with no concurrent changes in GH. No measurements of body mass or caloric balance were conducted, and a control group was not included. Nonetheless, the study presented a significant reduction in IGF-1 with only 3 days of intensive training. Previous investigations suggested that carbohydrate availability could affect IGF-1 secretion (63) but also thyroid hormones, or increase in GH could be responsible (62, 64, 65).

In a follow-up study, adolescent girls went through a 5-week endurance training period with no caloric restriction and demonstrated a decrease in IGF-1 in the intervention

but not in the control group (66). This study also showed no change in GH or its binding protein in the intervention group (66). Roemmich and Sinning also evaluated changes in GH, GH binding protein (GHBP), and IGF-1 in undernourished male adolescents throughout a wrestling season (67). They showed increases in GH concentrations and concomitant decreases in GHBP and IGF-1 concentrations during the competitive season (3–4 months long) (67). During the postseason, all hormones returned to baseline concentrations. The authors speculated that GH receptors decreased, and in response, GH secretion increased to compensate for the partial GH resistance possibly triggered by the negative caloric balance. In a later study, Eliakim et al. studied male adolescents who went through a 5-week exercise training program and a matched control group (68). In the training group, IGF-1 and GHBP concentrations decreased similarly to the study on wrestlers (about 12 and 21% respectively). However, the adolescents in this latter study were in eucaloric state and experienced no changes in body mass. Eliakim et al. also speculated of a transient decrease in GH sensitivity induced by exercise training (66).

In a recent review article on the topic, Eliakim and Nemet explain that there may be two phases in the response of the GH-IGF-1 axis to exercise training (51). The initial phase is characterized by decreased IGF-1 and transient resistance to GH action (51), with IGF-1 perhaps produced locally and not as much systemically. In the second phase, if energy balance is sustained, there is a rebound of systemic IGF-1 as well as an increase in GHBP. Therefore, there seems to be enough evidence to support that exercise training does not have a negative impact in the GH-IGF-1 axis if caloric balance and adequate carbohydrate are sustained. Equally important, short periods of training with negative caloric balance can cause transient disruptions in GH and IGF-1.

SEX HORMONES

The increased number of children and adolescents participating in competitive sports has captured the attention of the medical discipline. The high level of exercise training completed by these young athletes and the possible risks associated with it has gained considerable attention. In particular, delayed menarche and alterations to the hypothalamic–pituitary–gonadal (HPG) axis have been documented in female young athletes such as runners (69). The pediatric exercise science community undertook several studies to determine the possibility of alterations and their severity in both sexes.

Rowland et al. failed to show that an 8-week training season in cross-country male runners affected either total testosterone or free testosterone (70). Later, the functioning of the HPG axis in undernourished wrestlers was evaluated before season, late season, and after the season ended (67). The study included a group of age-matched healthy male controls. The wrestlers' total testosterone and free testosterone concentrations decreased during the season and returned to baseline during the postseason. These changes were unrelated to estradiol, prolactin, or cortisol, which modulate the activity of the HPG axis. Only the levels of free testosterone fell below the normal range. The evidence from both studies indicates that in pubertal males, exercise training of short duration may not affect the functioning of the HPG axis negatively; however, a longer training season accompanied with caloric deficit may affect the HPG axis, specifically testosterone release. Nevertheless, it appears that once the athletes are in the postseason period, the levels of these male sex hormones return back to normal.

In girls, Jahreis et al. presented an increase in testosterone concentration during short (3-day) and longer (7-week) training periods (58). It was shown in young female gymnasts that over 3 days of intensive training (3.5–5.5 h a day), concentrations of total testosterone increased by approximately 25% with no significant change in dehydroepiandrosterone (DHEA) and its sulfate form (DHEAS) (58). Similar findings were shown by Kraemer et al. during a 7-week competitive season for track and field in female adolescents (36). Changes in the hormones were evaluated at rest and in response to a graded discontinuous exercise protocol to volitional fatigue. No effect of training was found for DHEA or DHEAS. In contrast, testosterone concentrations increased over time. At the end of the season the adolescents presented a larger exercise-induced increase in testosterone compared to baseline, similar to data presented by Viru (9). Overall, exercise training appears to influence testosterone concentrations both in boys and girls. Although it may affect the concentrations of testosterone negatively in boys, this effect is transient and reverses during the off-season. The data of one study suggest that changes in testosterone can occur in girls after 3 days of training (58). Pubertal maturation appears to affect baseline testosterone levels in girls and their response to exercise (9).

LEPTIN

Obesity increases resting levels of leptin in children (37, 71). Exercise training by itself may not change leptin unless the training program results in negative caloric balance, improvement in fitness, and fat mass loss (72, 73). For example, a study in normal weight female adolescents showed no change in basal leptin concentrations after a 7-week running competitive season (36). Kraemer and colleagues also did not see differences pre-/post-training season in the acute leptin response to exercise. In contrast, in a 4-month training study in obese 7–11-year-old children, a decrease in baseline leptin was reported (71). The decrease in basal leptin concentrations was greater in those children who had the highest concentrations at the beginning of the program. The change in leptin was not only related to changes in body mass but also the training protocol itself (71). When children stopped participating in the training program their leptin concentrations increased. Several hypotheses explain aspects of leptin regulation. One possibility is that improvements in insulin sensitivity and thus lower basal insulin in response to training lead to lower leptin concentrations (71). Several studies have now followed results showed by Gutin and colleagues presenting decreases in leptin concentrations in obese children or adolescents completing physical activity or lifestyle interventions at least 3-month long (61, 74). Conversely, Pedrosa et al. showed no significant changes in leptin in overweight children (body mass index [BMI] > 85th percentile) either completing an individualized lifestyle protocol or a group lifestyle intervention at either 6 or 12 months (75). No measurements of body fat were included in this study besides BMI z-scores, so it is hard to determine if there were substantial reductions in body fat. From the available evidence, it appears that children or adolescents who have excess adiposity have higher leptin concentrations compared to healthy counterparts. If youth participates in at least 2-month programs including exercise that induces fat mass loss, likely decreases in leptin are seen.

Resistance Training

One debate in the pediatric literature is related to the contribution of neural vs. hormonal adaptations resulting from strength training. The fact that significant improvements in strength with resistance training are observed in children in the absence of hypertrophy indicates that neurologic mechanisms likely play a large role in strength development during childhood (3). These factors include, but are not limited to, increases in motor unit firing rate, recruitment, or conduction velocity. Strength levels increase similarly in girls and boys until the onset of puberty. Once puberty begins, anabolic hormones and growth factors play a large role in muscular enlargement in boys (45). In this section we will discuss the role of resistance training on insulin, cortisol, GH, and testosterone because of their clinical relevance or their anabolic or catabolic properties.

INSULIN

A few studies have evaluated chronic changes in insulin concentrations in response to resistance training (76). In a randomized controlled trial (RCT) Shaibi et al. demonstrated that in overweight males, two resistance-training sessions a week for 16 weeks increased insulin sensitivity by 45% in 90% of the participants (49). Fasting insulin concentration also decreased in the intervention group, but the change was not statistically significant. Van Der Heijden also showed improved hepatic insulin sensitivity in obese adolescents completing a resistance-training program but no change in peripheral insulin sensitivity or fasting insulin (77). A follow-up RCT in 54 obese adolescent girls and boys showed no effect of the resistance-training protocol in insulin concentrations or insulin sensitivity (78). Davis et al. suggested that perhaps the later study lacked statistical power as the exercise dose used translated into gains in muscle strength but no significant change in insulin parameters. More studies are needed to demonstrate the effectiveness of resistance training in insulin in children or adolescents. Possibly, the twice a week may not be sufficient frequency of sessions a week given that exercise stimulated insulin sensitivity lasts up to 48 h postexercise (79). Future studies evaluating this paradigm should use 3–4 days of training a week to determine the efficacy of such training in increasing insulin sensitivity and decreasing insulin concentrations.

CORTISOL, GH, AND TESTOSTERONE

A few studies, to date, have evaluated changes in hormonal concentrations in response to resistance training. Cross-sectional data comparing trained vs. untrained boys (11–12 years) show no differences in baseline GH or testosterone concentrations (80). Two longitudinal studies evaluated changes in cortisol, GH, and testosterone after 1 year of resistance training. Mero et al. showed no differences in cortisol or GH because of training (81). In contrast, these authors showed increases in basal testosterone from 2.92 ± 1.04 to 5.81 ± 1.33 nmolL⁻¹ in the junior male athletes (10–12 years) participating in an athletic program but not in the nonathletic peers (81). Tsolakis also studied the effect of long-term resistance training (12 months) on hormonal levels of 12 to 13-year-old males and a control group (82). The results indicated that the training program had no effect on serum GH or testosterone concentrations. The performance variables such as handgrip strength and jump height were also similar between the fencers and the control group, which questions the stress of the training program. However, the fencers demonstrated a significant increase in leg cross-sectional area, while the

control group did not. It is possible that an increase in cross-sectional leg area unaccompanied by an increase in resting testosterone levels may have occurred due to acute increases in testosterone, encouraging anabolism in response to acute exercise stress.

Last, Tsolakis et al. also evaluated hormonal responses after 2 months resistance training and 2 months of detraining in prepubertal (11–13 years) and pubertal boys (14–16 years) (83). As expected, pre-training mean testosterone levels were approximately three times greater in the older boys (14.6 ± 4.2 nmolL⁻¹) compared to the younger boys (4.9 ± 5.7 nmolL⁻¹). In response to the training program, testosterone increased in the young boys (124%) and in the old boys (32%). Following the 2-month detraining period, mean testosterone levels did not significantly change in either group when compared to the post-training levels. The changes in testosterone may have been so dramatic in the young boys as the effect of the resistance training may have been coupled with normal increase in testosterone due to puberty. In a follow-up study using a similar design with a control group, Tsolakis et al. concluded that after 2 months of resistance training, boys (11–13 years) experienced a doubling of basal testosterone (4.9 ± 5.7 to 10.9 ± 6.2 nmolL⁻¹) independent of changes in testosterone because of maturation (84). As expected, muscle strength decreased after detraining, but testosterone levels were maintained throughout the detraining period.

The literature regarding resistance-training-related changes in anabolic and catabolic hormones shows no effect on basal concentrations of cortisol and GH in boys. Some short- and long-term studies suggest that testosterone increases independent from the increases that naturally occur due to puberty, but the evidence is conflicting. Authors speculate that increases in muscle strength during childhood and adolescence depend on neural adaptations (84), but studies evaluating changes during training were not long enough to have GH or testosterone-mediated increases in cross-sectional area. Therefore, at this point, it would be premature to conclude that anabolic hormones such as GH or testosterone are unrelated to gains in muscle mass or strength during childhood and early adolescence.

SUMMARY AND FUTURE DIRECTIONS

The studies conducted have just begun to quantify the effects of different types of exercise on the hormonal responses of children and adolescents as it is noted in Table 1. There are numerous hormonal responses to varying exercise conditions, for which there is no descriptive data. The majority of endocrine studies have been completed on short-term aerobic exercise. No information exists on prolonged aerobic exercise, possibly because naturally children do not choose to participate in this form of exercise. There is some data on aerobic exercise training hormonal adaptations at rest, but very little knowledge on how the aerobically trained children respond to exercise. Data are starting to accrue on resistance exercise, but there is little information on the adaptations that occur with resistance training. Furthermore, there are very limited studies on the mechanisms causing the specific exercise responses or what is the significance of the responses. In some cases, like GH, catecholamine, or cortisol, one can speculate as to the mechanisms based on the adult literature. However, a number of the hormonal responses are interactive, and there is little or no understanding of these interactions in children. For example, insulin is influenced by the catecholamine and the SNS. Both the SNS and the

Table 1
Summary of hormonal responses of children to acute and chronic exercise

Hormone	Aerobic exercise			Resistance exercise	
	Training effects			Training effects	
	GXT	Submaximal	Rest	Rest	During exercise
Catecholamine	↑	↑	↓	↓	↑
Insulin	NC	↓	↓	?	?
Glucagon	NC	NC	?	?	?
Cortisol	↑	↑ or NC	↓ NC	?	↑ ↓
Growth hormone	↑	↑	NC	↑	?
IGF-1	NC	NC		?	?
Testosterone	↑ ?	?		↑ NC	?
Estrogens	?	?	?	?	?
Leptin	NC	NC	↓ NC	?	↓ NC

GXT graded exercise test; ↑ increase; ↓ decrease; NC no change; ? presently unknown

catecholamine responses to exercise are less in children than adults, yet the decline in insulin during exercise approximates the adult response. Resting GH concentrations differ with pubertal status, but postexercise concentrations appear similar among pubertal stages, so why the similarities?

Obesity appears to modify the hormonal responses to exercise. Could these modifications partially explain why obese children have difficulties losing weight via an exercise program? Could the modification be genetic and partially explain why the weight gain occurs? Quite interestingly, obese children typically have GH and IGF-1 levels similar or slightly lower than normal weight youth. So the question becomes, why does the obese child typically have a faster linear growth, faster rate, and earlier pubescence than a normal weight child? There are many important questions in need of future study. The study of pediatric exercise endocrinology is clearly in its “infancy.”

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Exercise in Older Adults: The Effect of Age on Exercise Endocrinology

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The world's population is aging at an unprecedented rate. In 2000, 606 million people were aged 60 and over, and the number of older adults is expected to triple by the year 2050, resulting in a global population of two billion senior citizens. The rate of growth for the older adult population is significantly greater than total population growth, meaning that approximately one in five people in the world will be over the age of 60 by 2050 (1). This dramatic demographic change will have a significant economic and social impact on society.

Characterized by a decline physical in well-being, aging is marked by changes in body composition (increase in body fat, loss of muscle mass and bone density), decreased muscular strength and power, decreased aerobic power, and increased risk of chronic disease. Sarcopenia, the loss of muscle mass with aging, is associated with diminished functional capacity and increased frailty among older adults, which threatens independence. This issue is particularly important in light of our increasing life expectancy—there are clear social, economic, and even ethical implications to continually reducing adult mortality without concomitantly striving to increase disability-free life

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expectancy at a similar rate. Thus, understanding the aging process is becoming an increasingly important agenda and even more critical is understanding how best to promote “successful aging.” The concept of successful aging was introduced by Rowe and Kahn (2) and is defined as a low probability of disease, high cognitive and physical function, and active engagement with life. The goal of this chapter is to examine the relationship between exercise and successful aging and, more specifically, the possible role of the endocrine system in mediating that relationship.

THE AGING ENDOCRINE SYSTEM

Aging has been described as a “time dependent functional decline that converts healthy adults into frail ones” (3), an “inexorable physiological deterioration which eventually leads to the development of frailty” (4), and “the accumulation of diverse deleterious changes in the cells and tissues with advancing age that increase the risks of disease and death” (5). Aging impacts many body systems including the endocrine system, and in the course of normal aging, several changes in endocrine function are observed. There is a progressive loss of secretory cell mass, a decline in the rate of hormone degradation, alterations in end-organ and tissue sensitivity to hormones, and changes in the modulation of endocrine feedback mechanisms (6). Consequently, there are age-specific changes in various hormone levels, although any discussion of aging must distinguish between “normal” aging and the effects of age-related disease. For example, the prevalence of impaired glucose tolerance and type 2 diabetes increases among the elderly, resulting from a combination of age-related insulin resistance, genetic predisposition, and environmental factors such as poor diet (7). Thyroid dysfunction is also more common among older adults, with overt and subclinical hypothyroidism occurring in 0.6–7.8% of elderly people, more commonly in women than men (8). The causes of hypothyroidism in the elderly are similar to the causes among younger adults, primarily resulting from autoimmune disease (8, 9). Both diabetes and hypothyroidism are clinically relevant conditions that require pharmacological intervention, and although prevalence is increased among the elderly, these are not universal characteristics of the aging process.

On the other hand, three key aspects of the endocrine system are altered to varying degrees throughout the course of so-called normal aging. These include the changes in sex steroids that characterize menopause and/or andropause, changes in dehydroepiandrosterone (DHEA) known as adrenopause, and changes in the growth hormone/insulin-like growth factor I (GH/IGF-I) system, known as somatopause (10).

Menopause and Andropause

Probably the most familiar age-related change in endocrine function is female menopause. The progressive loss of ovarian follicles during aging leads to the absence of follicular function resulting in the cessation of menses (11). Three estrogens that occur endogenously are estradiol, estrone, and estriol; estradiol and estrone are interconvertible in the liver. The primary ovarian steroid hormone change that occurs as a result of menopause is a marked reduction of estradiol from the normal reproductive mean level of 257 pmol L⁻¹ to a level of 40 pmol L⁻¹. Similarly, the mean circulating estrone level decreases from 211 to 100 pmol L⁻¹ (12). Estrone, produced both in the ovary and

by peripheral conversion of androstenedione in adipose tissue, becomes the primary circulating estrogen after menopause (12). Following the menopause transition, there does not appear to be any effect of increasing age on estrogen levels in women (13). Circulating levels of progesterone, produced by the corpus luteum, do not appear to show any clear change with increasing age (11).

The loss of ovarian function at menopause also results in changes in hypothalamic and pituitary function. The pituitary gonadotropins (follicle-stimulating hormone (FSH) and luteinizing hormone (LH)) stimulate the ovarian secretion of estradiol and inhibin from follicular granulosa cells. As aging progresses, the secretion of inhibin falls, leading to a slow rise in FSH and LH (14). As the menopause approaches, the loss of ovarian feedback leads to a profound rise in the gonadotropins, reaching a peak 2–3 years after menopause, after which they progressively decline with age (11, 15).

Men experience a decline in testosterone with age that is sometimes referred to as “andropause”; however, decreases in circulating testosterone in males are more subtle and gradual than the decline in estrogen at menopause. The Massachusetts Male Aging Study reported a 1.6% decline in total testosterone per year in men between the ages of 40 and 70 (16), and Harman (5) reported that 28% of men in their 70s in the Baltimore Longitudinal Study of Aging met the criteria for hypogonadism. Testosterone secretion is higher in the morning in young men, and this diurnal pattern appears to be blunted in older men (17). The age-related decline in testosterone in men may result partly from changes in the hypothalamic–pituitary axis with decreased GnRH and subsequently reduced LH secretion (18). A decrease in Leydig cell mass and function has been observed in older men which likely also plays a role in diminished testosterone levels (18). It is important to note that unlike menopause in women, not all men experience “andropause” and there is some debate about whether the decline in testosterone observed in aging men is a true androgen deficiency. Several studies have shown an age-related decline in estradiol and bioavailable estradiol in men, which may be a result of the decrease in testosterone, as testosterone is the main precursor to estradiol in men (19, 20). However, this is not a consistent finding as Muller et al. (21) reported no age-related differences in estradiol among 400 men between the ages of 40 and 80.

In men, the testes are the primary source of testosterone while in women, testosterone is secreted by both the ovaries and adrenal glands. Women, also experience a decline in testosterone levels with age, independent of menopause (22, 23). The expected testosterone concentration of a 40-year-old woman would be 0.61 nmol L^{-1} , about half the level of a 21-year-old woman at 1.3 nmol L^{-1} . Menopause itself does not result in a dramatic change, and in postmenopausal women serum testosterone is only slightly lower than in premenopausal women (22, 24). There is evidence to suggest the ovary is still a source of testosterone after menopause (25), although this is a somewhat controversial finding (26).

Most testosterone is transported in blood by sex hormone-binding globulin (SHBG). While SHBG increases with age in men (27, 28), the change in serum SHBG levels in women is unclear. Free testosterone, bioavailable testosterone, and the free androgen index decline dramatically with age in men as a result of a decrease in total testosterone and an increase in SHBG (29, 30). In postmenopausal women, however, it is still unresolved whether there are systematic age-related changes in free testosterone or free androgen index, as SHBG levels in women have been reported to increase, decrease, or show no change after menopause (24, 31, 32).

Adrenopause

Dehydroepiandrosterone (DHEA) is secreted from the adrenal glands and is the most abundant steroid hormone in the body. It is a weak androgen that plays a role in many body tissues either by conversion to more potent sex steroids or by direct action on target tissues. The serum levels of DHEA and its sulfate conjugate, DHEAS, peak at 20–24 years in men and 15–19 years in women. The concentration of DHEAS in women is about 25–30% less than men, and the gender difference persists at all ages (33). There is a progressive decline in the circulation of DHEA(S) starting in the early twenties with a decrease of 1.5% per year until levels diminish to 10–20% of the peak by the age of 70 (33, 34). In women there is no relationship with menopausal status because DHEA and DHEAS are secreted exclusively by the adrenal glands in both premenopausal and postmenopausal women (22). The pronounced age-related decline in DHEA(S) appears to result from a decrease in the activity of specific steroidogenic enzymes as well as diminished size of the zona reticularis in the adrenal gland (35, 36). It is important to note that the adrenopause refers specifically to adrenal androgens as there is no consistent age-related change in cortisol concentrations (35).

Somatopause

The activity of the growth hormone/insulin-like growth factor I (GH/IGF-I) axis declines significantly with age. Iranmanesh et al. (37) demonstrated a 14% decline in growth hormone (GH) secretion per decade in men, and circulating GH over the age of 70 is approximately one-third of the level found in young adulthood (38). GH is released in a pulsatile manner and the older men show decreased frequency of secretory bursts and increased metabolic clearance (37). In women, there is a threefold higher mean serum GH concentration over 24 h when compared to men that appears to result from a greater GH secretory burst mass, as opposed to a gender difference in the frequency of GH bursts (39, 40). The age-related decline in daily GH secretion is less significant in premenopausal women compared to men (41), but menopause results in a significant decline in GH secretion in women, likely due to the fact that estrogen is a dominant regulator of GH secretion (42). However, Lieman et al. (43) compared age-appropriate menopausal women to women with premature ovarian failure and found that age and body composition had greater effects on GH than lack of estrogen.

The age-related decline in GH may result from numerous factors including decreased sex steroids, increased somatostatin, increased body fat, and a decrease in the production of ghrelin (40, 44). Ghrelin is a peptide secreted by the stomach that is an endogenous ligand of the GH secretagogue receptor found in the brain. Ghrelin stimulates GH secretion and also stimulates appetite (45). Circulating concentrations of ghrelin have been shown to be lower in older adults compared to younger adults, which could contribute to both the lowered GH secretion and diminished appetite that occurs with advancing age (46). However, several other studies have found no effect of age on ghrelin concentrations and suggest that age- and gender-related differences in ghrelin are explained by a negative correlation between ghrelin and total skeletal muscle mass (47).

Growth hormone has direct effects on metabolism and the growth of various body tissues although many of its anabolic and metabolic effects are mediated by IGF-I. The liver is the primary source of circulating IGF-I, and the actions of IGF-I are

mediated by a family of IGF-binding proteins that regulate the half-life and bioavailability of IGF-I in circulation. There are at least six binding proteins, with the majority (75%) of circulating IGF-I bound in a ternary complex with IGFBP-3 and an acid-labile subunit (48). IGFBPs mediate IGF-I actions by increasing the half-life of circulating IGF-I and by regulating IGF-I availability to bind with IGF receptors in the target tissues, in addition to exerting their own independent effects on tissues (49). There are also at least three variants of IGF-I produced locally in skeletal muscle that have autocrine and paracrine effects on muscle tissue (50). One of the locally produced variants of IGF-I, known as mechano growth factor (MGF), is upregulated in muscle in response to physical activity (50). Both GH and IGF-I are important for stimulating protein synthesis and increasing muscle mass, and IGF-I stimulates satellite cell proliferation and inhibits apoptosis (51).

Serum levels of IGF-I are generally higher in men than women (52), and are inversely related to age in both men and women, likely as a result of the age-related decline in GH (52–57). Ruiz-Torres and Kirzner (58) reported that the decline in IGF-I with age is exponential, with a much greater slope between the ages of 20 and 50 and a slower decline beyond age 55. Several studies have also reported a negative association between age and IGFBP-3, although the decline in IGFBP-3 is not as great as that of IGF-I, such that older adults typically have a lower ratio of IGF-I:IGFBP-3 (54, 55, 59) which may indicate lower IGF-I bioavailability. There is also evidence that aging results in loss of MGF expression in response to physical activity (50).

Hormones and Health in Older Adults

The potential clinical implications of diminished hormone levels among the elderly are varied. The side effects associated with the menopausal transition, such as vasomotor symptoms and the negative impact on quality of life are well documented, although these are generally transient effects that diminish over time. A more long-term effect from loss of estrogen is reduced bone mineral density and increased risk of osteoporosis after menopause (60). Studies have shown circulating estradiol is strongly associated with bone mineral density in men (61), thus declining estrogen levels could also have implications to the health of aging men. Sipila et al. (62) found that low serum estradiol was a significant predictor of fall-related fractures among 75-year-old women, independent of bone mineral density. They speculate this relationship could be explained by the effects of estrogen on the central nervous system, which may impact motor control and thus influence the risk of falls. Diminished testosterone levels may have negative effects on body composition, muscle mass and strength, bone mineral density, and sexual function in both men and women (17, 18, 25, 63). Baumgartner et al. (27) examined data from the New Mexico Aging Process Study and reported that free testosterone was significantly correlated with muscle mass and grip strength in elderly men, although the same associations were not found in elderly women.

DHEA(S) may have significant effects on many diseases and conditions of aging, including osteoporosis, atherosclerosis, diabetes, and dementia (35, 64, 65). DHEA inhibits interleukin-6 (IL-6) production from peripheral blood mononuclear cells; thus the decrease in DHEA with age may be a significant factor for the manifestation of inflammatory and age-related diseases (66). The apparent myriad effects of DHEA on human health and aging have led some to refer to it as the “fountain of youth” (67).

The age-related changes in the GH/IGF-I system and IGF-I bioavailability have also been associated with a plethora of health outcomes. Serum IGF-I and GH are related to strength, lean body mass, and bone mineralization in elderly men and women (27, 58, 68–70), and it has been suggested that declining levels of these hormones contribute to the musculoskeletal atrophy and pathogenesis of sarcopenia (51, 71). Two recent cross-sectional studies have found circulating IGF-I and IGF-I bioavailability to be inversely related to metabolic risk factors and the metabolic syndrome, including dyslipidemia, obesity, and hypertension (52, 54). Furthermore, circulating IGF-I has also been tentatively linked to cognitive decline and dementia (72). Low IGF-I bioactivity has been associated with increased mortality among older men (73), and this is supported by studies showing a positive association between circulating IGFBP-1, IGFBP-2, and mortality among elderly men and women (74).

In contrast to the apparently positive effects of sex steroids, GH, and IGF-I on musculoskeletal health and physical function among the elderly, these hormones have been associated with increased risk of various types of cancer. Among postmenopausal women there is a positive association between circulating estrogens and development of cancer of the breast and endometrium (75–77). Androgens, specifically circulating testosterone, have been associated with increased risk of prostate cancer (78) although other studies have found no association (79). DHEA(S) has also been implicated in the development of breast cancer (80) and endometrial cancer (76), possibly by activating estrogen receptors (80). IGF-I can alter cell behavior to promote unregulated cell growth and subsequently increase risk of cancer of the colon, breast, lung, and prostate (81). IGFBP-3 seems to be inversely related to cancer risk, possibly by decreasing the availability of IGF-I to bind to the IGF-I receptor (82).

Age-related disease and dysfunction are undoubtedly the result of multiple factors. Although there is no clear consensus about the clinical implications of circulating hormone concentrations late in life, the decline in hormones corresponds noticeably with increasing risk of disease and frailty which has led to the development of “antiaging” strategies targeted at the endocrine system (71). This is not a new concept, and in fact, interest in the relationship between hormones and the negative effects of aging dates back to the nineteenth century, when Brown-Séquard enthusiastically described the beneficial effects he experienced from self-administering canine testicular extracts, including improved physical function, stamina, and sexual performance (83). This led to an entire industry of hormone “rejuvenation” treatments that, in many ways, continues today (84).

While pharmacological intervention may be beneficial, that is not always the case. Supplementation with growth hormone, ghrelin mimetics, and steroids may have positive effects on body composition, muscle mass, bone mineral density, and caloric intake (85), all effects which should theoretically translate to improved functional outcomes. However, changes in strength and overall quality of life as a result of hormone interventions have been inconsistent at best (85). Furthermore, there may be risks associated with the use of exogenous hormone supplements, such as increased risk of cancer, that outweigh any potential benefits (85). This was underscored by the highly publicized results of the Women’s Health Initiative trial, which demonstrated an increased risk of breast cancer and coronary heart disease events for women using a combined estrogen/progestin therapy (86).

PHYSICAL ACTIVITY AND ENDOCRINE FUNCTION IN OLDER ADULTS

The difficulty distinguishing between physical changes that result from primary aging and those that are exacerbated by environmental and lifestyle factors presents a challenge to aging research. The influence of lifestyle may partly explain why some individuals age more successfully than others (2). For example, a significant decrease in physical activity occurs with aging and the decline in muscle function with aging has structural and functional similarities to the loss of function observed during disuse (87). Older adults are the least active of any group, and among Canadians, 62% of people 65 years or older are inactive (88) compared to 40% of individuals between 20 and 24 years (89). This pattern of lower activity among older adults holds in many other nations, including the USA and Sweden (90).

Physical activity is a potent stimulus of the endocrine system; thus it seems reasonable that many of the changes in hormones associated with age may be impacted by a decline in habitual activity. Figure 1 illustrates the many complex interrelationships among primary age-related changes in physical and endocrine function and lifestyle factors. Numerous researchers have attempted to identify potential determinants of hormone levels among older adults. Physical activity is a variable of interest in many observational studies, based on the theory that one of the mechanisms by which physical activity promotes successful aging and reduces the risk of cancer is by altering the hormonal milieu.

Physical Activity and Sex Steroids

It has been suggested that the age-related decline in levels of DHEA is attenuated in older men who engage in long-term endurance training (91). Ravaglia et al. (92) found that DHEAS was 80% higher among active compared to sedentary elderly men. Similarly, among elderly women, Bonnefoy et al. (93) reported that circulating levels of DHEAS were positively correlated with habitual physical activity, sport participation, and aerobic power, although they did not find a similar relationship among men. These studies all employed relatively small sample sizes between 17 and 96 subjects. Larger observational studies have found no relationship between DHEA(S) and physical activity among postmenopausal women (94) or older men (21, 95).

Cauley et al. (13) studied 176 postmenopausal women and found the most active women had the lowest estrogen levels, while testosterone was not associated with physical activity. More recently, and in a much larger study, Chan et al. (96) also found an inverse relationship between physical activity and estradiol among 2,082 postmenopausal women, with the most active quartile of subjects having 6% lower estradiol than the least active quartile. Unlike Cauley et al. (13), Chan et al. (96) did find an association between activity and testosterone, with the most active women having testosterone concentrations that were almost 20% lower compared to the least active women. SHBG was also significantly higher among active women (96). Results from the Penn Ovarian Aging Study (94) confirmed the inverse association between physical activity and both estradiol and testosterone in a longitudinal study of 391 women over 10 years. Testosterone and estrogen were 15 and 19% lower, respectively, among the most active postmenopausal women (94). They found a greater effect of physical activity on

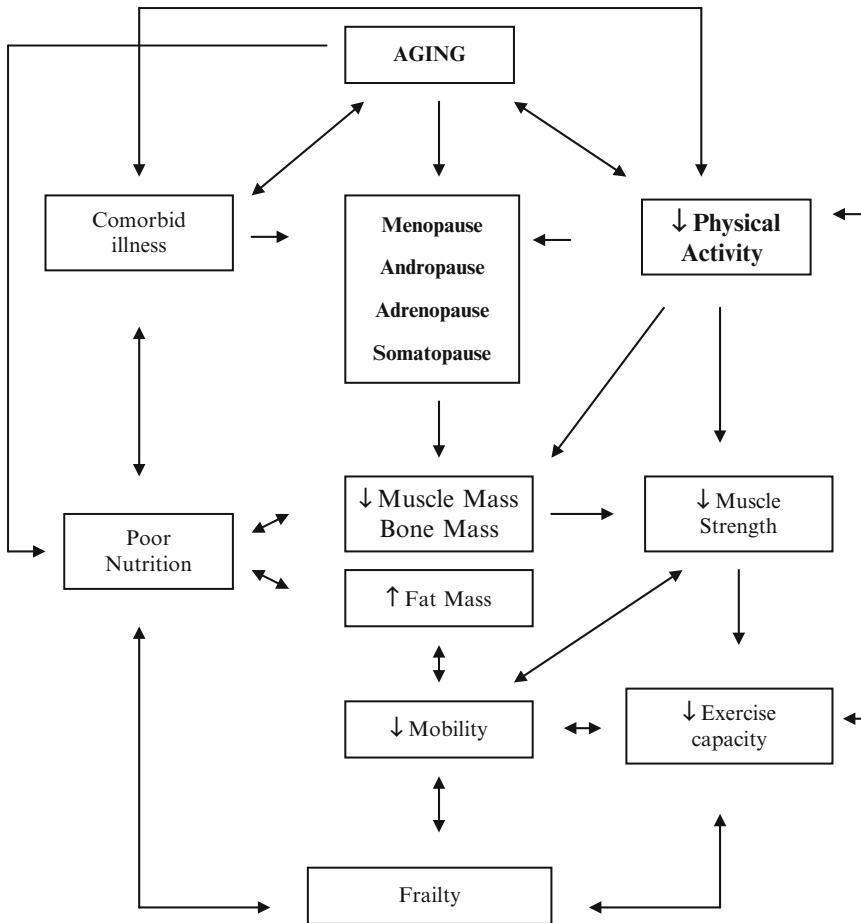


Fig. 1. A model of the relationships among aging, hormones, and physical function. Adapted from Copeland, J.L. (2004) Anabolic hormones in aging women: effects of supplementation vs. physical activity. *Canadian Journal of Applied Physiology*, 29(1): 76-89, NRC Research Press.

hormone levels among women in the late phase of the menopausal transition, with 54% lower estradiol and 47% lower testosterone among active women who had been amenorrheic for only 3–11 months.

Several studies have also found an association between physical activity and sex steroid hormone levels among older men. Muller et al. (21) reported a positive association between physical activity and total testosterone as well as SHBG among 40–80-year-old men. Other studies have reported a similar association among men across a broad age range (97, 98). Ari et al. (99) compared ten male master athletes with ten sedentary age-matched controls and found the athletes had significantly higher levels of testosterone. In contrast, Ravaglia et al. (92) examined 48 active men and 48 sedentary controls between the ages of 50 and 74 years and found no difference in testosterone levels. Slowinska-Lisowska et al. (20) also found testosterone was not associated with physical activity among older males, but observed that estrogen was

significantly lower among older men with the highest level of activity. In contrast, Shiels et al. (98) found men who engaged in vigorous activity four times per week actually had higher estradiol, although their analyses included younger men as well.

The observed effect of activity on endogenous sex steroids among older adults is partially mediated by changes in body fat. Postmenopausal estrogen is primarily formed by the aromatization of adrenal androgens such as DHEA and androstenedione (32), and Cauley et al. (13) found a strong relationship between estrogens and obesity among postmenopausal women. It is important to note that even after controlling for obesity, the inverse relationship between physical activity and estrogen persisted (13), suggesting body fat may be only partly mediating the effects of activity. Similar to women, Muller et al. (21) found that higher levels of body fat were associated with higher estradiol levels among older men. Furthermore, Schmitz et al. (94) observed that activity-related differences in testosterone were greatest among women in the highest BMI category.

The balance of the cross-sectional evidence suggests that physical activity is associated with lower testosterone and estradiol among older women, higher testosterone among older men, and higher DHEAS and SHBG in both sexes. These results have generally been confirmed by randomized controlled trials of aerobic exercise interventions. Hawkins et al. (100) monitored circulating sex hormones in 172 older men who were assigned either an aerobic exercise program or control group. After 3 months they found a 14% increase in SHBG in exercisers and a 14.5% increase in dihydrotestosterone (DHT), with no changes in testosterone, free testosterone, or estradiol. Hawkins et al. (100) also noted the greatest increases in DHT occurred among men (both exercisers and controls) who lost the greatest amount of body fat over the 12-month period and among exercisers who saw the greatest improvements in aerobic fitness. As insulin is known to inhibit SHBG production, it is assumed that the effect of exercise on SHBG is mediated by decreases in body fat and/or decreases in insulin. However, Hawkins et al. (100) found no effect of body fat on SHBG and saw the largest increases in SHBG among men who had the greatest improvements in fitness. Similar results in older women were reported by Friedenreich et al. (101), who found significant increases in SHBG after a year of aerobic exercise that were unrelated to insulin but appeared to be moderated by improvements in fitness. Friedenreich et al. (101) also found significant decreases in total and free estradiol in their training group compared to the control group although the differences were attenuated after adjusting for changes in body fat. Taken together, these data suggest that in older men and women, training-induced alterations in circulating estrogens are largely mediated by loss of body fat while the increases in androgens and SHBG appear to be more strongly related to changes in fitness. The mechanism by which increasing physical fitness impacts circulating androgens and SHBG among older adults remains unclear.

Physical Activity and the GH/IGF-I System

The GH/IGF-I axis in older adults is also impacted by lifestyle factors, including physical activity (102). Ari et al. (99) observed higher levels of GH among male masters athletes compared to sedentary controls. In contrast, Deuschle et al. (103) compared ten

older male marathoners (50–78 years) and ten age-matched sedentary men and found no difference in circulating GH between the two groups. Growth hormone is secreted in a pulsatile manner from the anterior pituitary and a single sample may be insufficient to assess the impact of physical activity on the pattern of GH release. Many more observational studies have focused on establishing the determinants of IGF-I among older adults, as it is the main downstream mediator of GH actions and is much less variable than GH with no significant diurnal variation in systemic concentrations (48).

Studies of the relationship between physical activity and IGF-I among the elderly have produced inconsistent results, with some reporting a positive relationship (91–93) and others finding no association (53, 55, 103, 104). Orenstein and Friedenreich (105) published a systematic review of all studies of physical activity and IGF-I and of the cross-sectional studies among older adults, there were approximately equal numbers showing a positive association between physical activity and IGF-I and those showing no association. The largest epidemiological study to date that examined lifestyle determinants of circulating IGF-I concentrations was recently reported by Parekh et al. (52). Using the NHANES III data, Parekh et al. (52) examined the impact of diet and physical activity on IGF-I among 6,058 men and women over the age of 20, and they found no relationship between IGF-I and physical activity. The apparent lack of association between habitual physical activity and circulating IGF-I is supported by the controlled trial reported by McTiernan et al. (106). They compared the effects of 12 months of moderate intensity aerobic exercise to a stretching program in 173 postmenopausal women, and found no change in IGF-I or IGFBP-3 with the aerobic exercise, despite reporting excellent adherence to the program. Their results were not influenced by age, body mass index, or changes in aerobic power.

It is interesting to note that some of the studies that found no relationship between physical activity and IGF-I did observe significantly higher levels of IGFBP-1 among active older adults (103, 104). This effect is possibly a result of lower insulin levels among more active older adults as insulin suppresses the production of IGFBP-1 (48). Higher IGFBP-1 among active older adults may decrease the activity of IGF-I by forming IGF-I/IGFBP-1 complexes that cannot bind to cell surface receptors (48).

Clearly, much of the evidence describing the relationship between physical activity and hormones among the elderly is contradictory. This is exemplified by the fact that some researchers have proposed physical activity as a way to *increase* circulating levels of steroids and IGF-I to promote musculoskeletal health and function in the elderly (107), while others suggest physical activity will *decrease* hormones and thus reduce the risk of cancer (108). This apparent paradox requires further research.

One of the main challenges facing observational studies is the accurate measurement of physical activity. The majority of the aforementioned studies relied on self-reported physical activity, and lack of standardization is a significant problem associated with using self-report to measure a complex exposure like physical activity (109). Furthermore, self-report questionnaires are extremely limited in their ability to objectively classify intensity and provide detailed information about the pattern of habitual physical activity. There may also be issues with the use of questionnaires specific to an older population, including vision and hearing impairments or disturbances to cognition and

short- or long-term memory (109). There can be problems with accurately reporting the intensity of exercise, as perceptions of what is “hard” activity or “light” activity depends on the tolerance and fitness level of the individual, both of which are affected by age (109). These issues pose a significant problem to the examination of physical activity and endocrine function as it will be difficult to detect differences if activity levels among subjects are homogeneous. This is illustrated by the results of Ravaglia et al. (92), who found no relationship between physical activity and testosterone in older men, but they also found no differences in body fat or lean body mass between their most active and least active subjects, which suggests they did not vary greatly in habitual activity.

It is interesting to note the few studies that employed an objective measure of physical activity or fitness, such as a motion sensor (13) or maximal aerobic power (31, 91), found significant relationships between physical activity and hormone levels. In fact, Cauley et al. (13), who used both self-report and objective measures of activity, reported that the inverse relationship they observed between physical activity and estrogen was stronger with the motion sensor data than it was with the self-report data. As technological advances now allow more robust and detailed assessments of physical activity (110, 111), future research may be able to determine if certain patterns or intensities of activity are associated with changes in endocrine function among the elderly.

Although the measurement of hormone concentrations is, in theory, simpler than measuring a complex behavior such as physical activity, there are still many challenges to be overcome. Most studies assess hormone levels with a single blood sample and many hormones have a circadian rhythm as well as seasonal variability that can significantly influence results (112). Furthermore, there is often no mention of the time span between the blood draw and the most recent exercise session, and acute exercise can have an impact on hormone levels that could persist for hours or even days, depending on the intensity and duration of the exercise session. Prior exercise could be a major confounding variable when comparing hormone levels between sedentary and active older adults.

Assessing age- and activity-related changes in hormone levels is further complicated by the interactions among different hormones and by changes in binding proteins that may impact free hormone levels with no discernible effect on total hormone levels. For example, SHBG has been shown to be positively associated with both age and physical activity in older men (21), which may result in decreased free or bioavailable testosterone. Age- and exercise-related increases in IGFBP-1, IGFBP-2, and IGFBP-3 may similarly impact levels of free IGF-I and IGF-I activity (55, 104). Furthermore, improvements in technology have allowed identification of multiple forms of protein hormones, both in circulation, such as the over 100 isoforms of GH that have been identified in serum (113), and in muscle, such as the locally produced splice variants of IGF-I (50). The development of more advanced analytical techniques will undoubtedly yield new insights into the effect of exercise on the endocrine system.

Of course, observational studies that show a relationship between circulating hormone levels and physical activity cannot infer causation. It is certainly plausible that the relationship between hormone levels and physical activity is mediated by health.

Healthy older adults with a lower “biological age” may have fewer age-related changes in endocrine function and may also be more likely engage in an active lifestyle due to higher functional capacity. One also has to consider the possibility that hormones—which have both physiological and behavioral effects—are influencing physical activity as opposed to the other way around.

Although there are no human data to support this idea, Bowen et al. (114) recently proposed the theory that gender differences in physical activity could be explained by sex hormones. Bowen and colleagues base the idea on extensive animal literature that shows a significant sex effect on patterns of physical activity. If we extend this to an aging population, perhaps individuals who maintain higher levels of anabolic hormones, due to either genetics or the impact of lifestyle factors, are more likely to engage in regular physical activity. If this were the case, then we would expect that hormone replacement among older adults might impact physical activity behavior. Although few studies have examined this question specifically, various studies of estrogen replacement therapy in women have also measured physical activity and physical function. Andersen et al. (115) examined the association between physical activity and HRT use in NHANES III data and found a higher prevalence of sedentary behavior among women who had never used HRT compared to those who had used HRT. Unfortunately this finding is limited by the cross-sectional design of the study and experimental studies have found no impact of HRT on physical activity among women (116, 117). Comparable studies of testosterone replacement and the subsequent effect on physical activity are scarce but perhaps warranted. Ibebunjo et al. (118) recently conducted an experiment in male mice to determine the effects of androgen depletion and androgen replacement on voluntary wheel running. They found testosterone had a significant impact on wheel running behavior as androgen-depleted mice ran more slowly and significantly less distance than mice that received testosterone replacement after orchiectomy. Ibebunjo et al. (118) therefore suggest that voluntary physical activity could be at least partially centrally mediated. If this were also true in humans, it may offer insight into the age-related decline in physical activity.

ACUTE EXERCISE-INDUCED HORMONE RESPONSES IN OLDER ADULTS

A significant number of intervention studies have been undertaken to examine the effect of a bout of exercise on hormone levels in older adults. Typically acute exercise will stimulate significant changes in serum concentrations of many hormones in young men and women, although the nature of the response will vary depending on the mode, duration, and intensity of the exercise, as well as the training status of the individual (119, 120). However, given the age-related changes in the endocrine system outlined at the beginning of this chapter, one might reasonably expect exercise-induced hormone responses to be different among elderly individuals.

Both acute endurance and resistance exercise have been shown to increase levels of testosterone and DHEA(S) in older men and women (121–127), although studies examining the steroid hormone response to resistance exercise are far more numerous than those examining endurance exercise. Few studies have examined the estrogen response to exercise among older adults. Kemmler et al. (124) reported a 20% increase

in estradiol after 60 min of combined endurance and resistance exercise in postmenopausal women. Copeland et al. (121) also reported an increase in serum estradiol after both endurance and resistance exercise in women across a wide age range, including older adults.

The exercise-induced increases in circulating steroids range between 10 and 40% and are highly transient, with concentrations typically returning to pre-exercise values within 30–120 min following exercise. In general, steroid hormone responses to exercise in elderly subjects are comparable to those of younger subjects, but this is not a universal finding. Aldred et al. (128) found a diminished DHEA(S) response to cycling exercise in older adults, and others have reported lower testosterone responses to resistance exercise in older men and women (125, 129). There is evidence that blunted steroid hormone responses to exercise may relate more to exercise intensity and/or training status than age. This is supported by studies that have shown exercise-induced increases in DHEA(S) or testosterone among older women only *after* a period of regular training (122, 130), when subjects had a higher exercise intensity, as evidenced by greater post-exercise lactate concentration (130). Craig et al. (129), however, did not measure lactate or heart rate, and Aldred et al. (128) used different durations and intensities of exercise for their young and older subjects, which limits interpretation of their findings.

Exercise of at least moderate intensity will stimulate secretion of growth hormone (GH) such that plasma GH concentrations increase within 10–15 min of the start of exercise (102, 131), and this response is greater in women than men (132). Although exercise will still induce an increase in GH among older adults (121, 125, 133, 134), the response is typically attenuated (102). Pyka et al. (135) measured GH concentrations during a circuit of 12 resistance exercises performed by a group of old (72 ± 0.8 years) and young (27 ± 1.6 years) men and women. The young subjects showed a significant rise in GH throughout the session while the older subjects demonstrated no significant increase. This blunted GH response to acute exercise among the elderly has been confirmed by others (122, 132, 136), and GH release during exercise is often between four and sevenfold lower among older adults (131). Aging also appears to eliminate the gender difference in exercise-induced GH responses with older men and women showing similar 4-h integrated GH concentrations after aerobic exercise of varying intensities (132).

It has been suggested that lower exercise intensity in the older subjects explains the blunted GH response to exercise. Indeed, many of the studies that found a lower GH response to exercise in older subjects also reported a significant age-related difference in exercise intensity (122, 135). Weltman et al. (132) found that only exercise intensities above the individual lactate threshold increased GH output in older subjects, and even at the highest exercise intensities, the integrated GH response was significantly diminished compared to the younger subjects. Gulka et al. (137) found that the GH response to treadmill exercise was significantly lower in older versus younger women but that age differences were minimized in fit older women. It appears that a greater relative exercise intensity may be required to stimulate GH in older adults.

In addition to exercise intensity there are other factors that may influence the GH secretion, including relative adiposity and fat distribution, sleep, and physical fitness—all factors that are influenced by increasing age (102, 138). Vahl et al. (139) reported

that abdominal fat and fitness were more important than age as determinants of GH responsiveness to multiple provocative stimuli, and Hartman et al. (102) concluded that abdominal visceral fat, fasting insulin, and IGF-I are the best predictors of GH secretion. Given this, losing weight and exercising may restore GH secretion to some degree, although there is likely a primary age effect on the hypothalamus that cannot be reversed (102).

IGF-I is the primary mediator of GH actions and can regulate GH secretion by negative feedback. While both GH and IGF-I decrease with age, peripheral responsiveness to GH remains the same (140), and thus it seems logical that if exercise impacts GH concentrations it would also impact IGF-I concentrations. However, the IGF-I response to exercise is highly inconsistent and appears to be independent of changes in growth hormone. Several studies have shown an increase in IGF-I in elderly subjects after resistance exercise (141–143) and after a 30-s Wingate test (144), while others have found no change in the IGF-I in response to endurance or resistance exercise (121, 133). Most of these studies measured total IGF-I, although Bermon et al. (141) also measured free IGF-I and found a 94% increase after resistance exercise, which is much greater than the ~10–15% increase that is typically observed for total IGF-I. Orenstein and Friedenreich (105) reviewed 115 studies of exercise and IGF-I, and of the 47 studies that examined the IGF-I response to acute exercise specifically among older adults, 18 reported an increase in IGF-I, 26 reported no change, and three reported a decrease. This ratio of positive and negative findings was not notably different in any other age group. Thus, age does not appear to independently impact IGF-I responsiveness to exercise, as it does for GH.

Exercise may indirectly influence IGF-I activity by altering the circulating concentrations of IGF-binding proteins, but in comparison to IGF-I, a fairly limited number of studies have examined the effect of acute exercise on IGF-binding proteins in older adults. In the review by Orenstein and Friedenreich (105), three studies reported increased IGFBP-3 with exercise while 13 found no change. Chadan et al. (133) found that moderate intensity activity in older women did not impact levels of IGF-I, but it did increase IGFBP-2 and IGFBP-3 and decreased levels of IGFBP-1. IGFBP-1 has been shown to inhibit IGF-I action, while IGFBP-2 and IGFBP-3 appear to potentiate the effects of IGF-I; thus Chadan et al. (133) concluded that exercise may enhance IGF-I action in elderly women. However, the decrease in IGFBP-1 is a somewhat surprising result as IGFBP-1 typically increases with exercise in a dose–response manner (145, 146), presumably as a result of decreased insulin since insulin directly inhibits hepatic IGFBP-1 synthesis (145).

Despite the large number of studies, it is difficult to draw firm conclusions about the effect of exercise on circulating hormone concentrations among the elderly. As with younger populations, the response appears dependent on the type, duration, and intensity of exercise; the age, gender, and fitness level of the subjects; the sampling interval used; whether free or total hormone concentrations are assessed; and which isoforms of various ligands are being measured. Overall, it appears that the endocrine system of older adults is still responsive to an exercise stimulus, although the response may be diminished to some degree.

The mechanism of exercise-induced changes in hormone concentrations is not clearly understood, but changes in circulating hormone levels likely result from some combination of altered secretion, decreased hepatic clearance, and hemoconcentration.

Regardless of the mechanism, changes in the concentration of a given hormone will increase or decrease the probability of ligand–receptor interactions, and the biological activity of hormones is ultimately dependent on the availability of receptors in the target tissue. Bamman et al. (147) reported a 100% increase in androgen receptor (AR) mRNA in exercised muscle following an acute bout of resistance exercise in young male subjects; however, this response has yet to be demonstrated in older subjects. Roberts et al. (148) found that older men had greater AR gene expression at rest than young men, but 24 h following an acute bout of resistance exercise, there was no change in AR expression in either age group (148). Ahtiainen et al. (149) also found no effect of either an acute heavy resistance exercise protocol or 21 weeks of resistance training on the AR protein concentration of young or old male subjects. Interestingly, they did note that individual changes in muscle AR protein concentration after exercise were predictive of increases in lean body mass and muscular strength after resistance training (149). Increased ARs in exercised muscle may be related to exercise-induced increases in serum testosterone, as androgens are known regulators of AR protein expression (148, 149). Thus, older adults experiencing “andropause” may subsequently have diminished responsiveness in downstream regulators of androgen activity.

SIGNIFICANCE OF EXERCISE-INDUCED HORMONE RESPONSES IN OLDER ADULTS

As studies of the endocrine response to various exercise protocols continue to accumulate, a fundamental question remains: what is the significance of these transient changes in hormone levels to the older adult? A majority of studies cite the potential beneficial effects of anabolic hormones on the elderly as the rationale for examining exercise-induced endocrine responses. This is based on the theory that increases in systemic anabolic hormones will facilitate training adaptations by increasing protein synthesis, lean body mass, and strength (150) and subsequently reduce the risk of sarcopenia, osteoporosis, and frailty. This theory is supported by several studies that have found a significant correlation between acute increases in GH and testosterone and increased muscle cross-sectional area with resistance training in men (151, 152). However, Wilkinson et al. (153) challenged the view that increases in anabolic hormones stimulate muscular hypertrophy following resistance training. Ten young male subjects completed a unilateral resistance training program that resulted in significantly increased thigh muscle cross-sectional area (CSA) despite having no exercise-induced increases in circulating GH, IGF-I, or testosterone (153). Furthermore, using an animal model, Matheny et al. (154) showed that mice with a deficiency in serum IGF-I still exhibit muscular hypertrophy following resistance training. It seems that changes in systemic hormones are not required for muscular adaptations to occur, but one could speculate that they may enhance or accelerate the adaptations. That possibility has been both refuted and supported in the literature. West et al. (155) found no difference in rates of protein synthesis after training that elicited large increases in circulating GH and testosterone compared to training with no increases in circulating endogenous hormones. However, Ronnestad et al. (156) recently demonstrated that training of the elbow flexors with elevated endogenous GH and testosterone induced superior adaptations in muscle strength and CSA when compared to similar training with no increases

in these hormones. Clearly further research is needed to clarify the importance of acute increases in anabolic hormones on training adaptations. Furthermore, all of these studies used young male subjects and it is not known if exercise-induced hormone responses might be more relevant in an elderly population with lower basal hormone levels.

West and Phillips (157) conclude that exercise-induced increases in systemic testosterone or GH are of minimal importance to the muscular hypertrophic response and suggest that locally produced androgens are more relevant to muscle anabolism. Although activation of local androgen metabolism has been shown in response to exercise in rodents (158, 159), this has yet to be confirmed in human studies. Pollanen et al. (160) compared circulating and muscle concentrations of steroids in pre- and postmenopausal women. They found the expected differences in circulating hormones, with postmenopausal women having significantly lower concentrations of estrogens and androgens, but noted that *muscle* concentrations of estradiol and testosterone were actually higher in the postmenopausal women, with no difference in muscle DHEA between the two age groups. Despite having higher muscle hormone concentrations, the postmenopausal women had lower muscle size and strength compared to the premenopausal women, and systemic hormone concentrations were positively related to muscle quality (160). It seems that peripheral and systemic hormones may have differing roles in the regulation of skeletal muscle and further research is needed to elucidate the effects of aging and exercise on intracrine versus endocrine steroid hormone activity.

Although research on the effect of exercise on local steroid synthesis is limited, there is substantial evidence to suggest that locally produced IGF-I is upregulated in response to exercise (161). In addition to the hepatic synthesis of systemic IGF-I, three splice variants of locally produced IGF-I have been identified in skeletal muscle, the so-called IGF-I E peptides: IGF-IEa, IGF-IEb, and IGF-IEc (also known as mechano growth factor (MGF)) (161). The IGF-I E peptides appear to have different roles, with IGF-IEa increasing muscle protein synthesis while MGF initiates muscle repair processes after damage (161, 162). Animal studies have shown that muscle IGF-I expression increases in response to loading and contributes to muscle hypertrophy, even in animals with low systemic IGF-I (162). In humans, muscle IGF-I has been shown to increase significantly after a single bout of resistance exercise in young subjects (147). In elderly subjects, Singh et al. (163) found that muscle IGF-I increased by almost 500% following 10 weeks of resistance training. The increases in muscle IGF-I paralleled training-induced increases in muscle damage and in developmental myosin (163). Those results were supported by Hameed et al. (164) who found increases in MGF in both young and elderly men in response to damaging eccentric exercise. These studies suggest that age does not impair the local growth factor response to exercise, which appears to be critical for mediating training adaptations. This local response may also explain the training-induced increase in muscle size without a change in systemic hormone levels, as seen in several studies described above (153–155). More research is needed to understand the physiological effects of exercise- or training-induced changes in systemic and peripheral hormones in order to understand the conflicting evidence regarding the health benefits and risks of increasing concentrations of hormones and growth factors. It is possible that circulating hormones and locally produced hormones have differing roles in the health and function of elderly individuals.

In conclusion, it is indisputable that exercise is beneficial to the health and quality of life of older adults (165). Regular physical activity can minimize the age-related decline in functional capacity and associated disability, and reduce the risk of many chronic diseases. It has also been clearly demonstrated that exercise has potent effects on the endocrine system and can influence circulating hormone concentrations, locally produced hormone expression, and hormone receptor expression. The balance of evidence seems to suggest that these endocrine system responses to exercise are still possible in elderly individuals if a sufficient dose of activity is achieved. Yet the link between the beneficial effects of exercise and changes in endocrine function is much more tenuous. Given the demographic shift mentioned at the beginning of this chapter, there is considerable incentive to clarify the relationships between exercise, endocrine function, and successful aging.

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Oncology Patients and Aerobic Exercise: Immune System, Endocrine System, and Soluble Factor Responses

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CONTENTS

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INTRODUCTION

The American Cancer Society (ACS) projects that in 2012, over 1.6 million Americans will be diagnosed with some form of cancer and that nearly 580,000 will die from their cancer (1). Earlier detection and advances in newer treatments have improved disease prognosis, although cancer survivors are still faced with challenges and sequelae associated with the disease itself, the cancer treatments, or other comorbid conditions that may predate the cancer diagnosis (2, 3). Additionally, many cancer survivors are at risk for developing a recurrence of their disease or a second malignancy (2, 3). Therefore, interventions that may decrease the risk for developing a cancer recurrence or new malignancy while also improving survival are warranted.

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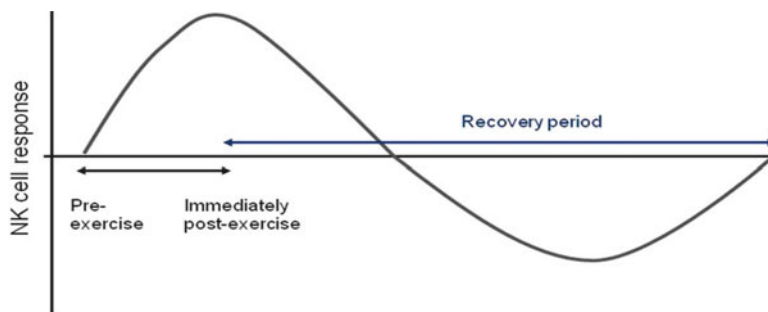


Fig. 1. Biphasic shift in immune cell counts which may be seen in NK cells, helper T lymphocytes, cytotoxic T lymphocytes, and B lymphocytes.

Aerobic exercise may be an attractive adjunct therapy for cancer patients and survivors because of its potentially positive influence on biologic systems involved in disease protection and anticancer defense (4). Numerous reviews have comprehensively described the effect of acute and chronic aerobic exercise on the cellular components of innate and adaptive immunity in healthy individuals (5–13). In response to acute aerobic exercise, many leukocyte subpopulations including natural killer (NK) cells, helper T lymphocytes (CD4⁺), cytotoxic T lymphocytes (CD8⁺), and B lymphocytes (CD19⁺) can experience a biphasic shift in circulating cell counts, where cell counts may increase during exercise and may decrease below pre-exercise values during recovery (see Fig. 1) (6–8). In contrast, cell counts for neutrophils and monocytes may increase during exercise as well as during recovery (6, 8, 9). When examining the impact of acute aerobic exercise on leukocyte subpopulation function, results are varied, with some immune cells showing increased function during exercise and decreased function during recovery (NK cells), increased function during exercise and recovery (neutrophils, lymphocyte apoptosis), or decreased function during exercise and recovery (lymphocyte proliferation response to mitogens) (6). The magnitude of the immune response is largely affected by exercise intensity and duration, such that moderate intensity and shorter duration exercise generally leads to smaller changes in cell counts and function, and higher intensity and longer duration exercise generally leads to greater alterations in cell counts and function, relative to pre-exercise levels (6, 7, 9). When considering the effect of aerobic exercise training on immune function, results are variable. Some parameters such as NK cell activity may be increased in physically active individuals compared to sedentary individuals (6). Other parameters such as lymphocyte proliferation and neutrophil function may be decreased, increased, or not significantly different in physically active individuals compared with sedentary individuals (6). Exercise training intensity and volume may also influence cellular immune function, where periods of moderate intensity/volume exercise may lead to enhanced immune cell numbers and function, and periods of high intensity/volume exercise may lead to suppressed cell numbers and function (6, 8, 12).

Several investigations regarding the relationship between exercise intensity/volume and disease risk have led exercise immunologists to develop a “J-shaped” hypothesis, which postulates an inverse relationship between disease risk/cancer susceptibility

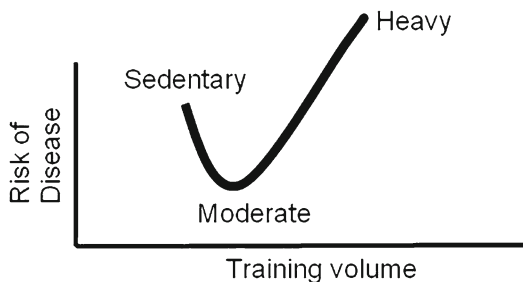


Fig. 2. The J-shaped curve, illustrating the hypothesis that exercise training volume and immune function are inversely related (4, 6, 7, 9).

(see Fig. 2) (4, 6, 7, 9). In general, the hypothesis states that regular, moderate exercise may lead to enhanced immune function and therefore a decreased risk of disease and cancer, whereas repeated bouts of exhaustive exercise and overtraining could lead to immunosuppression and therefore an elevated risk of disease and cancer (4, 6, 7, 9). This hypothesis is largely based on epidemiological investigations of exercise intensity and development of upper respiratory tract infections (URTI), where athletic individuals have reported higher incidences of URTI during periods of strenuous training or in the days following a major event such as a marathon (6, 8, 13), and individuals participating in moderate exercise have reported fewer URTI symptoms (6, 13). However, it should be noted that decreases in immune function related to strenuous exercise do not necessarily confer clinical immunodeficiency, and no conclusive evidence currently exists linking exercise intensity/training volume with immunological mechanisms and cancer risk in humans (6, 12, 13).

Since aerobic exercise has been shown to influence cellular immune system responses in healthy individuals, studies regarding the effect of aerobic exercise on the immune system in cancer patients and survivors have begun to appear in the literature. A review by Fairey et al. (4) was the first to systematically examine the current literature; at which time there were six published studies. The purpose of this current review is to (1) briefly describe the role of aerobic exercise as an intervention for improving functioning in cancer patients and survivors, (2) provide a comprehensive review of the impact of acute aerobic exercise and aerobic exercise training on cellular immune system function in cancer patients and survivors, (3) discuss the impact of aerobic exercise on endocrine and soluble factor responses in cancer patients and survivors, and (4) outline several areas on which future research should focus.

AEROBIC EXERCISE AS AN INTERVENTION FOR ONCOLOGY PATIENTS

Through epidemiological studies, physical activity has shown to be strongly associated with prevention for colon and breast cancer, and is recognized as a probable or possible means of prevention for prostate, endometrium, and lung cancer (14). Biological mechanisms governing the relationship between increased physical activity and decreased cancer risk are varied, and include factors such as decreased colorectal

transit time, decreased ratio of prostaglandins, decreased bile acid secretion and enhanced acid metabolism (colon cancer), decreased exposure to estrogen and testosterone (breast cancer and prostate cancer respectively), decreased percent body fat, increases in antitumor defenses, improved antioxidant defense systems, decreased insulin and glucose levels, and decreased levels of insulin-like growth factors (all cancers) (14). As a result, organizations including the ACS and the American Institute for Cancer Research have recommended physical activity guidelines with the goal of reducing cancer rates in the general population (15, 16). In general, individuals are encouraged to be physically active as part of everyday life, such as engaging in at least 150 min of moderate intensity activity per week, 75 min of vigorous physical activity per week, or an equivalent combination, and the chosen activities should be spread throughout the week (15, 16).

Over the past 20 years, much research has emerged regarding the effect of exercise on physiological and psychological functioning in cancer patients and survivors (17–25). This body of “exercise oncology” literature has shown that exercise interventions can improve quality of life, cardiorespiratory fitness, muscular strength and endurance, body composition, and self-esteem, and can also decrease fatigue, anxiety, and depression (17, 19, 23). Many of these “first-generation” studies have shown promising results: that exercise training appears to mitigate many treatment-related side effects, patients are able to make significant improvements in cardiorespiratory fitness, and that regular exercise seems to be safe, well tolerated, and associated with few adverse events. To this end, the ACS and the American College of Sports Medicine (ACSM) have both issued guidelines regarding appropriate physical activity levels for cancer patients and survivors (2, 26, 27). In the most recent set of ACSM guidelines published in 2010, Schmitz et al. (2) outline that the goals of an exercise prescription should include regaining and improving physiological parameters such as aerobic capacity, strength flexibility, body composition, and neurological, endocrine, muscular, and cardiorespiratory outcomes. Additionally, exercise prescriptions should also allow cancer patients and survivors to regain and improve psychosocial parameters such as quality of life, self-esteem, and body image (2). Taken together, these improved outcomes would hopefully give cancer patients and survivors tools and knowledge to help reduce the risk of recurrence, second malignancy, or late effects of cancer treatment, as well as the ability to withstand any current or future cancer treatments if necessary. Regarding aerobic exercise, the ACSM recommends that cancer patients and survivors follow guidelines that are very similar to those already in place for the general population, where individuals are encouraged to exercise for 150 min per week at a moderate intensity, or 75 min per week at a vigorous intensity, or for an equivalent combination (2, 27–29). Since some cancer treatments may pose challenges for physical activity, the ACSM also gives further guidance for specific cancer sites such as the breast, prostate, and colon. For example, patients with breast cancer or prostate cancer who are receiving endocrine therapy and may be at risk for decreased bone density may be advised to alter their activities to decrease the risk of fracture. Patients with colon cancer who may have an ostomy may be advised to obtain physician consent before participating in contact sports. Additionally, patients with peripheral neuropathy may be advised to use exercise modes such as stationary cycling that do not rely on balance. Patients with cardiac

complications may require close supervision during exercise, and may need some modifications to their exercise prescriptions. Lastly, patients who have received treatments that may compromise the immune system may be advised to avoid activities and facilities that could pose a risk of infection, such as very vigorous exercise or using public gym equipment (2). Considering all of these guidelines and recommendations, the overall statement by the ACSM is that cancer patients and survivors should be as physically active as their conditions allow and avoid inactivity, and that exercise seems to be safe for most in-treatment or off-treatment patients cleared by their physicians to participate in regular exercise (2). Just as importantly, exercise prescriptions should be individualized, taking into account a cancer patient/survivor's pretreatment aerobic fitness, the presence of other medical conditions, the response to treatment, and any negative treatment side effects that could be experienced acutely or chronically (2). Even though it appears that the benefits of exercise outweigh the risks of an adverse event, little data exists to confirm this possibility.

As mentioned previously, physical exercise, particularly aerobic exercise, may be an attractive adjunct therapy for cancer patients and survivors because of the potential for positive influence over biologic factors involved in disease protection and anticancer defense (4). Several cellular immune system components are associated with anticancer defense, including NK cells, neutrophils, macrophages, and T lymphocytes (4). NK cells, neutrophils, and macrophages are all part of the innate immune system, which is the body's first line of defense against pathogens. These immune cells are able to destroy tumor cells by various mechanisms including apoptosis, production of peroxides and free radicals, and production of cytokines which can act on immune cells to enhance their cytotoxic capabilities (4). T lymphocytes are part of the adaptive immune system, which follows the innate immune response and is able to target specific pathogens. In particular, cytotoxic T lymphocytes are able to destroy tumor cells, also by apoptosis (4). Cancer treatments may often have considerable effects on the immune system of cancer patients and survivors, particularly by decreasing cell number and function of total lymphocytes, T lymphocytes, B lymphocytes, T lymphocyte ratio (CD4⁺/CD8⁺), and NK cells (4). Evidence suggests that decreases in immune function in cancer patients and survivors after treatment may be associated with disease relapse, poorer prognosis, and decreased survival rates (30–35). Additionally, increased levels of proinflammatory and decreased levels of anti-inflammatory cytokines could be associated with treatment-related side effects such as muscle wasting, which can be a significant concern in patients undergoing treatment for acute leukemia (23). Other cytokines including cutaneous T cell-attracting chemokine (CTACK) and interleukin (IL-15) may affect immune responses at either peripheral or mucosal sites or could be associated with increased activity of cytotoxic T lymphocyte and lymphocyte-activated killer cells (36). Regarding the endocrine system, cancer patients and survivors experiencing fatigue may also experience dysregulation in cortisol levels, which may affect various components of health-related quality of life (HRQOL) (37). Therefore, research aiming to understand how aerobic exercise may modify cellular immune system, endocrine, and soluble factor responses in cancer patients and survivors is clinically relevant.

AEROBIC EXERCISE AND ITS INFLUENCE ON THE IMMUNE SYSTEM OF ONCOLOGY PATIENTS

A comprehensive literature search up to early (March) 2012 identified original research articles that examined the effects of aerobic exercise on cellular immune system responses in cancer patients and survivors. These articles were determined from online searches through PubMed. Key terms that were combined during the searches included aerobic exercise, acute aerobic exercise, aerobic exercise training, low intensity, moderate intensity, high intensity, cancer, leukocyte, lymphocyte, neutrophil, natural killer cell, monocyte, T cell, and B cell. Studies were included in the review if they met the following criteria. First, studies had to be conducted using human subjects who were performing aerobic exercise during or after cancer treatment. Second, studies had to identify a cellular immune system component as an outcome variable in the data analysis and had to provide findings (i.e., cell counts and/or functional activity) in text, table, or graphical form. Third, studies had to identify the methods used to measure and quantify immune responses.

Overall, 13 studies published between 1994 and 2010 met these inclusion criteria. These studies will be discussed in two major groups: the first comprising investigations using acute aerobic exercise and the second comprising studies using aerobic exercise training interventions. Where appropriate, the discussion will be further subdivided so that findings among studies may be more easily compared.

Immune Responses to Acute Aerobic Exercise

To date, only two studies have examined the influence of acute aerobic exercise on cellular immune responses, both using pediatric cancer patients (38, 39). For the purpose of this discussion, acute aerobic exercise is defined as a single bout of aerobic exercise. Shore and Shepard (38) examined the immune response to exercise at anaerobic threshold in 6 children with a history of cancer (predominantly acute lymphoblastic leukemia (ALL)) and compared the findings to those of 11 healthy children. The six patients had been diagnosed within 5 years of study enrollment, and were either receiving chemotherapy or had completed chemotherapy. Similarly, Ladha et al. (39) studied the effect of acute moderate-to-vigorous aerobic exercise on immune function in four pediatric patients with ALL who were receiving maintenance therapy and six matched healthy controls. In both studies, the subjects performed a progressive exercise test to exhaustion to determine peak aerobic capacity ($VO_{2\text{ peak}}$), and both tested the immune response to an acute aerobic exercise session lasting 30 min in duration. In the study by Shore and Shepard (38), the subjects performed the acute exercise bout at an intensity corresponding to anaerobic threshold (exercise mode is not specified). In the study by Ladha et al. (39), subjects alternated 10 min of running at 85% of $VO_{2\text{ peak}}$, 10 min of walking at 70% of $VO_{2\text{ peak}}$, and 10 min of running, again at 85% of $VO_{2\text{ peak}}$. Immune parameters were measured from blood samples drawn pre-exercise, immediately post-exercise, and during recovery at 30 min postexercise (38), 1 h postexercise (39), and 2 h postexercise (39). Shore and Shepard (38) examined leukocyte and lymphocyte cell counts (total leukocytes, total lymphocytes, monocytes, granulocytes, T cells (CD3⁺,

CD4⁺, CD8⁺, CD4⁺/CD8⁺ ratio), B cells (CD19⁺), and NK cells (CD56⁺) as well as cytolytic activity and lymphocyte proliferation. In the Ladha et al. (39) study, neutrophil count and neutrophil function were the primary outcome variables, although total leukocyte, total lymphocyte, monocyte, eosinophil, and basophil cell counts were also measured.

The main immune changes reported by Shore and Shepard (38) that occurred in response to the acute exercise were an overall leukocytosis and lymphocytosis that followed profiles similar to those seen in healthy children. The authors also reported that cell counts were lower in the subjects who were currently receiving chemotherapy, and that exercise did not seem to cause changes in T cell counts (CD3⁺, CD4⁺, or CD8⁺). The pre-exercise CD4⁺/CD8⁺ ratio was lower compared to that seen in healthy children, dropping to below 1.0 at 30 min postexercise. Similarly, the NK cell count (CD56⁺), cytolytic activity, and lymphocyte proliferation activity in the subjects currently receiving chemotherapy were lower compared to healthy children but not for the subjects who had completed chemotherapy. However, the authors do not describe detailed immune responses to the aerobic exercise bouts, such as the magnitude of change in cell counts and activity postexercise relative to pre-exercise levels.

In the Ladha et al. (39) study, the authors observed a significant main effect for time for neutrophil count, in that it increased significantly from pre-exercise to immediately postexercise ($p=0.011$). At 1 h postexercise, neutrophil count decreased significantly compared to neutrophil count immediately postexercise ($p=0.045$). At 2 h postexercise, neutrophil count increased significantly compared to neutrophil count at 1 h postexercise ($p=0.052$). Similar changes in other immune components were observed. There were no significant differences in neutrophil counts between the two study groups at any time point, although significant main effects for group were observed for total lymphocyte and eosinophil cell counts ($p<0.0005$ and $p=0.006$, respectively). Unstimulated neutrophil oxidative burst (i.e., oxidative burst measured before stimulation with phytohaemagglutinin (PMA)) was significantly higher in the control group across all time points compared to the patient group ($p=0.029$), although no significant main effect for time was observed. When examining the ratios of PMA-stimulated neutrophils at 5, 10, and 15 min to unstimulated neutrophils across time (pre-exercise to 2 h postexercise), the patient group displayed a considerably greater increase in neutrophil oxidative burst compared to the control group ($p=0.048$ – 0.074).

The results of these two studies suggest that acute aerobic exercise seems to produce generally similar immune responses in pediatric cancer patients compared to healthy children. Ladha et al. (39) suggest that the decreased unstimulated neutrophil function observed in the cancer patients across time compared to the healthy controls may be related to immunosuppressive effects of chemotherapy. When examining the ratio of stimulated neutrophils to unstimulated neutrophils across time, the patients with ALL experienced a greater per-cell activity compared to the control group, which could be in part due to the low neutrophil oxidative burst activity observed in the unstimulated state. The authors of both studies caution that further research is needed to understand the effects of acute exercise on immune function in cancer patients in order to determine optimal prescriptions of aerobic exercise, particularly for individuals whose immune systems may already be weakened by chemotherapy.

Immune Responses to Aerobic Exercise Training

Twelve studies have examined the effect of aerobic exercise training on cellular immune responses (38, 40–50). The discussion in this section will be divided into two groups: cellular immune responses in oncology patients who are undergoing some form of major cancer treatment such as chemotherapy, bone marrow transplant (BMT), or blood stem cell transplant (during treatment) and cellular immune responses in oncology patients who have completed their major cancer treatments (after treatment). In this fashion, studies with similar patient characteristics will be discussed together in order to more easily compare results and conclusions.

DURING CANCER TREATMENT

Five studies have examined the effect of aerobic exercise training on cellular immune system responses during treatment (38, 43, 45, 48, 49). Table 1 summarizes the information from these five studies, including characteristics of the study sample, study design, baseline exercise testing method used to quantify aerobic fitness, mode, intensity, and duration of the acute exercise sessions that made up the exercise training interventions, duration of the interventions, immune component(s) and their assessment method(s), time points of immune assessment, and study findings. Two studies used only adult cancer patients (43, 48). Two studies used only pediatric cancer patients (38, 49). One study used both adolescent and adult cancer patients (45). Types of cancers included both hematological and solid tumors, and the exercise interventions were performed when patients were receiving chemotherapy, autologous peripheral blood stem cell transplant (PBSCT), allogenic BMT, allogenic hematopoietic stem cell transplant (HSCT), or a combination of chemotherapy and PBSCT. The study designs fell into four categories: a pretest-posttest design with patients in an exercise training group and matched healthy controls also in an exercise training group (38), randomized controlled trial with patients divided into an exercise training group and a non-exercising control group (43, 48), a non-randomized trial with patients divided into either an exercise training group or a non-exercising control group (45), and a non-randomized trial with patients specifically recruited into an exercise training group and a group of matched “historical” controls (49). Exercise intervention modes varied and included cycle ergometry, relaxation and stretching exercises, walking on a treadmill, soccer, skating, cross-country skiing, and swimming. Exercise intensities were light to vigorous, ranging from 50% of cardiac reserve (i.e., 220-age-resting heart rate) (43) or 50–90% of maximum heart rate (38, 45, 49). One study did not specify the exercise intensity, but the activities performed by the patients during their sessions (relaxation, stretching, and bending exercises in the supine position) were most likely of light intensity (48). Exercise sessions were performed for durations ranging from 10 to 40 min and frequencies of 3 to 7 days/week. Total intervention duration ranged from 2 weeks to 3 months. Immune parameters were measured at rest from blood samples taken at the beginning and end of the exercise interventions, and occasionally at time points during the interventions.

Dimeo et al. (43), Kim and Kim (48) examined the effect of aerobic exercise training in adult cancer patients undergoing treatment. Changes in immune parameters were examined over the intervention period, and results were compared within and between the study groups. The immunological outcome variable in the Dimeo et al. (43) study

Table 1

Summary of studies examining cellular immune system responses to aerobic exercise training in cancer patients and survivors during cancer treatment

Author	Sample	Design	Baseline exercise testing method	Exercise intervention	Immune component(s)	Method(s) of immune assessment	Time points of immune assessment	Results
Dimeo et al. (43)	Adult cancer patients (various solid tumor types) receiving HDC and autologous PBSCT (n=70)	RCT, with 33 patients in the training group and 37 patients in the control group	Treadmill; incremental stress test to exhaustion to determine peak aerobic performance (defined as maximal speed reached during test)	Mode: bed ergometer to simulate biking Intensity: at least 50% of cardiac reserve (220-age-resting heart rate) Session duration: 30 min (alternate 1 min of exercise with 1 min of rest) Session frequency: 7 days/week Duration of intervention: ~2 weeks	Neutrophils	Complete blood counts	Daily, at least 12 h post-exercise	Significantly shorter duration of neutropenia in the training group compared to the control group
Shore and Shepard (38)	Pediatric cancer patients mostly treated for ALL receiving chemotherapy (n=3) Healthy controls (n=11)	Pretest-posttest with matched controls	Progressive cycle ergometer test to exhaustion to determine VO _{2 peak}	Mode: cycling, soccer, skating, cross-country skiing, swimming Intensity: 70–85% of heart rate maximum Session duration: 30 min Session frequency: 3 days/week Duration of intervention: 12 weeks	Cell counts: total leukocytes, total lymphocytes, monocytes, granulocytes, CD3 ⁺ , CD4 ⁺ , CD8 ⁺ , CD4 ⁺ /CD8 ⁺ ratio, CD19 ⁺ , CD56 ⁺ Cytolytic activity Lymphocyte proliferation	Cell counts: complete blood count, flow cytometry Cytolytic activity: determined against K562 human myeloid tumor cells before and after addition of IL-2 Lymphocyte proliferation: stimulation with PHA and PWM	Before and after intervention	Baseline cell counts lower in cancer patients compared to the healthy controls; significantly lower for total leukocytes, total lymphocytes, CD3 ⁺ , CD4 ⁺ , CD8 ⁺ , and CD19 ⁺ Baseline cytolytic activity and lymphocyte proliferation lower in cancer patients compared to healthy controls; significantly lower for PHA-induced lymphocyte proliferation Cell counts decreased from baseline to post-intervention in cancer patients Cytolytic activity increased from baseline to post-intervention in cancer patients Lymphocyte proliferation decreased from baseline to post-intervention in cancer patients

(continued)

Table 1
(continued)

<i>Author</i>	<i>Sample</i>	<i>Design</i>	<i>Baseline exercise testing method</i>	<i>Exercise intervention</i>	<i>Immune component(s)</i>	<i>Method(s) of immune assessment</i>	<i>Time points of immune assessment</i>	<i>Results</i>
Hayes et al. (45)	Adolescent and adult cancer patients (breast cancer and various hematological cancers) receiving HDC and autologous PBSCT (<i>n</i> = 12)	Initially a RCT, with six patients in the training group and six patients in the control group	Maximal graded treadmill test to determine maximum heart rate and training heart rate range	Mode: treadmill walking and stationary cycling Intensity: 70–90% maximum heart rate Session duration: 20–40 min Session frequency: 3 days/week Duration of intervention: 3 months	Cell counts: total leukocytes, total lymphocytes, CD3+, CD4+, CD8+, CD4+/CD8+ ratio T cell proliferation with PHA	Cell counts: complete blood count and flow cytometry T cell proliferation: stimulation with PHA	Pre-PBSCT (PI), 17–21 days post-PBSCT, 1 month post-PI (I2), 2 months post-PII (I2), 3 months post-PIII (PIII)	No significant group × time interaction for any immune component No significant changes in total leukocyte counts across time Significant decrease in total lymphocyte counts from PI to PII; counts return to PI levels by I1 CD3+, CD4+, and CD8+ counts were significantly lower compared to normative data at PI; counts decreased further at PII. CD3+ counts returned to PI levels by I1, although counts at PIII were significantly lower compared to normative data. CD4+ counts remained below PI levels at I1, I2, and PIII, and counts continued to be significantly lower compared to normative data. CD8+ counts at PII were significantly lower compared to normative data, but returned to PI levels by I1. CD4+/CD8+ ratio was lower at PII, I1, I2, and PIII compared to PI, and counts at I1, I2, and PIII were significantly lower compared to normative data No significant change in total T-cell function across time, but was significantly lower compared to normative data at all time points. T cell function per CD3+ cell significantly increased from PII to PIII. T cell function per CD3+ cell was significantly lower compared to normative data at PII, I1, and I2

Kim and Chamorro-Viña et al. (48) (49)	Adult cancer patients with leukemia or severe aplastic anemia receiving allogeneic BMT ($n=35$) Pediatric cancer patients (rhabdomyosarcoma, neuroblastoma, and various types of leukemia) receiving allogeneic HSCT ($n=20$)	RCT, with 18 patients in the exercise group and 17 patients in the control group Non-randomized controlled trial with 7 patients in the exercise group and 13 patients in a matched "historical" control group	Does not specify Does not specify	Mode: relaxing, stretching, and bending exercises performed in the supine position Intensity: does not specify Session duration: 30 min Session frequency: 7 days/week Duration of intervention: 6 weeks Mode: cycle ergometer Intensity: 50–70% of age-predicted maximum heart rate Session duration: mean of 25–30 min (range of 10–40 min) Session frequency: 5 days/week Duration of intervention: 3 weeks	Cell counts: total lymphocytes, CD3+, CD4+, CD8+, CD4+/CD8+ ratio Cell counts: total leukocytes, total lymphocytes, T monocytes, T cells, NK cells, NK-T cells, CD4+, CD8+, dendritic cells	Complete blood count and flow cytometry Complete blood count and flow cytometry	Before and after the intervention Before the intervention, 15 days post-HSCT, 30 days post-HSCT	Significant group×time interaction effect for total lymphocyte counts, where cell counts slightly increased in the exercise group and significantly decreased in the control group No significant group×time interaction effects or group and time main effects for CD3+, CD4+, CD8+, or CD4+/CD8+ ratio No significant group×time interaction effects or group and time main effects for any variable after adjustment for multiple comparisons
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HDC high dose chemotherapy; *PBSCT* peripheral blood stem cell transplant; *ALL* acute lymphoblastic leukemia; *RCT* randomized controlled trial; *IL* interleukin; *PHA* phytohemagglutinin; *PWM* poke-week mitogen; *PMA* phorbol myristate acetate; *BMT* bone marrow transplant; *HSCT* hematopoietic stem cell transplant; *NK* natural killer; *MKCA* natural killer cell activity; *ConA* concanavalin A

was duration of neutropenia during the intervention period, defined as an absolute neutrophil count of $<5 \times 10^9$ cells/L. The authors found that the patients in the exercise training group experienced significantly days of neutropenia compared to patients in the control group ($p=0.01$). In the Kim and Kim (48), the immunological outcome variables were changes in total lymphocyte and T cell subsets before and after the intervention period. The authors found that total lymphocyte counts did not significantly differ between the two study groups or from pre-intervention to post-intervention. However, the authors did find a significant group-by-time interaction effect where the total lymphocyte counts for the control group decreased and the total lymphocyte cell counts for the exercise training group increased ($p=0.031$). When examining the T cell responses to the intervention (i.e., CD3⁺, CD4⁺, CD8⁺, and CD4⁺/CD8⁺ ratio), the authors do not report significant differences in cell counts between the study groups or across time. Similarly, there were no significant group-by-time interaction effects for percentages of CD3⁺ and CD4⁺ cells or the CD4⁺/CD8⁺ ratio.

Shore and Shepard (38) and Chamorro-Viña et al. (49) examined the effect of aerobic exercise training in pediatric cancer patients undergoing treatment. The immunological outcome variables in the Shore and Shepard (38) study were changes in cell counts (total leukocytes, total lymphocytes, monocytes, granulocytes, T cells, B cells, NK cells), cytolytic activity, and lymphocytes proliferation before and after the intervention. The authors found that pre-intervention cell counts for total leukocytes, total lymphocytes, and T cells (CD3⁺, CD4⁺, and CD8⁺), as well as phytohaemagglutinin (PHA)-induced lymphocyte proliferation, were significantly lower in the cancer patients compared to the healthy controls ($p<0.05$). In the control group, total leukocyte cell count and CD3⁺ cell count significantly decreased pre-intervention to post-intervention ($p<0.05$). Changes in other cell counts, cytolytic activity, and lymphocyte proliferation did not change significantly in the control group over the course of the intervention. In the patient group, there were no significant changes in any immune parameter across time, although lymphocyte proliferation and cell counts except B cell counts did decrease from pre-intervention to post-intervention. Cytolytic activity did increase over the course of the intervention in the patient group, but the change was not significant. The immunological outcome variables in the Chamorro-Viña study (49) were changes in cell counts (total leukocytes, total lymphocytes, monocytes, T cells, NK cells, NK-T cells, dendritic cells) measured pre-intervention, 15 days post-HSCT, and 30 days post-HSCT. No significant changes were observed for any immune parameter across time or between study groups. Cell counts were generally decreased at 15 days post-HSCT compared to pre-intervention levels, and increased at 30 days post-HSCT compared to 15 days post-HSCT in both study groups. A time effect was found for total T cells (CD3⁺), CD4⁺, and dendritic cells ($p=0.04$, 0.032 and 0.001, respectively). Additionally, a group-by-time interaction effect was found for dendritic cells ($p=0.045$). However, the low p -values reported for these changes were not reported as significant because of adjustment for multiple comparisons.

Lastly, Hayes et al. (45) examined the effects of an aerobic exercise intervention in a sample of adolescent and adult cancer patients undergoing chemotherapy and autologous PBSCT. Type of cancer was varied, and included several types of hematological cancers,

breast cancer, and rhabdomyosarcoma. The immunological outcome variables in this study were cell counts for total leukocytes, total lymphocytes, and T cell subsets (CD3⁺, CD4⁺, CD8⁺, CD4⁺/CD8⁺ ratio), as well as T cell function. Results were compared across the study time points, between the two study groups, and to previous normative age- and sex-matched data. Cell counts were measured at five time points: before receiving PBSCT (PI), 17–21 days after receiving PBSCT (PII), 1 month post-PI (I1), 2 months post-PII (I2), and 3 months post-PII (PIII). From PI to PII, the effect of the PBSCT was being assessed, and all patients were considered to be in the same group for those two time points. The exercise training intervention took place between PII and PIII, and therefore immunological differences between study groups were assessed during this time. The authors found that there was no significant group-by-time interaction effect for any immunological parameter. When the results for the two study groups were pooled, significant changes across time were observed for total lymphocyte, CD3⁺, CD4⁺, and CD8⁺ cell counts ($p < 0.05$); the CD4⁺/CD8⁺ ratio; and T cell proliferation index per CD3⁺ cell ($p < 0.01$). Cell counts decreased from PI to PII (the period of time pre-PBSCT to post-PBSCT), but increased again to near- or above-PI levels at I1, I2, and PIII. Only the CD4⁺ cell count remained significantly below PI levels at I1 and PIII ($p < 0.05$). Likewise, the CD4⁺/CD8⁺ ratio decreased over time, and remained significantly below PI levels at I1, I2, and PIII ($p < 0.05$). Total T cell proliferation did not change significantly across time, although T cell proliferation per CD3⁺ cell was significantly higher at PIII compared to PII ($p < 0.01$). Significant differences also existed for cell counts and T cell proliferation between the patients in this study and normative data. T cell counts (CD3⁺, CD4⁺, and CD8⁺) were significantly lower compared to normative data at PI and PII ($p < 0.01$). CD4⁺ cell counts remained significantly lower compared to normative data throughout I1, I2, and PIII ($p < 0.01$). Total lymphocyte and CD3⁺ cell counts returned to normal levels at I1, but became significantly decreased again at PIII ($p < 0.01$). The CD4⁺/CD8⁺ ratio remained significantly below normative values throughout I1, I2, and PIII ($p < 0.01$). Total T cell proliferation remained significantly below normative values across all study time points, whereas T cell proliferation per CD3⁺ cell was significantly depressed only at PII, I1, and I2 ($p < 0.01$).

The general conclusions reached by the authors of these five studies were that aerobic exercise training during cancer treatment is likely safe, does not seem to significantly hinder immune function compared to non-exercising cancer patients or healthy controls, and may even help to increase immune cell counts or reduce the number of days of immunosuppression during hospitalization. In the two studies that compared their findings to normative data or healthy controls, the authors observed that pre-intervention values for many immune parameters in the cancer patients were significantly depressed (38, 45). Furthermore, immune cell counts and function may decrease further over the course of the exercise intervention, although differences between pre-intervention and post-intervention values were largely nonsignificant (38, 45). Shore and Shepard (38) advises that exercise interventions should be prescribed cautiously, on an individual basis, and with careful monitoring of immune function, especially when considering a cancer patient whose immune system has already been weakened by cancer treatments.

AFTER CANCER TREATMENT

Seven studies have examined the effect of aerobic exercise training on cellular immune system responses after treatment, all in the adult cancer survivor population (40–42, 44, 46, 47, 50). Table 2 summarizes the information from these seven studies as previously described. Five studies used breast cancer survivors (40–42, 46, 47). Two studies used stomach cancer survivors (44, 50). The amount of time that had elapsed from completion of cancer treatment to the beginning of the exercise intervention varied among the studies, from 2 days to several years.

Peters et al. (40, 41), Nieman et al. (42), Fairey et al. (46), and Hutnick et al. (47) examined the effect of aerobic exercise training in post-treated breast cancer patients and survivors. The amount of time that had elapsed between the completion of cancer treatment to the beginning of the exercise intervention was clearly stated in four studies as 2 weeks–2 months (47), at least 6 months (40, 41), and 3.0 ± 1.2 years (42). One study stated that patients had completed cancer treatment between January 1999 and June 2000, but did not clearly define when the exercise intervention began (46). Study designs fell into three categories: a one-group pretest-posttest design (40, 41), a randomized-controlled trial design with subjects divided into an exercise training group and a non-exercising control group (42, 46), and a non-randomized trial where subjects were specifically recruited into either an exercise training group or a non-exercising control group (47). Exercise intervention modes included cycle ergometry, and running or walking on a treadmill or track. Exercise intensities were moderate to vigorous, ranging from 60 to 86% of heart rate maximum (40–42), 70 to 75% of $VO_{2\text{ peak}}$ (46), or 60 to 75% of functional capacity (47). Exercise sessions were performed for durations ranging from 10 to 40 min and frequencies of 2 to 5 days/week. Total duration of the interventions ranged from 8 to 29 weeks. Immune parameters were measured at rest from blood samples taken at the beginning and end of the exercise interventions, and occasionally at time points during the intervention.

The immunological outcome variables for these five studies include cell counts for total leukocytes, total lymphocytes, granulocytes, monocytes, NK cells, neutrophils, T cells, and B cells. Additionally, NK cell activity (NKCA), phagocytic activity of monocytes, lymphocyte proliferation, and neutrophil function were also assessed. The effects of aerobic exercise training on immune parameters seen in these studies are similar to those previously described for cancer patients during treatment. Peters et al. (40), Nieman et al. (42), Fairey et al. (46), and Hutnick et al. (47) did not observe significant changes in immune cell counts across time in the exercise training group compared to the control group. When looking at percentages of immune cell type rather than absolute cell counts, Peters et al. (41) did observe a significant increase in percentage of granulocytes and a significant decrease in the percentage of monocytes post-intervention compared to values at pre-intervention and at 5 weeks into the intervention ($p < 0.05$). The authors also observed that the percentage of total lymphocytes was significantly decreased post-intervention compared to the value at 5 weeks into the intervention ($p < 0.05$). Hutnick et al. (47) found that the percentage of $CD4^+ CD69^+$ cells (activated T helper cells) was significantly higher in the exercise training group compared to the control group at the end of the intervention ($p < 0.05$). When looking at the functional capacity of the immune parameters, more differences were

Table 2
Summary of studies examining cellular immune system responses to aerobic exercise training in cancer patients and survivors after cancer treatment

<i>Author</i>	<i>Sample</i>	<i>Design</i>	<i>Baseline exercise testing method</i>	<i>Exercise intervention</i>	<i>Immune component(s)</i>	<i>Method(s) of immune assessment</i>	<i>Time points of immune assessment</i>	<i>Results</i>
Peters et al. (40)	Adult breast cancer survivors (n=24)	One group, pre-test-posttest	Progressive cycle ergometer test to exhaustion	Mode: cycle ergometer Intensity: 60–86% of heart rate maximum Session duration: 30–40 min Session frequency: 5 days/week for first 5 weeks, then 2–3 days/week for 6 months Duration of intervention: 29 weeks	NK cell count (CD56 ⁺) NKCA	NK cell count: flow cytometry NKCA: ⁵¹ Cr release assay	Before the intervention, at the end of 5 weeks, at the end of the intervention	NK cell counts did not significantly change across time Post-intervention NKCA was significantly increased compared to NKCA measured pre-intervention and at 5 weeks

(continued)

Table 2
(continued)

<i>Author</i>	<i>Sample</i>	<i>Design</i>	<i>Baseline exercise testing method</i>	<i>Exercise intervention</i>	<i>Immune component(s)</i>	<i>Method(s) of immune assessment</i>	<i>Time points of immune assessment</i>	<i>Results</i>
Peters et al. (41)	Adult breast cancer survivors (n = 24)	One group, pre-test-posttest	Progressive cycle ergometer test to exhaustion	Mode: cycle ergometer Intensity: 60–86% of heart rate maximum Session duration: 30–40 min Session frequency: 5 days/week for first 5 weeks, then 2–3 days/week for 6 months Duration of intervention: 29 weeks	Cell counts: total leukocytes, total lymphocytes, granulocytes, monocytes Phagocytic activity of monocytes	Cell counts: complete blood count Phagocytic activity of monocytes: treatment with receptor destroying enzyme-treated sheep erythrocytes and Anti-D-loaded human erythrocytes	Before the intervention, at the end of 5 weeks, at the end of the intervention	Total lymphocyte cell counts significantly decreased post-intervention compared to cell counts at 5 weeks Percentage of granulocytes significantly increased post-intervention compared to percentages at pre-intervention and 5 weeks Percentage of lymphocytes significantly decreased post-intervention compared to percentage at 5 weeks Percentage of monocytes significantly decreased post-intervention compared to percentages at pre-intervention and 5 weeks Phagocytic activity of monocytes to receptor destroying enzyme treated sheep erythrocytes significantly increased at 5 weeks and post-intervention compared to pre-intervention level

Nieman et al. (42)	Adult breast cancer survivors (<i>n</i> = 12)	RCT, with six survivors in the exercise training group and six survivors in the control group	Symptom-limited treadmill test and a 6 min-walk test	Mode: walking on an indoor track Intensity: ~75% of heart rate maximum Session duration: 30 min Session frequency: 3 days/week Duration of intervention: 8 weeks	Cell counts: total leukocytes, neutrophils, total lymphocytes, CD3 ⁺ , NK cells (CD16 ⁺ CD56 ⁺) NKCA	Cell counts: complete blood counts and flow cytometry NKCA: ⁵¹ Cr release assay	Before and after the intervention	No significant group × time interaction effects for any immune component
Na et al. (44)	Post-treated adult stomach cancer patients (<i>n</i> = 35)	RCT, with 17 survivors in the exercise training group and 18 survivors in the control group	Does not specify	Mode: arm and cycle ergometers Intensity: 60% of maximum heart rate Session duration: 30 min Session frequency: twice per day for 5 days/week Duration of intervention: 2 weeks	NKCA	⁵¹ Cr release assay	Days 1, 7, and 14 of the intervention	NKCA decreased from Day 1 to Day 14 in the control group and increased in the exercise training group. NKCA at Day 14 was significantly higher in the exercise training group compared to the control group

(continued)

Table 2
(continued)

<i>Author</i>	<i>Sample</i>	<i>Design</i>	<i>Baseline exercise testing method</i>	<i>Exercise intervention</i>	<i>Immune component(s)</i>	<i>Method(s) of immune assessment</i>	<i>Time points of immune assessment</i>	<i>Results</i>
Fairey et al. (46)	Adult postmenopausal breast cancer survivors (n=52)	RCT, with 24 survivors in the exercise training group and 23 survivors in the control group	Incremental cycle ergometer test to determine $VO_{2\text{ peak}}$	Mode: recumbent or upright cycle ergometers Intensity: ~70–75% of $VO_{2\text{ peak}}$ Session duration: 15–35 min Session frequency: 3 days/week Duration of intervention: 15 weeks	Cell counts: T cells, B cells, granulocytes, monocytes, NK cells Lymphocyte proliferation Neutrophil function NKCA	Cell counts: immunofluorescence assays Lymphocyte proliferation: stimulation with PHA and PWM Neutrophil function: flow cytometry to examine oxidative burst post-stimulation with PMA NKCA: ^{51}Cr release assay	Before and after the intervention	No significant difference between study groups for changes in cell counts or neutrophil function across time Significant increase in spontaneous lymphocyte proliferation across time in the exercise training group compared to the control group Significant increase in NKCA across time in the exercise training group compared to the control group

Humnick et al. (47)	Post-treated adult breast cancer patients (n=36)	Non-randomized controlled trial; patients specifically recruited into either the exercise training group (n=21) or the control group (n=15)	Does not specify	Mode: treadmill running and walking Intensity: 60–75% of functional capacity Session duration: 10–20 min Session frequency: 3 days/week Duration of intervention: 6 months	Cell counts: CD3 ⁺ , CD4 ⁺ , CD8 ⁺ , B cells, NK cells, activated CD4 ⁺ cells, CD4 ⁺ also expressing CD69 ⁺ Lymphocyte proliferation	Cell counts: immunohistochemistry and flow cytometry Lymphocyte stimulation with PHA, ConA, and PWM	Post-treatment but before beginning the exercise intervention (T2); 3 months into the exercise intervention (T3); post-intervention (T4)	No significant difference in CD3 ⁺ , CD4 ⁺ , CD8 ⁺ , B cell, or NK cell counts between study groups at any time point Mean CD3 ⁺ , CD8 ⁺ , B cell, and NK cell counts were below the interquartile range for healthy individuals at all time points Percentage of CD4 ⁺ CD69 ⁺ cells significantly increased in the exercise training group compared to the control group post-intervention Significantly higher lymphocyte proliferation in the exercise training group compared to the control group post-intervention
Lee et al. (50)	Adult stomach cancer survivors (n=21)	One group, pre-test-posttest	Does not specify	Mode: Tai Chi Intensity: light to moderate Session duration: 30–40 min Session frequency: 1 day/week Duration of intervention: 24 weeks	Cell counts: total leukocytes, total lymphocytes, monocytes, CD4 ⁺ , CD8 ⁺ , CD4 ⁺ /CD8 ⁺ ratio, NK cells	Flow cytometry	Before and after the intervention	Significant increases in percentages of total leukocytes and monocytes at the end of the intervention

HDC high dose chemotherapy; *PBSCT* peripheral blood stem cell transplant; *ALL* acute lymphoblastic leukemia; *RCT* randomized controlled trial; *IL* interleukin; *PHA* phytohemagglutinin; *PWM* pokeweed mitogen; *PMA* phorbol myristate acetate; *BMT* bone marrow transplant; *HSCt* hematopoietic stem cell transplant; *NK* natural killer; *NKCA* natural killer cell activity; *ConA* concanavalin A

observed between study groups. Peters et al. (40, 41) observed that NKCA was significantly increased post-intervention compared to values measured pre-intervention and at 5 weeks into the intervention ($p < 0.05$), and that monocyte phagocyte activity was significantly increased at 5 weeks into the intervention and post-intervention compared to pre-intervention levels ($p < 0.05$). Nieman et al. (42) did not observe significant differences in NKCA across time between exercising and non-exercising participants, but Fairey et al. (46) observed that NKCA did increase across time in exercising participants compared to non-exercising controls ($p < 0.001$ – 0.39). Fairey et al. (46) did not observe significant differences in neutrophil function across time between study groups, but did observe significant increases in spontaneous lymphocyte proliferation across time in the exercise intervention group compared to the control group ($p = 0.007$). Likewise, Hutnick et al. (47) also observed significantly higher lymphocyte proliferation in the exercise training group post-intervention compared to the control group ($p < 0.05$).

Lastly, Na et al. (44) and Lee et al. (50) examined the effect of aerobic exercise training in post-treated stomach cancer patients and survivors. The amount of time that had elapsed between the completion of cancer treatment to the beginning of the exercise intervention was 2 days (44) and at least 2 years (50). Na et al. (44) used a randomized-controlled trial design with subjects divided into an exercise training group and a non-exercising control group while Lee et al. (50) used a one-group pretest-posttest design. Exercise intervention modes included arm and cycle ergometry and Tai Chi. Exercise intensities were light to moderate, and Na et al. (44) explicitly states that the exercise intensity used in their intervention was 60% of maximum heart rate. Exercise sessions were performed for 30–40 min and frequencies of 1 day/week (50) and twice daily for 5 days/week (44). Total duration of the interventions were 2 weeks (44) and 24 weeks (50). Immune parameters were measured at rest from blood samples taken at the beginning and end of the exercise interventions (50) and on days 1, 7, and 14 of the intervention (44).

Na et al. (44) examined changes in NKCA across the intervention period and found that NKCA measured in the control group decreased from day 1 to day 7 to day 14, while NKCA increased over the three time points in the exercise training group. Additionally, NKCA measured at day 14 was significantly higher in the exercise training group compared to the control group ($p < 0.05$). Lee et al. (50) examined changes in cell counts (total leukocytes, total lymphocytes, monocytes, NK cells, CD4⁺, CD8⁺, CD4⁺/CD8⁺ ratio) before and after the intervention. The authors found that the percentages of total leukocytes and monocytes were significantly increased post-intervention compared to pre-intervention ($p = 0.011$ and 0.02 , respectively), but no other immune changes were observed across time.

The general conclusions reached by the authors of these seven studies were that aerobic exercise training either does not significantly affect immune function in cancer survivors or that it could lead to improvements in resting immune function. The specific immune function parameters that seemed to be affected by aerobic exercise training were NKCA (40, 44, 46), monocyte phagocytic activity (41), and lymphocyte proliferation (46, 47). In the two studies that did not report changes in immune function, Nieman et al. (42) suggested that 8 weeks may not be enough time to elicit marked changes in

resting NKCA. Lee et al. (50) noted that immune markers were within the normal ranges at the beginning of the intervention, and therefore there may not have been much room for improvement even after exercise training. Even so, improvements in resting immune function after exercise training may be beneficial to the cancer survivor population, in that it may lead to an increased capacity for clearance infectious microorganisms and neoplastic cells, as well as improvements in immune function beyond what may be expected with normal recovery after cancer therapy (41, 46, 47).

AEROBIC EXERCISE AND ITS INFLUENCE ON THE ENDOCRINE SYSTEM AND SOLUBLE FACTOR RESPONSES IN ONCOLOGY PATIENTS

A recent literature was used to identify original research articles that examined the effects of aerobic exercise on endocrine and soluble factor (e.g., cytokines and C-reactive protein (CRP)) responses in cancer patients and survivors. Again, these articles were determined from online searches through PubMed. Key terms that were combined during the searches included acute aerobic exercise, aerobic exercise training, cancer, stress hormones, cortisol, insulin, estrogen, testosterone, cytokine, interleukin, and CRP. Overall, 18 studies published between 2003 and 2011 were considered, three of which have been discussed in the above sections (46, 47, 50). One study examined the influence of acute aerobic exercise and 17 studies examined the effect of aerobic exercise training on endocrine and soluble factors in oncology patients. As was done previously, these studies will be discussed in two major groups: the first comprising investigations using acute aerobic exercise and the second comprising studies using aerobic exercise training interventions. Where appropriate, the discussion will be further subdivided so that findings among studies may be more easily compared.

Endocrine and Soluble Factor Responses to Acute Aerobic Exercise

Jones et al. (51) compared levels of fasting insulin and CRP in a group of 47 breast cancer survivors who had been receiving hormonal therapy (aromatase inhibitor or tamoxifen) for at least 6 months with a group of 11 postmenopausal, age-matched healthy women. All subjects in the breast cancer survivor group had received anthracycline-based chemotherapy. Subjects also completed a cardiopulmonary exercise test to assess $VO_{2\text{ peak}}$ and were also assessed for a number of other cardiovascular disease risk factors (body composition, heart rate, blood pressure, stroke volume, cardiac output, a- VO_2 difference, systemic vascular resistance, cardiac power output, cardiac reserve, fasting cholesterol, and fasting glucose). There was no significant difference between CRP levels or insulin levels in the breast cancer survivor group compared to the control group ($p=0.268$ and 0.438 , respectively), although $VO_{2\text{ peak}}$ was significantly lower in the breast cancer survivor group ($p=0.004$). Breast cancer survivors also showed decreased cardiovascular function on a number of other factors including exercise stroke volume, cardiac output, cardiac power output, and cardiac power output reserve ($p<0.05$). In the breast cancer survivor group alone, $VO_{2\text{ peak}}$ was negatively associated with CRP levels ($r=-0.33$, $p=0.023$), insulin levels ($r=-0.31$, $p=0.036$), as well as

glucose levels ($r=-0.37$, $p=0.011$). Additionally, breast cancer survivors who were receiving aromatase inhibitors displayed significantly greater CRP and insulin levels compared to breast cancer survivors receiving tamoxifen ($p<0.05$). These findings may indicate that breast cancer survivors who exhibit greater impairments in cardiorespiratory fitness ($VO_{2\text{ peak}}$) may also have greater impairments in cardiovascular disease risk profile as measured by both traditional and novel (i.e., insulin, glucose, and CRP) biochemical markers. Further, the higher glucose, insulin, and CRP levels displayed by the breast cancer survivors who were receiving aromatase inhibitors compared with tamoxifen could indicate that aromatase inhibitors might be associated with greater cardiovascular impairments compared with tamoxifen.

Endocrine and Soluble Factor Responses to Aerobic Exercise Training

DURING TREATMENT

Eight studies have examined the effect of aerobic exercise training programs on endocrine and/or soluble factor responses in oncology patients currently undergoing treatment (23, 37, 51–57). Table 3 summarizes the information from these eight studies as previously described. All studies involved adult cancer patients with various types of cancers including breast, lung, acute myelogenous leukemia (AML), and prostate cancer who were currently undergoing chemotherapy, hormonal therapy, or radiation therapy. In one study, patients were studied prior to undergoing surgical resection (53). The study designs utilized were similar to those previously described, with five studies using a randomized controlled trial design with patients allocated to either a usual-care control group or an exercise group (37, 52, 54–56), two studies using a one-group pretest-posttest design (23, 53), and one study using a non-randomized design where patients were stratified by the type of therapy they were receiving (57). Exercise interventions varied and included both home-based as well as supervised exercise sessions. Exercise modes included walking (at home or on a treadmill), jogging, cycle ergometry, and the elliptical trainer. Exercise intensities varied from moderate to vigorous, ranging from 50 to 100% of $VO_{2\text{ peak}}$, 40 to 50% of HRR, 60 to 85% of maximum heart rate, and/or 11 to 13 on the Borg 6–20 point scale. Three studies did not specify exact exercise intensities (37, 52, 54). Exercise session durations varied from 15 to 45 min of continuous exercise per day. In one study, patients exercised twice per day for 5–10 min per session (23), and in another study, patients alternated 30 s of high-intensity intervals with 60 s of active recovery for 10–15 sets of intervals (53). Exercise session frequencies ranged from 2 to 7 days per week, and the total duration of the interventions ranged from 4 to 24 weeks. One study did not specify the exact intervention duration but stated that patients completed an average of 30 ± 25 sessions (53). Endocrine variables measured included the hormones insulin, cortisol, and testosterone, while the soluble factor variables measured included the cytokines interleukin (IL)-1 β , IL-6, IL-8, IL-10, tumor necrosis factor (TNF)- α , and interferon (IFN)- γ . Other soluble factors measured included CRP, intracellular adhesion molecule (ICAM)-1, macrophage inflammatory protein (MIP)-1 α , and monocyte chemoattractant protein (MCP)-1. All endocrine and soluble factor variables were measured from plasma or serum from blood samples taken at

Table 3

Summary of studies examining endocrine and soluble factor responses to aerobic exercise training in cancer patients and survivors during cancer treatment

Author	Sample	Design	Baseline exercise testing method	Exercise intervention	Endocrine/soluble factor component(s)	Method(s) of endocrine/soluble factor assessment	Time points of endocrine/soluble factor assessment	Results
Demark-Wahnefried et al. (52)	Adult premenopausal breast cancer patients receiving adjuvant chemotherapy (n=82)	RCT, with 27 patients in the control group (calcium-rich diet), 26 patients in the calcium rich diet plus exercise group, and 29 patients in the calcium rich diet plus exercise plus high fruit/vegetable low fat diet	Does not specify	Mode: home-based intervention; does not specify Intensity: does not specify Session duration: ≥ 30 min per day Session frequency: ≥ 3 days per week Duration of intervention: 6 months	Insulin, IL-1 β , CRP	ELISA	Before and after the intervention.	No significant differences observed for blood biomarkers between study time points
Jones et al. (53)	Adult lung cancer patients prior to surgical resection (n=12)	One group, pretest-posttest	Incremental cycle ergometer test to determine $VO_{2\text{peak}}$ and a 6-min walk test	Mode: cycle ergometer Intensity: 60–100% of $VO_{2\text{peak}}$ Session duration: 20–30 min at 60–65% of $VO_{2\text{peak}}$, 20–30 min at VT, intervals of 30 s at $VO_{2\text{peak}}$ and 60 s of active recovery for 10–15 intervals Session frequency: 5 days per week Duration of intervention: patients completed 30 ± 25 exercise sessions (mean \pm SD)	ICAM-1, MIP-1 α , IL-6, IL-8, MCP-1, TNF- α , CRP	ICAM-1, MIP-1 α , IL-6, IL-8, MCP-1, TNF- α , ELISA CRP: radioimmunoassay	Before and after the intervention	Significant reduction in ICAM-1 level across time

(continued)

Table 3
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<i>Author</i>	<i>Sample</i>	<i>Design</i>	<i>Baseline exercise testing method</i>	<i>Exercise intervention</i>	<i>Endocrine/soluble factor component(s)</i>	<i>Method(s) of endocrine/soluble factor assessment</i>	<i>Time points of endocrine/soluble factor assessment</i>	<i>Results</i>
Battaglini et al. (23)	Adult patients with AML receiving induction chemotherapy (n=8)	One group, pretest-posttest	Total time spent cycling on a recumbent cycle ergometer at 60% of HRR until an RPE of seven on the CR10 modified Borg Scale was reached, or the patient requested to terminate the test	Mode: recumbent cycle ergometer or treadmill walking Intensity: 40–50% of HRR Session duration: 5–10 min, two times per day in-hospital, 10–30 min per day at home during the last 2 weeks of the intervention Session frequency: 3–4 days per week Duration of intervention: approximately 8 weeks	IL-6, IL-10, IFN- γ	Luminex assay	Before the intervention, midway through the intervention, after the intervention	Marginally significant decrease in IL-6 levels across time
Payne et al. (54)	Adult breast cancer patients ages ≥ 55 years receiving hormonal therapy (n=20)	RCT, with ten patients in the usual care control group and ten patients in the exercise group	Does not specify	Mode: home-based walking intervention Intensity: moderate Session duration: 20 min Session frequency: 4 days per week Duration of intervention: 12 weeks	Cortisol, IL-6	Radioimmunoassay	Before and after the intervention	No significant differences between study groups or across time

Sprod et al. (37)	Adult breast and prostate cancer patients receiving radiation therapy (n = 38)	RCT, with 19 patients in the usual care control group and 19 patients in the exercise group	Does not specify	Mode: home-based walking intervention using pedometers Intensity: moderate Session duration/frequency: patients increased number of steps each day by 5–20% Duration of intervention: 4 weeks	IL-6, TNF- α	ELISA	Before and after the intervention	No significant differences in baseline biomarker levels between groups Serum IL-6 was significantly lower in the exercise group post-intervention compared to the control group Significant associations between IL-6 and various measures of sleep quality
Segal et al. (55)	Adult prostate cancer patients receiving radiation therapy (n = 121)	RCT, with 41 patients in the usual care control group, 40 patients in the resistance exercise group, and 40 patients in the aerobic exercise group	Incremental treadmill test to determine $VO_{2\text{peak}}$	Mode: cycle ergometer, treadmill, or elliptical trainer Intensity: 50–75% of $VO_{2\text{peak}}$ Session duration: 15–45 min Session frequency: 3 days per week Duration of intervention: 24 weeks	Testosterone	Does not specify	Before the intervention, midway through the intervention, after the intervention	Significant main effect for time and a significant group by time interaction effect for testosterone levels. Smaller decreases in testosterone levels observed in the resistance training group compared to the aerobic exercise and control groups

(continued)

Table 3
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Author	Sample	Design	Baseline exercise testing method	Exercise intervention	Endocrine/soluble factor component(s)	Method(s) of endocrine/soluble factor assessment	Time points of endocrine/soluble factor assessment	Results
Galvao et al. (56)	Adult prostate cancer patients undergoing androgen suppression therapy (n=57)	RCT, with 28 patients in the usual care control group and 29 patients in the exercise group	400-meter walk	Mode: cycling and walking/jogging Intensity: 65–80% of maximum heart rate and 11–13 on the Borg 6–20 point scale Session duration: 15–20 min Session frequency: 2 days per week Duration of intervention: 12 weeks	Testosterone, insulin, CRP	Does not specify	Before and after the intervention	Significant decrease in CRP across time for the exercise group, while increasing across time in the control group
Galvao et al. (57)	Adult prostate cancer patients undergoing androgen suppression therapy (n=50)	Non-randomized, with 16 patients receiving acute androgen suppression therapy and 34 patients receiving chronic androgen suppression therapy	400-meter walk	Mode: cycling and walking/jogging Intensity: 65–80% of maximum heart rate and 11–13 on the Borg 6–20 point scale Session duration: 15–20 min Session frequency: 2 days per week Duration of intervention: 12 weeks	Testosterone, insulin, CRP	Does not specify	Before and after the intervention	No significant differences between groups for any biomarker

RCT randomized controlled trial; IL interleukin; CRP C-reactive protein; ELISA enzyme-linked immunosorbent assay; VT ventilatory threshold; SD standard deviation; MIP macrophage inflammatory protein; MCP monocyte chemoattractant protein; TNF tumor necrosis factor; AML acute myelogenous chemotherapy; HRR heart rate reserve

rest before and after the intervention periods, with some studies also measuring these markers in the middle of the intervention.

Demark-Wahnefried et al. (52), Payne et al. (54), and Sprod et al. (37) examined the effect of aerobic exercise training in breast cancer patients undergoing adjuvant chemotherapy, hormonal therapy, and radiation therapy. Sprod et al. (37) also examined the effect of aerobic exercise training on prostate cancer patients undergoing radiation therapy. Demark-Wahnefried et al. (52) measured changes in insulin, IL-1 β , and CRP levels along with various body composition, physical activity, and quality of life parameters. Payne et al. (54) measured changes in cortisol and IL-6 levels along with various markers of fatigue, sleep disturbances, and depressive symptoms. Similarly, Sprod et al. (37) measured changes in IL-6 and TNF- α along with various markers of sleep quality. All markers were measured before and after the intervention period; all three studies included a usual-care control group as well as an intervention group. Demark-Wahnefried et al. (52) and Payne et al. (54) did not observe any significant changes in hormone, cytokine, or CRP levels between study groups or across time. Additionally, the authors did not perform any analyses to examine whether changes in hormone, cytokine, or CRP levels were associated with other study endpoints. Sprod et al. (37) found that there were no significant differences in cytokine levels between study groups at baseline, but that serum IL-6 levels were significantly lower in the exercise group post-intervention compared with the control group ($p=0.04$), although in both groups, IL-6 levels did increase over the course of the intervention period. The authors found that at baseline, higher levels of plasma IL-6 were associated with measures of poorer sleep quality in the exercise group ($r=-0.54$ to -0.63 , $p=0.004-0.017$). Post-intervention, there was a negative association between plasma IL-6 levels and sleep efficiency in the exercise group ($r=-0.49$, $p=0.034$), whereas in the control group, there was a negative association between plasma IL-6 levels and sleep duration ($r=-0.49$, $p=0.036$). Additionally in the control group, there was a positive association between changes in TNF- α levels across time and changes in sleep latency and use of sleep medications ($r=0.50-0.58$, $p=0.009-0.031$), whereas no significant associations were observed between changes in sleep measures and changes in cytokine levels across time in the exercise group.

Jones et al. (53) examined the effect of aerobic exercise training in a group of lung cancer patients prior to undergoing surgical resection. The authors measured changes in several inflammatory markers and cytokines including ICAM-1, MIP-1 α , IL-6, IL-8, MCP-1, and TNF- α before and after the intervention period. The authors found that the only significant change that occurred across time was a decrease in ICAM-1 ($p=0.041$), although all other inflammatory markers and cytokines except TNF- α did decrease following the exercise intervention (changes were nonsignificant). Additionally, changes in the inflammatory markers and cytokines were not significantly correlated with changes in markers of cardiorespiratory fitness such as $VO_{2\text{ peak}}$.

Battaglini et al. (23) examined the effect of aerobic exercise training on three cytokines (IL-6, IL-10, and IFN- γ) in a group of patients with AML undergoing in-hospital chemotherapy. Changes in cytokine levels were measured before the intervention, midway through the intervention, and at the end of the intervention. The authors found that IL-6 levels decreased in a marginally significant fashion across time ($p=0.059$), while there were no significant changes in either IL-10 or IFN- γ .

levels across time, although patients did experience significant improvements in cardiorespiratory endurance, reductions in total fatigue, and reductions in depression scores across time ($p=0.009-0.023$).

Lastly, Segal et al. (55) and Galvao et al. (56, 57) studied the impact of aerobic exercise training in prostate cancer patients currently receiving either radiation therapy or androgen suppression therapy. Segal et al. (55) measured changes in testosterone levels before the intervention, midway through the intervention, and after the intervention, and Galvao et al. (56, 57) measured changes in testosterone, insulin, and CRP levels before and after the intervention. Segal et al. (55) found that testosterone levels significantly decreased over time in the control group and in the aerobic exercise group ($p<0.001-0.021$), while the decrease in testosterone levels for the resistance training group was not significant over time. Resistance and aerobic exercise training also lead to significant improvements in fatigue, quality of life, aerobic fitness, muscular strength, body composition, and triglyceride levels ($p<0.001-0.052$). The two studies by Galvao et al. (56, 57) did not show any significant changes in testosterone or insulin across time, while a significant decrease in CRP level was seen across time in the exercise group compared to the control group ($p=0.008$) (56). The authors also found that there were no significant differences in testosterone, insulin, or CRP levels between patients in the exercise group who had received acute androgen suppression therapy vs. patients in the exercise group who had received chronic androgen suppression therapy (57). Similar to other studies, the authors also found that various markers of quality of life, fatigue, aerobic fitness, lean mass, and muscular strength improved after the exercise intervention ($p<0.001-0.047$).

The general conclusions reached by the authors of these eight studies similar to those previously reported are that aerobic exercise can lead to significant improvements in aerobic fitness, quality of life, fatigue, and body composition, and that aerobic exercise interventions are feasible and well tolerated in a variety of oncology patients undergoing various forms of treatment. Similar to those studies examining the effect of aerobic exercise on cellular immune responses, aerobic exercise interventions in oncology patients undergoing therapy may lead to improvements in hormonal, cytokine, and other soluble factor levels, or these parameters may be unchanged over the course of the intervention. Several of these parameters are also considered markers of systemic inflammation, such as CRP, IL-6, TNF- α , and IFN- γ , while others such as IL-10 are considered anti-inflammatory. The effect of aerobic exercise in potentially lowering the levels of systemic inflammatory makers and raising the levels of anti-inflammatory markers could be associated with other health outcomes including decreased cardiovascular disease, decreased muscle wasting, and improved sleep quality (23, 37, 51). However, the fact that not all inflammatory markers are affected by an aerobic exercise intervention could indicate that only certain inflammatory markers may be responsive to exercise (53).

AFTER TREATMENT

Ten studies have examined the effect of aerobic exercise training on endocrine and soluble factor responses in oncology patients after the completion of treatment (36, 46, 47, 50, 58-63). Nine of these studies utilized adult cancer survivors, whereas one study used a mixed sample of adolescent and adult cancer survivors (63). Table 4 summarizes the information from these ten studies as previously described. Eight studies used breast

Table 4

Summary of studies examining endocrine and soluble factor responses to aerobic exercise training in cancer patients and survivors after cancer treatment

Author	Sample	Design	Baseline exercise testing method	Exercise intervention	Endocrine/soluble factor component(s)	Method(s) of endocrine/soluble factor assessment	Time points of endocrine/soluble factor assessment	Results
Fahey et al. (58)	Adult postmenopausal breast cancer survivors (n=52)	RCT, with 24 survivors in the exercise training group and 23 survivors in the control group	Incremental cycle ergometer test to determine VO _{2peak}	Mode: recumbent or upright cycle ergometers Intensity: ~70–75% of VO _{2peak} Session duration: 15–35 min Session frequency: 3 days/week Duration of intervention: 15 weeks	Insulin, IGF-I, IGF-II	Insulin: radioimmunoassay IGFs: ELISA	Before and after the intervention	No significant differences between groups for changes in insulin or IGF-II levels across time Significant difference between groups for change in IGF-I level across time. IGF-I level decreased in the exercise group and increased in the control group
Fahey et al. (46)	Adult postmenopausal breast cancer survivors (n=52)	RCT, with 24 survivors in the exercise training group and 23 survivors in the control group	Incremental cycle ergometer test to determine VO _{2peak}	Mode: recumbent or upright cycle ergometers Intensity: ~70–75% of VO _{2peak} Session duration: 15–35 min Session frequency: 3 days/week Duration of intervention: 15 weeks	IL-1 α , IL-4, IL-6, IL-10, TNF- α , TGF- β	ELISA	Before and after the intervention	No significant differences between groups for change in cytokine levels across time
Fahey et al. (59)	Adult postmenopausal breast cancer survivors (n=52)	RCT, with 24 survivors in the exercise training group and 23 survivors in the control group	Incremental cycle ergometer test to determine VO _{2peak}	Mode: recumbent or upright cycle ergometers Intensity: ~70–75% of VO _{2peak} Session duration: 15–35 min Session frequency: 3 days/week Duration of intervention: 15 weeks	CRP	ELISA	Before and after the intervention	Marginally-significant difference between groups for change in CRP levels across time. CRP level decreased in the exercise group and increased in the control group

(continued)

Table 4
(continued)

<i>Author</i>	<i>Sample</i>	<i>Design</i>	<i>Baseline exercise testing method</i>	<i>Exercise intervention</i>	<i>Endocrine/soluble factor component(s)</i>	<i>Method(s) of endocrine/soluble factor assessment</i>	<i>Time points of endocrine/soluble factor assessment</i>	<i>Results</i>
Humick et al. (47)	Post-treated adult breast cancer patients (n = 36)	Non-randomized controlled trial; patients specifically recruited into either the exercise training group (n = 21) or the control group (n = 15)	Does not specify	Mode: treadmill running and walking Intensity: 60–75% of functional capacity Session duration: 10–20 min Session frequency: 3 days/week Duration of intervention: 6 months	IL-6, IFN- γ	ELISA	Post-treatment but before beginning the exercise intervention (T2); 3 months into the exercise intervention (T3); post-intervention (T4)	No significant differences in IL-6 levels between groups or across time Significant difference between groups for change in IFN- γ levels between the T2 and T3 time points. IFN- γ level decreased in the exercise group and increased in the control group Significantly higher IFN- γ /IL-6 ratio in the exercise group compared to the control group at the T2 time point. Significant increase in the IFN- γ /IL-6 ratio in the exercise group from the T3 to the T4 time point
Hughes et al. (60)	Hispanic breast cancer survivors (n = 25)	One group, pretest-posttest	Does not specify	Mode: primarily walking Intensity: does not specify Session duration: 60 min Session frequency: does not specify Duration of intervention: 10 weeks	Cortisol	Does not specify	Approximately every 2 weeks during the intervention	Cortisol levels significantly decreased throughout the intervention

Ligibel et al. (61)	Adult breast cancer survivors ($n=82$)	RCT, with 42 survivors in the usual care control group and 40 survivors in the exercise group	Does not specify	Mode: does not specify Intensity: 55–80% of maximum heart rate Session duration/frequency: 90 min per week Duration of intervention: 16 weeks	Insulin	Immunochemiluminometric assay	Before and after the intervention	Significant decrease in insulin levels in the exercise group across time
Gomez et al. (36)	Adult breast cancer survivors ($n=16$)	RCT, with eight survivors in the usual care control group and eight survivors in the exercise group	Incremental cycle ergometer test to determine $VO_{2\text{ peak}}$	Mode: cycle ergometer Intensity: 70–80% of maximum heart rate Session duration: 20–30 min Session frequency: 3 days per week Duration of intervention: 8 weeks	Large battery of cytokines	Bio-Plex human cytokine immunoassay	Before and after the intervention	Significant group by time interaction effect for CTACK levels Marginally-significant group by time interaction effect for IL-15 and MIF levels as well as for the IL-10/TNF- α ratio
Sprod et al. (62)	Adult breast cancer survivors ($n=19$)	RCT, with ten survivors in the standard support therapy group and nine survivors in the exercise group	Does not specify	Mode: Tai chi chuan Intensity: light to moderate Session duration: 60 min Session frequency: 3 days per week Duration of intervention: 12 weeks	Insulin, cortisol, IGF-1, IL-6, IL-8	ELISA and radioimmunoassay	Before and after the intervention	No significant changes in cortisol, IGF-1, IL-6, or IL-8 levels in either group across time Marginally-significant increase in insulin levels across time in the standard support therapy group Significant associations between changes in cortisol, IGF-1, and IL-8 with changes in various components of HRQOL across time

(continued)

Table 4
(continued)

<i>Author</i>	<i>Sample</i>	<i>Design</i>	<i>Baseline exercise testing method</i>	<i>Exercise intervention</i>	<i>Endocrine/soluble factor component(s)</i>	<i>Method(s) of endocrine/soluble factor assessment</i>	<i>Time points of endocrine/soluble factor assessment</i>	<i>Results</i>
Lee et al. (50)	Adult stomach cancer survivors (n=21)	One group, pretest-posttest	Does not specify	Mode: Tai Chi Intensity: light to moderate Session duration: 30–40 min Session frequency: 1 day/week	IL-6, TNF- α	ELISA	Before and after the intervention	No significant difference in cytokine levels across time
Järvelä et al. (63)	Adolescent and adult survivors of childhood ALL (n = 17)	One group, pretest-posttest	Incremental cycle ergometer test to determine VO _{2 peak}	Duration of intervention: 24 weeks Mode: Walking and jogging (home-based) Intensity: does not specify Session duration: 30 min Session frequency: ≥ 3 days per week Duration of intervention: approximately 16 weeks	Insulin	Immunochemiluminometric assay	Before and after the intervention	Significant decrease in insulin level across time

RCT randomized controlled trial; *IGF* insulin-like growth factor; *IL* interleukin; *TNF* tumor necrosis factor; *TGF* transforming growth factor; *ELISA* enzyme-linked immunosorbent assay; *CRP* C-reactive protein; *IFN* interferon; *HRQOL* health related quality of life; *CTACK* cutaneous T cell-attracting chemokine; *MIF* macrophage migration inhibitory factor; *ALL* acute lymphoblastic leukemia

cancer survivors (36, 46, 47, 58–62). One study used stomach cancer survivors and one study used long-term survivors of pediatric ALL (50, 63). The amount of time that had elapsed from completion of cancer treatment to the beginning of the exercise intervention varied among the studies, from immediately posttreatment to over 20 years posttreatment.

Fairey et al. (46, 58, 59) completed a series of three investigations looking at the effect of an exercise intervention on insulin, insulin-like growth factors (IGF)-I and II, CRP, and a variety of cytokines (IL-1 α , IL-4, IL-6, IL-10), tumor necrosis factor (TNF)- α , and transforming growth factor (TGF)- β in a group of 52 postmenopausal breast cancer survivors. Study participants were randomly assigned to either a non-exercising control group or the exercise intervention group. Endocrine and soluble factor levels were measured from resting blood samples taken at the beginning and end of the intervention period. The authors found that there was a significant difference in IGF-1 levels between groups across time, in that IGF-1 decreased in the exercise group and increased in the control group ($p=0.045$). There was a marginally significant difference in CRP levels between groups across time, in that CRP decreased in the exercise group and increased in the control group ($p=0.066$). There were no significant differences in insulin, IGF-II, or any cytokine levels between groups over the course of the intervention period.

Hutnick et al. (47), Hughes et al. (60), Ligibel et al. (61), Gomez et al. (36), and Sprod et al. (62) also examined the effect of exercise training in breast cancer survivors on a variety of hormones and soluble factors including cortisol, insulin, IGF-I, as well as an extremely wide variety of cytokines. Ligibel et al. (61), Gomez et al. (36), and Sprod et al. (62) examined changes in their study outcomes at the beginning and end of the intervention period, and all three randomized their samples of breast cancer survivors into a usual-care or standard therapy control group and an exercise intervention group. Hutnick et al. (47) and Hughes et al. (60) also examined the effects of their exercise interventions at various points throughout the intervention period, although study participants were not randomly assigned to the control or intervention group. When considering endocrine study endpoints, Hughes et al. (60) found that salivary cortisol levels decreased in their study group of Hispanic breast cancer survivors across time, when measured at regular intervals before, during, and after the exercise intervention period ($p<0.001$). However, Sprod et al. (62) found that exercise did not significantly change serum cortisol levels across time. Ligibel et al. (61) found that insulin levels significantly decreased in the exercise group over the course of the intervention period ($p=0.03$), whereas Sprod et al. (62) observed a marginally significant increase in insulin levels in the control group over the course of the intervention period ($p=0.08$). Sprod et al. (62) did observe that changes in cortisol levels during the course of the intervention period were associated with two components of HRQOL, namely changes in physical role limitations ($r=0.074$, $p<0.05$) and changes in health perceptions ($r=0.46$, $p<0.05$). Similarly, Sprod et al. (62) observed associations between changes in IGF-I during the course of the intervention period and changes in overall HRQOL ($r=-0.56$, $p<0.05$), changes in physical role limitation ($r=-0.68$, $p<0.05$), and social functioning ($r=-0.56$, $p<0.05$), although no significant differences were observed for changes in IGF-1 levels between study groups across time. When considering cytokine study

endpoints, results were similar to those for the endocrine study endpoints. Hutnick et al. (47) did not observe significant differences in IL-6 levels between study groups across time, but did observe that IFN- γ levels significantly decreased in the exercise group and increased in the control group over the course of the first 3 months of the intervention period ($p < 0.05$). There was also a significantly higher IFN- γ /IL-6 ratio in the exercise group compared to the control group 3 months into the exercise intervention period, and the IFN- γ /IL-6 ratio significantly increased in the exercise group from the 3-month to the 6-month time point during the intervention period ($p < 0.05$) (47). Sprod et al. (62) did not find significant changes in either IL-6 or IL-8 levels between study groups across the intervention period, but did find that there was an association between changes in IL-8 level and changes in emotional role limitations ($r = 0.59$, $p < 0.05$). Gomez et al. (36) examined the impact of exercise on a battery of over 40 cytokines. However, the authors only found a significant group-by-time interaction effect for levels of CTACK ($p = 0.016$), where CTACK level did not change in the exercise group but did increase over time in the control group (36). The authors did find a marginally significant group-by-time interaction effect for IL-15 ($p = 0.058$) and macrophage migration inhibitor factor (MIF) levels ($p = 0.070$), as well as for the IL-10/TNF- α ratio ($p = 0.064$). Specifically, the levels for all three of these markers decreased across time in the exercise group and increased across time in the control group (36).

As described previously, Lee et al. (50) examined the effect of a Tai Chi intervention program on a group of Korean stomach cancer survivors, looking at the effect of the 24-week exercise program on IL-6 and TNF- α levels. The authors did not observe any significant change in either cytokine level over the course of the intervention. Lastly, Järvelä et al. (63) examined the effect of exercise training on insulin levels in a group of adolescents and adults who were long-term survivors (median time since diagnosis = 15.9 years) of childhood ALL. The authors observed that a simple home-based exercise intervention program significantly reduced insulin levels across time ($p = 0.01$), while also decreasing other cardiovascular and metabolic risk factors including insulin resistance ($p = 0.002$), waist-to-hip ratio ($p = 0.002$), and percent body fat ($p = 0.04$) while improving $VO_{2\text{ peak}}$ ($p = 0.01$).

The general conclusions reached by the authors of these ten studies are similar to those previously stated. Aerobic exercise interventions appear to be safe, feasible, well tolerated, and can lead to improvements in various markers of physical fitness and quality of life. As seen before, aerobic exercise interventions can lead to improvements in endocrine and soluble factor levels. Decreases in CRP levels could be associated with decreased cardiovascular disease risk, as well as changes in pro- and anti-inflammatory cytokine production by blood mononuclear cells and NK cell cytotoxic activity (46). Similarly, decreases in insulin and other factors associated with cardiovascular and metabolic diseases could be relevant for survivors of childhood ALL, as these individuals can be at a higher risk for developing cardiovascular disease as a result of their treatment (63). Exercise interventions may aid in decreasing stress, as seen through decreasing salivary cortisol levels (60), and might also act to decrease low-grade inflammation and treatment-related side effects through increasing levels of anti-inflammatory and decreasing levels of proinflammatory cytokines (36, 62). However, reasons for not observing significant changes in hormone or soluble factor levels after

an exercise intervention can be varied, and may be related to short intervention duration, interventions that are not geared towards weight loss, or the fact that the local levels of cytokines (i.e., many cytokines act locally) may not be comparable to systemic circulating levels of these cytokines (47).

FUTURE RESEARCH DIRECTIONS

The previous review by Fairey et al. (4) has comprehensively outlined the limitations of previous exercise immunology literature in the cancer patient/survivor population, as well as recommendations for future research. Common limitations in previous exercise immunology research have included issues pertaining to sample size and sampling methods; use of heterogeneous populations (i.e., mixed ages, gender, and cancer sites, pathologies, and treatment types); lack of randomized control trial designs with usual-care or wait-list controls; short durations of interventions (i.e., less than 12 weeks); use of partially unsupervised training interventions; limited reporting of the type of physical fitness assessment used as well as the time points of physical fitness assessment with respect to the intervention period; lack of discussion of immune system changes with respect to clinical outcomes for cancer survivors; differences among studies in how blood samples were collected, how much time had elapsed between blood sampling and exercise, and whether subjects were asked to follow any pre-assessment guidelines prior to blood collection; lack of reporting of immune system components at other time points besides pre- and post-intervention; inconsistencies in how immune system results are presented; and a lack of information regarding the effect of exercise on the wide variety of leukocyte subsets (phenotypes and functional activities), cytokines, acute phase proteins, and neuroendocrine hormones (4). To address these limitations, Fairey et al. (4) offer recommendations including the use of larger randomized samples within the context of a randomized controlled trial; use of statistical power calculations during the planning stages of a trial to ensure that sample sizes will be large enough to detect significant differences between groups in the primary endpoints if they exist; use of usual-care or wait-list controls to help reduce the influence of confounding variables; use of more homogeneous samples of oncology patients; use of supervised exercise interventions lasting for longer than 12 weeks with close monitoring of exercise parameters, clearer reporting of the types of physical fitness assessments used, as well as the reporting of changes in physical fitness over the course of an intervention; correlation of changes in exercise-related immune function to meaningful clinical outcomes; standardizing of pre-assessment behaviors and guidelines that subjects should follow before blood sampling (i.e., behaviors related to diet, medications, sleep patterns, and smoking status); more frequent assessment of immune parameters during an intervention in addition to pre- and post-intervention; assessment of immune parameters at multiple time points pre-exercise and during recovery from exercise; comparison of immune system parameters using peripheral blood sampling, lymphoid tissue sampling, and sampling of other body fluids; more consistent methods on how immune system results should be reported; and the assessment of a wider variety of cancer-related immune system parameters (i.e., immune cell phenotype and functionality analysis, soluble factor analysis, and neuroendocrine hormone analysis). These limitations and recommendations

are still valid concerns for researchers in this field, and many of them also apply to the current literature surrounding endocrine and soluble factor responses in cancer patients and survivors. However, it should be noted that many of the more recent investigations have included randomized controlled trials with larger sample sizes, more homogeneous samples, more extensive analyses of cellular immune function, and evaluation of immune, endocrine, and soluble factor responses at additional time points besides pre- and post-intervention (44–49). The current review outlines three areas that should be especially addressed in future studies.

Firstly, future studies should continue to examine the effect of acute aerobic exercise on cellular immune responses, hormones, cytokines, and other soluble factors in cancer patients and survivors, particularly for aerobic exercise intensities and durations that are commonly used when constructing exercise prescriptions for this population. For example, the current body of exercise oncology literature regarding breast cancer patients and survivors generally uses moderate-vigorous intensities (50–85% of $\text{VO}_{2 \text{ max}}$, $\text{VO}_{2 \text{ peak}}$, heart rate reserve, or maximum heart rate) for durations of 1 h or less per session (17, 18, 43, 46, 64–69). It is necessary to investigate how the immune system and endocrine system of a cancer patient or survivor respond to and recover from acute aerobic exercise, as it is a series of acute aerobic exercise bouts that make up an aerobic exercise prescription. More specifically, investigations that profile the cellular immune response and hormonal responses before exercise, immediately postexercise, and at multiple time points during recovery are needed. Furthermore, examining the magnitude and duration of immunosuppression that can often occur during recovery from acute exercise, particularly higher intensity and longer duration aerobic exercise, may provide findings that could impact recommendations for the amount of time a cancer patient or survivor should rest postexercise before engaging in a subsequent bout of exercise.

Secondly, future studies should examine and compare exercise-induced immune, endocrine, and soluble factor responses of cancer patients and survivors with those of healthy controls, i.e., individuals who have never been diagnosed with or received treatment for cancer. The majority of the studies reviewed that included a control group utilized a usual-care control group of cancer patients or survivors drawn from the same population as the experimental group (42–49). Only two studies used a control group of matched healthy individuals who exercised in the same manner as the cancer patients in the experimental group (38, 39). As exercise prescriptions for cancer patients and survivors are modeled after those for healthy individuals (2), it is important to understand whether a cancer patient's physiological responses, including the immune and endocrine system responses, are truly similar to those of healthy individuals. If future studies do find significant differences in physiological responses between cancer patients and healthy individuals to exercise, then this knowledge could also impact guidelines regarding appropriate exercise prescription.

Thirdly, future studies should examine the relationships between cellular immune responses to aerobic exercise and other biomarkers such as hormones and cytokines. Three of the studies in the immune responses sections of this review also examined the effect of aerobic exercise on cytokine responses for IL-1 α , IL-4, IL-6, IL-10, IFN- γ , TNF- α , and TGF- β 1; however, associations between changes in the cellular immune

parameters and changes in cytokine levels were not analyzed (46, 47, 50). Examining the relationships between cellular immune parameters, hormones, cytokines, and other soluble factors of the immune system in response to exercise may be important, as many of these factors, such as CRP, IL-1, TNF- α , TNF- β , IFN- α , and IFN- γ , may function as mediators of the exercise-induced immune response and because of their potential roles in anticancer defense (4).

Additionally, future studies should compare immune and endocrine system responses to aerobic exercise of various intensities (i.e., low vs. moderate vs. high intensity), aerobic exercise vs. resistance exercise, and immune and endocrine responses to exercise between oncology patients with various cancer diagnoses, cancer patients within the same diagnosis but who may have received different treatment regimens, and between very long-term cancer survivors and individuals who have never been through cancer treatment. These issues can certainly be considered subsets of the issues and recommendations previously stated, and will further help to refine the current exercise prescription recommendations for oncology patients, as well as to understand if certain intensity levels are more beneficial to the immune and endocrine systems in the context of an exercise prescription, if there are certain exercises that oncology patients should avoid, if resistance exercise might have an additive effect on the immune and endocrine system, and if there is a point at which a long-term survivor's physiology can truly be considered the same as a healthy individual who has never undergone cancer treatment.

CONCLUSION

Aerobic exercise has been shown to be a beneficial adjunct therapy for oncology patients as it can improve both physiological and psychological functioning. Aerobic exercise may also affect immune and endocrine system functioning in cancer patients and survivors, which may lead to potential improvements in mechanisms associated with systemic inflammation, cardiovascular disease risk, anticancer defense, cancer prognosis, recurrence, second malignancy, and overall survival. Current studies show that aerobic exercise is feasible in both pediatric and adult cancer patients during and after treatment, and in some cases may lead to improvements in resting immune function, cytokine profiles, and stress and metabolic hormone levels over the course of training. However, it should be noted that cancer patients undergoing cancer therapies including chemotherapy, blood stem cell transplant, and BMT may experience decreased immune system cell counts and function compared to healthy individuals, and that exercise interventions should be individualized and with careful monitoring of immune function. Further research is needed to more fully understand the effects of acute exercise on the immune system, the immune system responses of oncology patients compared to those of healthy individuals, and the relationships between exercise-induced cellular immune responses, hormones, and cytokines, which may also play an important role in anticancer defense. With this further research, it is hopeful that safer and more specific aerobic exercise guidelines will emerge which can help guide clinicians on the use of exercise as a tool to improve their patients' physical functioning, quality of life, and overall survival.

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Diabetes and Exercise

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TYPE 1 DIABETES AND SPORT

The American Diabetes Association recommends regular exercise for all people with diabetes, including those with type 1 diabetes because all patients with diabetes should have the opportunity to benefit from the many valuable effects of physical activity (1). At present, there is no clear evidence about the benefits of regular physical activity on glucose control in type 1 diabetes; however, regular exercise ameliorates quality of life, body composition, blood pressure and, possibly, decreases the risk of diabetes-related complications and mortality. Type 1 diabetic individuals (DM1) can practice almost all sports, but they must be well educated to check their blood glucose before, during and after the exercise sessions in order to adjust carbohydrate intake and/or insulin dosage. These adjustments should be based on patients' knowledge of basic principles of substrate and hormone responses to exercise, and on their self-experience. In order to optimally manage their glucose levels, DM1 subjects should learn that the physiologic responses of insulin and of counter-regulatory hormones (glucagon, epinephrine, cortisol and growth hormone) are different to acute or prolonged exercise.

During acute, intense exercise (>85% of $\text{VO}_{2\text{max}}$), epinephrine response can augment hepatic glucose output by six to sevenfold (2). In normal individuals, the sharp increase

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in liver glycogenolysis results in a modest hyperglycemia which is counteracted by a rapid augment of insulin secretion (3). Since DM1 subjects do not have endogenous insulin secretion, acute exercise regularly leads to postexercise hyperglycemia that can be mitigated before the intense exercise sessions by the administration of 1–2 I.U. of short-acting insulin analogues (4). DM1 subjects should also be aware of the possibility of postexercise hypoglycaemia and staying alert for 48–72 h (1). Hypoglycemia might occur, especially, in 2–6 h following acute exercise training hypoglycaemia, due to delayed insulin sensitivity.

During prolonged exercise at moderate (40–60% of $VO_{2\max}$) or submaximal (60–85% of $VO_{2\max}$) intensity, insulin secretion in normal subjects is progressively reduced by ~50%, while counter-regulatory hormonal response activates lipolysis, glycogenolysis and gluconeogenesis to consent adequate supply of substrates for oxidative phosphorylation in contracting muscles (5). The physiologic reduction in insulin secretion serves to augment hepatic glucose output and to prevent a reduction in circulating blood glucose caused by increased muscle uptake (5). In DM1 subjects, glucose response to prolonged exercise is modulated by insulin availability (4, 6). Previous adequate insulin availability results in a progressive decline in blood glucose levels which is greater if carbohydrates are preferentially oxidized in comparison to fatty acids (4). In contrast, starting exercise in a state of insulin deprivation leads to a progressive increase in circulating blood glucose levels due to integrated counter-regulatory hormone action which exposes the patients also to the risk of ketosis (4). Thus, before starting exercise, DM1 subjects should check their blood glucose (1, 6). Optimal glycemic values before starting a prolonged exercise session are those ranging between 120 and 180 mg%. Values lower than 120 mg% likely result, 30–60 min after starting exercise, in a hypoglycemia, but that could be easily prevented by ingesting 20–60 g of simple sugars, at about 30 min intervals (Table 1). Values over 250 mg% might be the consequence of either postprandial hyperglycemia or insulin deprivation (1, 6). Prolonged exercise will reduce elevated blood glucose levels in the former option, whereas if the cause is insulin deficiency, it will exacerbate hyperglycemia and might result in a frank ketoacidosis. It is highly recommended to DM1 subjects with glycemic values over 250 mg% to check blood ketone bodies and avoid to exercise if these are increased, because a positive result indicates a state of insulin deprivation (1, 6).

During exercise lasting over than 1 h, DM1 athletes should check their blood glucose at 30 min intervals and use the trend of glycemic values to come to a decision about the ingestion of carbohydrates. The intensity of exercise (estimated by heart rate monitoring) is a good predictor of the risk of hypoglycemia. Physiological studies demonstrate that the oxidation of glucose and fatty acids is almost equivalent during exercise at 40–60% of maximal capacity. A greater intensity leads to larger glucose utilization (7). Thus, during a prolonged intense exercise by DM1 subjects, for instance, biking uphill in comparison to a flat course, is expected to reduce blood glucose concentration (climbing a hill of 4–5 km can reduce by more than 100 mg% blood glucose concentration of diabetic cyclists). Skilled DM1 athletes should anticipate, on the basis of the training/race course, carbohydrate intake in order to avoid hypoglycemia.

Optimal insulin therapy for DM1 sportspersons is that which better mimics physiological changes in insulin secretion. In this regard, the use of continuous subcutaneous insulin infusion devices is most advantageous, especially if combined with subcutaneous

Table 1
The Decalogue of physically active insulin-treated diabetic subjects

Inject regular insulin or fast-acting insulin analogues into abdominal subcutaneous region
Cut the dosage of short-acting insulin analogue by 10–40% before the exercise, dependent on duration, intensity of the session and your previous experience
Cut the dosage of basal insulin analogue by 30–50% before the exercise, dependent on duration, intensity of the session and your previous experience
Before starting the exercise session, check your blood glucose
Before starting, ingest 20–60 g of simple carbohydrates if your blood glucose is less than 120 mg%
Before starting, delay the exercise session if your blood glucose is less than 80 mg%
Before starting, delay the exercise session if your blood glucose is greater than 250 mg%; you can exercise only if your blood ketones are negative
During prolonged exercise check your blood glucose every 30 min of exercise
During prolonged exercise supplement with 20–60 g of simple carbohydrates, every 30 min (preferably, make a decision on the basis of blood glucose trend)
After exercise, cut your usual short-acting insulin dosage by 10–30%

glucose monitoring. These systems allow the reduction of the basal rate of insulin administration during prolonged physical activity (usually by 50–80% of regular dose), as well as the correction of hyperglycemic surges by bolus injections. Traditional insulin therapy by subcutaneous multiple injections requires adequate planning of daily sport activities in order to reduce insulin dosage prior to exercise (in particular basal insulin) and, eventually, after exercise (in particular preprandial insulin). The carbohydrate/insulin ratio, which is the weight in grams of carbohydrates metabolized by 1 unit of short-acting insulin, is influenced by the type of exercise, especially by the duration of moderate exercise sessions. Our experience with DM1 cyclists shows that the longer the distance, the greater the effect of exercise on insulin sensitivity. In Fig. 1, we present unpublished data on carbohydrate/insulin ratios before and during a 1 day 300 km course (Italy's coast to coast in 2005), two 7-day courses of 200 km/day (Italy–Holland tour) and a 100 km/day course (Sardinia Island tour). All tours were completed by well-trained DM1 bikers who had twice than normal basal carbohydrate/insulin ratios (about 22–24) and reached mean increments of 80 for distances over 200 km (more than 7–8 h of daily biking) and of 45 for distances of 100 km/day. In contrast, when exercise is performed in extreme conditions, such as during high altitude mountain climbing, insulin sensitivity significantly declines. In Fig. 2, we present unpublished data on mean blood glucose and daily insulin doses at different altitudes, recorded in a group of 8 DM1 mountain climbers during the 2005 expedition to Peak Lenin in Kyrgyzstan (7,135 m high). In these extreme conditions, the higher the altitude, the higher were blood glucose values and insulin requirements, indicating a state of insulin resistance, possibly sustained by counter-regulatory hormones.

On the basis of literature data (6) and of our practical experience with DM1 athletes, involved in several sports, the sport activities that can be suggested to a person with type 1 diabetes are those which allow to check blood glucose and to eventually to ingest carbohydrates. Examples of safe activities are trekking, Nordic walking, biking, running and team sports with intervals (i.e., volleyball, soccer and basketball). There are sport

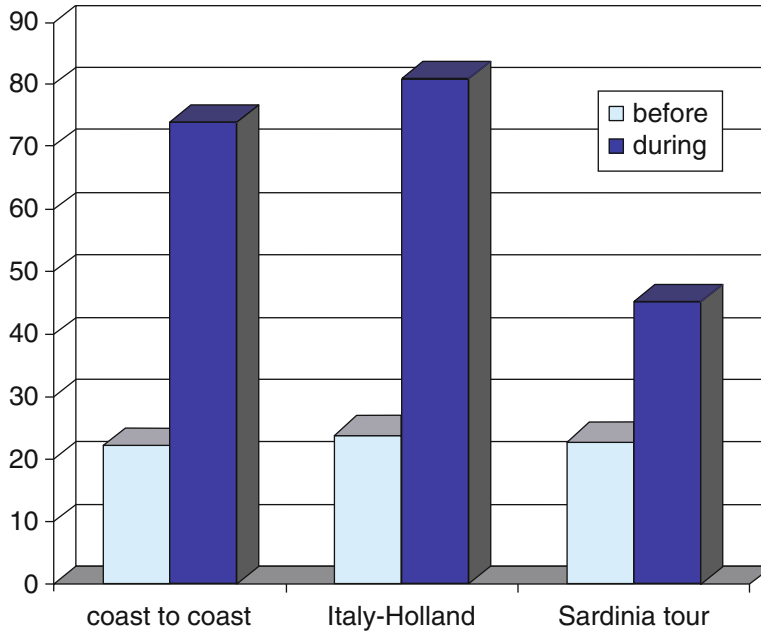


Fig. 1. Effects of 1 day 300 km course (Italy's coast to coast), of a week tour of 200 km/day (Italy-Holland tour) or of a week tour of 100/km/day (Sardinia tour) on carbohydrate/insulin ratio of well-trained bikers with type 1 diabetes mellitus.

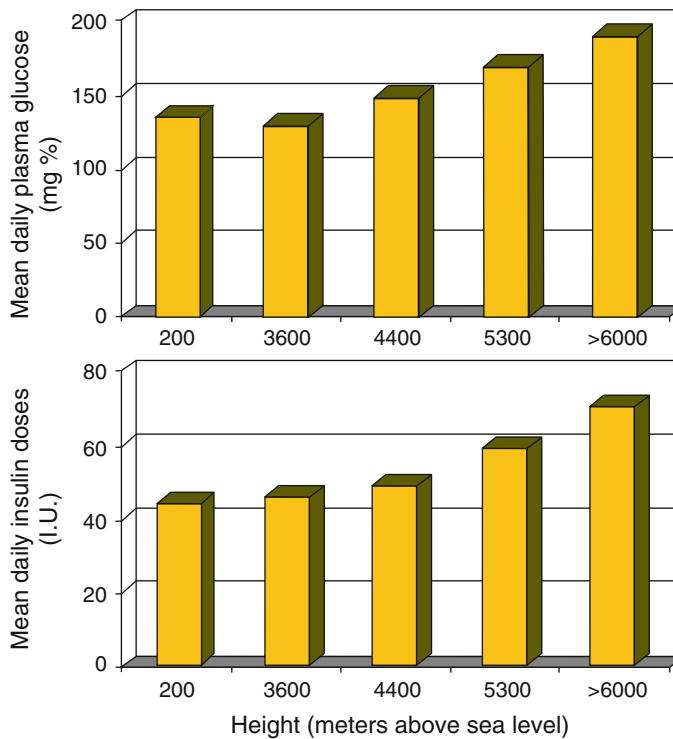


Fig. 2. Effects of altitude on mean daily plasma glucose levels and insulin requirements in 8 DM1 mountain climbers during the 2005 expedition to Peak Lenin in Kyrgyzstan (7,135 m high).

types in which an event of hypoglycemia might be harmful for the survival of patients and of their companions, like rock or high altitude climbing and scuba immersions. These sports, however, should be limited to a restricted number of responsible DM1 subjects, highly trained and motivated. For these subjects, recent advances in technical devices to check blood glucose are extremely useful to reduce the risk of accidents.

In conclusion, regular exercise and sports are useful to improve health status and quality of life of persons with DM1. There are no secure rules to adjust carbohydrate intake and insulin therapy before, during and after exercise. The optimal strategy to exercise safely is to follow general rules, as those reported in Table 1, and thanks to frequent self-glucose monitoring get experience on individual expected responses to different types and intensities of exercise.

TYPE 2 DIABETES AND EXERCISE

Today, more than 1,1 billion adults are overweight, 312 million of them are obese; the proportion of people with diabetes is projected to increase from 171 million to 366 million in 2030 (8). Diabetes has become a major cause of premature illness and death in most countries, mainly through the increased risk of cardiovascular disease (8). The World Health Organization (WHO), which is concerned about the rising incidence of obesity and diabetes worldwide consequent to the current sedentary lifestyle, states: “Each year, at least 1.9 million people die as a result of physical inactivity; without action to address the causes, deaths from non-communicable diseases will increase by 17% between 2005 and 2015” (9). In a recent joint document produced by the WHO and the World Economic Forum it is estimated that approximately 80% of cases of heart disease, stroke, type 2 diabetes and 40% of cancers could be prevented through inexpensive and cost-effective interventions addressing the primary risk factors (10). In particular, for type 2 diabetes mellitus (DM2) there is solid evidence regarding the efficacy and the cost-effectiveness of lifestyle intervention to prevent the disease in subjects with impaired glucose tolerance (11–13).

MITOCHONDRIAL DYSFUNCTION

The positive effects of exercise in DM2 prevention and care are mainly explained by the stimulation of exercise on mitochondrial biogenesis in skeletal muscle (14). Skeletal muscle represents about 80–90% of all insulin-sensitive tissues and accounts for about 50% of basal metabolic rate (15). Epidemiological studies have shown a significant inverse relationship between the level of physical fitness and the prevalence of metabolic syndrome either in adults (16) or in children (17). In rats genetic selection for low maximal oxygen uptake capacity ($VO_{2\max}$), an objective measure of physical fitness, leads to the typical features of the metabolic syndrome (18). Also in humans low values of $VO_{2\max}$ and of mitochondrial functional capacity are strictly related with reduced insulin sensitivity (19). Several studies have demonstrated mitochondrial dysfunction in subjects with insulin resistance, obesity and/or type 2 diabetes (19). There are also several studies demonstrating that regular aerobic exercise can increase $VO_{2\max}$ and partially reverse mitochondrial dysfunction by stimulating mitochondrial biogenesis and augmenting mitochondrial oxidative capacity (19–21).

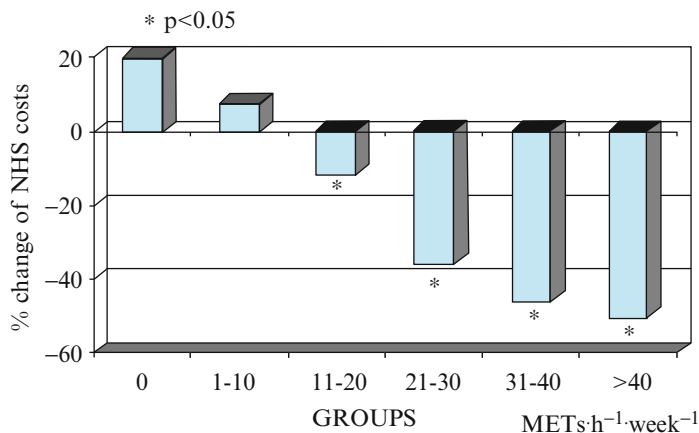


Fig. 3. Effects of increased METs-h-1-week-1 through leisure time physical activity, over 2 years, in subjects with type 2 diabetes mellitus on per cent reduction in costs paid by National Health Service (NHS) for drugs required to treat diabetes and hypertension (data from reference (22)).

EXERCISE PRESCRIPTION

The effectiveness of a training programme to treat diabetes is a function of age of subjects and of type, frequency, intensity and duration of exercise. In DM2 subjects there is a strict positive relationship between the amount of energy expenditure due to exercise and the improvements of several metabolic and anthropometric parameters (22). Thus, regarding the amount of physical activity it is recommended to start the training programme with reasonable targets, well matched with time availability and self-esteem of patients. Once subjects experience the beneficial effects of the lifestyle change, it is important to augment the amount of energy expenditure achievable through physical activity in order to maximize the benefits. It has been estimated that most of the beneficial effects in terms of improvement in metabolic and anthropometric parameters, reduction of cardiovascular risk and drug consumption are achieved with 35 METs·h⁻¹/week (22) (Fig. 3).

The intensity of exercise should be in the moderate range (3–6 MET) for several reasons. Usually, DM2 subjects are not familiar with high-intensity workouts and could easily drop out; the risk of acute cardiovascular events increases with the intensity of exercise; moderate intensity exercise can be sustained for prolonged time. A training planned on moderate intensity/long distances favours lipid consumption (5) and increases insulin sensitivity up to 14 days after the end of the last exercise session (23). Regarding type, amount and frequency of exercise for DM2 patients, the American Diabetes Association, the American Heart Association and the American College of Sports Medicine recommend at least 150 min per week of moderate aerobic physical activity that should be combined with three weekly sessions of resistance exercise to increase muscle strength (24–26). A meta-analysis of controlled studies that evaluated the effects of interventions based on structured physical activity in type 2 diabetes show an improvement in average HBA1c around 0.6–0.7% (27, 28). Recently, our group has shown that even a twice per week frequency of exercise at moderate intensity is

sufficient to improve glycemic control in type 2 diabetes mellitus (29). This weekly frequency of exercise is also sufficient to decrease of HBA1c at 12 months ($p=0.008$) by 0.45%, to increase the expression of skeletal muscle PPAR γ and PPAR α , to augment cardiovascular fitness ($VO_{2\max}$) and muscle strength and to reduce body weight, waist circumference, systolic blood pressure, total and LDL cholesterol levels (29). Types of exercise should include both aerobic and resistance exercise because it has been shown that combined aerobic and anaerobic exercise is more effective in reducing HBA1c levels of DM2 subjects in comparison to only aerobic exercise (30).

Despite the evidence about the benefits of exercise, many physicians do not spend time and efforts convincing DM2 subjects to practice physical activity. There is the need for simple and reproducible strategies of counselling to motivate these patients to exercise on a regular basis (31). It has been demonstrated that using an individual behavioural approach, it is possible for physicians to motivate the majority of type 2 diabetic subjects to long-term practice of exercise (32, 33).

MULTIDISCIPLINARY LIFESTYLE INTERVENTION

The efficacy of lifestyle intervention in DM2 is greatest when exercise is combined with therapeutic education, nutritional and psychological counselling. In our healthy lifestyle institute (CURIAMO), we are experimenting a multidisciplinary lifestyle intervention model for people with obesity and type 2 diabetes (Australian New Zealand Clinical Trials Registry: ACTRN12611000255987) (34). The CURIAMO model includes seven steps, involving the following experts: endocrinologists, sport medicine doctors or cardiologists, psychologists, dieticians, educators, nurses, exercise physiologists and promoters of outdoor activities. The intensive lifestyle intervention of the first 4 months includes the following steps: (1) initial medical examination by an endocrinologist, (2) interview by a psychologist, (3) assessment by a specialist in nutrition, (4) examination by a specialist in sport medicine, (5) a supervised programme of 24 sessions (2 per week) of structured indoor exercise with an exercise physiologist, (6) eight group therapy sessions with other patients (12 patients in each group) organized by an educator (a doctor of pedagogic sciences) and a nurse and (7) daily Nordic walking activity combined with weekend walking excursions planned by a manager for outdoor leisure time activities. For step 7, attendance by participants is optional, the choice to be made by participants being left open until the end of the study. Steps 1–4 are designed for achieving a clinical assessment and promoting behavioural changes in the participants. Steps 5–7 are designed to improve physical fitness and to use group power to support long-term lifestyle change. The exercise intervention is designed on the basis of the examination with the medical specialist in sports medicine, intended to measure aerobic capacity and muscle strength. The protocol test for maximal aerobic capacity, an incremental treadmill, starts with a 5 min warm up at a speed of 2–3 km/h; the load is then increased by 1 km/h, every 5 min until exhaustion. At the end of each step, the participant is given 20s' rest and capillary blood is drawn from the ear lobe for the determination of blood lactate using a lactate meter. The heart rate, continuously monitored by ECG, is used for obtaining the values corresponding to lactate concentrations of 2, 3 and 4 mM. These parameters are used to plan training and to monitor its effects.

The maximum dynamic force of extensor muscles of the leg and the flexor and extensor muscles of the arms is determined by means of the indirect method of extrapolation to 1, using one maximal repetition test on the leg press, and the muscle chest press machines. Tests of aerobic capacity and muscle strength are also used to heighten the participants' awareness of their physical status and to show them how to improve upon it. The exercise physiologist aims to achieve improvement in the participants' aerobic performance and muscle strength, and in their self-esteem, an indoor exercise programme is being used. The indoor exercise training consists of two sessions a week (3-day interval) in the gym for a total of 12 weeks (3 months). Each session, which lasts 90 min, is divided into a 60 min aerobic workout and 30 min of circuit training to enhance muscle strength and also to provide flexibility exercises. The aerobic workout is performed using ergometers for cardiovascular exercise (treadmill, bike, step and arm ergometers). The intensity of the workout is gradually increased (4 steps) from 50 to 65% of the heart rate reserve. The workout for muscular strength uses machines and isotonic free weights for the training of the lower and upper limbs; intensity is gradually increased, starting with 50 to reach 65% (there is one lower and one upper limit) of 1 repetition maximum (RM). Before and after each session, the resting heart rate, blood pressure and capillary blood glucose (participants with DM2) are measured. Training sessions are monitored by real-time heart rate telemetry and data are fed into a computer for subsequent analysis. The intensity of the training sessions is increased in four steps (50%, 55%, 60%, 65%) every 6 session.

The preliminary results, actually, obtained in 142 subjects with DM2 (age 56 ± 10 years, mean \pm SD) who completed the 3 month CURIAMO intensive lifestyle intervention, are very promising (Fig. 4) (35). Before and after the intensive intervention the following parameters significantly ($p < 0.05$) changed: HbA1c (7.2 ± 1 to $6.7 \pm 1\%$), plasma triglycerides (161 ± 97 to 149 ± 85 mg%), systolic (143 ± 15 to 133 ± 12 mmHg) and diastolic (83 ± 8 to 77 ± 8 mmHg) blood pressure, waist circumference (110 ± 12 to 106 ± 12 cm), fat mass ($32 \pm 1.1\%$ to $30 \pm 1.1\%$), cardiorespiratory fitness ($\text{VO}_{2\text{max}}$ 19.6 ± 2 to 26.1 ± 3 mL/kg⁻¹·min⁻¹) and blood lactate/walking speed ratio (1.7 ± 0.2 to 0.7 ± 0.1); daily defined doses and costs of antidiabetic and anti-hypertensive drugs were reduced by 10%, and energy expenditure, estimated using International Physical Activity Questionnaire (36), increased by 25 ± 4 MET·h⁻¹·week⁻¹. Using psychological and quality of life assessment tools, 20% of subjects with a pathological score for depression come back to normal values (Center for Epidemiologic Studies Depression Scale), whereas perceived quality of life (at baseline significantly lower vs. standard values, SF-36) reached the mean scores of healthy Italian population (37, 38). Thus, a lifestyle intervention delivered by a multidisciplinary approach significantly ameliorates body composition, metabolic control, blood pressure, cardiorespiratory fitness, energy expenditure and quality of life, while reduces depressive symptoms use and costs of drugs in type 2 diabetes. The impact of the intervention in terms of cost/efficacy and age adjusted years of quality of life needs to be documented with a prolonged follow-up.

In conclusion, exercise is a very effective therapy for DM2 patients, especially if delivered by a multidisciplinary approach that includes nutritional and psychological counselling. Respect to drug treatment, exercise therapy offers the distinctive opportunity

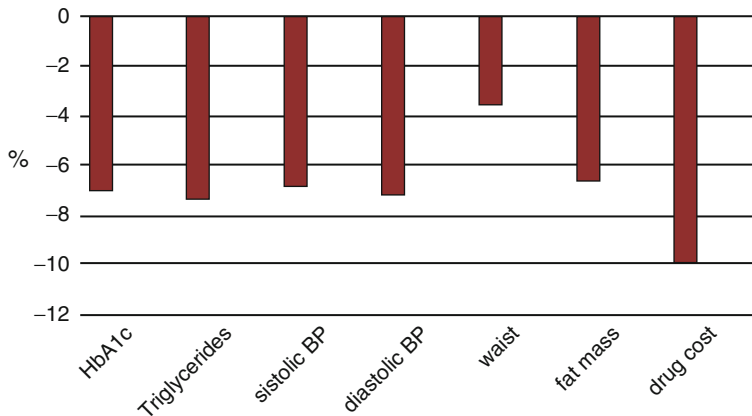


Fig. 4. Per cent reduction ($p < 0.05$) of HbA1c, plasma triglycerides, systolic and diastolic blood pressure, waist circumference, fat mass and costs of antidiabetic and anti-hypertensive drugs after the intensive CURIAMO lifestyle intervention, in 142 subjects with type 2 diabetes (age 56 ± 10 years, Mean \pm SD) (35).

of improving quality of life, mood status and the cardiorespiratory fitness (CRF) of patients. The beneficial effect on maximal oxygen transport capacity results in an increase likelihood of life span. Several prospective studies have demonstrated a significant inverse relationship between CRF and all causes risk of death in DM2 subjects (39, 40). These studies show that an improvement of 1 MET ($3.5 \text{ mL kg}^{-1} \text{ min}^{-1}$ of VO_2) reduces the risk of death by about 18% in DM2 subjects (39, 40). Since a 3 month individualized training programme can ameliorate CRF of DM2 subjects by about 2 MET (35), exercise therapy might reduce overall risk of mortality by about 40%. Considering the whole spectrum of beneficial effects of exercise in DM2 subjects, we urge to institute physical activity programmes in the prevention and cure of type 2 diabetes mellitus.

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Extreme Sports and Type 1 Diabetes Mellitus: An Oxymoron or a Growing Reality?

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INTRODUCTION

In recent years extreme sports events have gained a growing popularity throughout the world. These activities are not only popular with the adventurous elite athlete but are becoming so with even the everyday recreational sports enthusiasts. With this increasing number of persons becoming involved in extreme sports events, it is probable individuals with some serious medical conditions, which might be viewed as a contraindication to involvement in such an event, will be participants. Diabetes mellitus is one such medical condition.

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In less than a century, type 1 diabetes (insulin dependent) has turned from a rapidly fatal disease into a condition which, when adequately managed, is compatible with normal life and a full life expectancy. Ironically, before the advent of insulin, starvation diets and extreme physical exertion were the only means to prolong the lives of the young people affected with the disease. Nowadays, however, moderate recreational physical activity is recommended as part of a healthy lifestyle for those with the disease along with pharmacological treatment (1). The immense popularity and high visibility of top level sporting events, and the sizeable roster of world class athletes with type 1 diabetes (most notoriously three-time Olympian and swimming gold medal holder Gary Hall Jr.), together with improved therapeutic tools, are now setting the stage for the growing number of patients with type 1 diabetes to engage in extreme sports events. The Internet is replete with inspirational personal stories of people with type 1 diabetes achieving previously unthinkable goals, and taking part in extreme endurance competitions such as Ironman triathlons. Likewise there are a growing number of industry-supported sites promoting and organizing diabetic patients to train and participate in such events (<http://www.triabetes.org>, <http://www.teamtype1.org>). All of these factors carry the message that type 1 diabetes should not be an insurmountable obstacle to such activity. This may indeed be so; however, beyond fame and glitter, managing type 1 diabetes in the face of the demands of extreme physical activity is a formidable and serious challenge.

GLUCOSE HOMEOSTASIS DURING PHYSICAL ACTIVITY AND THE HURDLE OF TYPE 1 DIABETES

Optimal muscle function during physical activity, and thus performance, relies critically on adequate fuel supply. During the initial part of a prolonged aerobic physical activity, muscles rapidly use their glycogen depots, and thereafter muscle becomes dependent on blood glucose and free fatty acid supply as a source of energy. In the nondiabetic, the rapid rise of catecholamine secretion with the onset of exercise inhibits insulin secretion via α -adrenergic receptor stimulation (2, 3). In the setting of exercise, glucose uptake by the muscle is achieved via translocation of glucose transporter GLUT4 from the cytosol to the plasma membrane independently of insulin (4). The association of GLUT4 with the plasma membrane is inversely related to the level of glycogen depletion (5). Thus the decrease in circulating insulin does not hamper glucose influx and utilization by the muscle. At the same time, the fall in insulin alters the insulin/glucagon ratio in the portal circulation, which, combined with greater hepatic glucagon sensitivity (6), promotes augmented hepatic glucose output via glycogenolysis and later on gluconeogenesis (7). Additionally, with the rise of epinephrine and the fall in insulin, lipolysis rate intensifies, and the release of free fatty acids provides an important fuel source for the muscle (8). These coordinated responses ensure uninterrupted energy supply to the contracting muscle while preserving normoglycemia. Upon termination of exercise catecholamines decrease rapidly allowing renewed insulin secretion (together with voluntary carbohydrate ingestion) and muscle glycogen replenishing is gradually achieved due to the postexercise protracted location of

GLUT4 at the plasma membrane, and a heightened muscle glycogen synthase activity (9). This regulation of energy supply to the muscle, together with the preservation of normoglycemia during the entire duration of the event, is critically dependent on finely tuned insulin secretion regulation. Obviously, it is easy to see why this would be such a challenge in a person with type 1 diabetes.

Indeed, not being able to reduce the prevailing insulin level achieved by exogenous administration, patients with type 1 diabetes face the danger of hypoglycemia. This is worsened by the failure of counterregulatory glucagon response to hypoglycemia which is characteristic of patients with long-standing type 1 diabetes (10). Additionally, this may be compounded by the frequent occurrence of a failure to mount a catecholamine response to hypoglycemia in subjects with well-controlled diabetes, the so-called hypoglycemia-associated autonomic failure (11). Furthermore, antecedent exercise itself has been shown within hours to blunt the response to a subsequent hypoglycemia in type 1 diabetic patients (12).

Although hypoglycemia is potentially the most dreaded derangement during endurance sporting events in athletes with type 1 diabetes, it is by no means the only threat to glucose homeostasis with exercise. For example, hyperglycemia is likely to occur after short but intense anaerobic activities (e.g., sprinting) because of an extreme catecholamine secretion response which drives heightened hepatic glucose output, and impedes insulin action. In turn for the individual with type 1 diabetes, the inability to respond with an appropriate increased insulin secretion may result not only in hyperglycemia but even in ketoacidosis—particularly amidst poor general metabolic control (13).

TYPE 1 DIABETES AND EXTREME SPORTS IN PRACTICE: THE EVIDENCE

Realizing the challenge, and given the growing interest in the participation of individuals with type 1 diabetes in sports in general, several professional organizations have issued guidelines for the nutritional and medical management of this condition in recreational and competitive athletes (1, 14, 15). However recommendations for those taking part in extreme or hazardous events are still lacking. Nonetheless, a body of literature is emerging that sheds some light on the particular challenges these individuals will encounter. Furthermore, with the advent of some new avenues and technological developments these activities may be more accessible and appealing to people with type 1 diabetes.

Additionally, this accumulating evidence underscores areas of uncertainty for which well-designed studies might evolve into strategies that could minimize the risks. Unfortunately, for most of the disciplines, there are simply no data. Thus, for lack of published scientific evidence, health-care professionals asked to assist patients with type 1 diabetes who engage in such activities may have to apply their best judgment. The following sections will address some areas which have been the focus of published research on this topic. However, the discussion is delimited and excludes some of the extreme sports where there is a dominant psychological component (e.g., cliff jumping, paragliding) because of insufficient data at this time.

EXTREME ENDURANCE EVENTS: THE MARATHON PARADIGM

The first case of a person with type 1 diabetes completing a marathon run was reported in 1986 (16). This involved a 35-year-old man who had a 17-year history of insulin-dependent diabetes, and was a well-trained runner. Blood glucose concentrations were determined four times during the run and never came close to hypoglycemia levels; although, the authors mentioned a mild hypoglycemic reaction after completion of the run. Regrettably, they did not indicate the carbohydrate supply nor the insulin dose adjustment the subject had used. They concluded that it was possible for a patient with type 1 diabetes in a reasonable controlled state to complete a marathon. Subsequently, several small studies assessed the counterregulatory responses of type 1 diabetic marathon runners. By and large these studies found very similar responses to those observed in healthy subjects, with the exception of insulin levels that remained elevated in the diabetic subjects despite a pre-run dose reduction of approximately 50% (17–19). In general these studies were reassuring and helped to shape the notion that marathon running is compatible with type 1 diabetes. Furthermore, the advent of new insulin analogs is aiding in altering the dose adjustments made by the patients to prevent drastic glycemic alterations during or after such runs (20). Moreover, it has been demonstrated that continuous glucose monitoring is feasible throughout runs, and that it frequently reveals periods of unsuspected hypo- or hyperglycemia during and following the running (21). It is easy to envision how the use of such glucometer devices could help characterize the individual glycemic response, and plan future runs more appropriately. Finally, it is to be expected that integrated systems that combine a sensing device with an insulin pump fitted with a tailor-made algorithm will soon become standard practice for type 1 diabetic patients participating in such sporting events, providing added safety, and a sense of security for all.

Currently, there are no good estimates of the number of patients with type 1 diabetes who regularly engage in endurance events, but a recent Internet search reveals a multitude of sites and blogs with testimonials of individuals with type 1 diabetes who take part in marathons, triathlons, and extreme endurance events such as Ironman competitions and ultramarathons. It is thus obvious that such events are gaining in popularity among healthy individuals and the type 1 diabetic alike, making the lack of scientific guidance for diabetic athletes all the more acutely needed. Short of prospective studies that follow rigorous scientific protocols, a compilation of the current experience in the area by an international consortium of exercise physiologists, sports physicians, and trainers could provide the basis for practical guidelines and is awaited by all those facing this challenging medical condition.

ENDURANCE EVENTS IN EXTREME TOPOGRAPHIC AND ATMOSPHERIC CONDITIONS: THE CASE OF MOUNTAINEERING

An interest in the participation of type 1 diabetes individuals in mountain climbing events started to make headlines almost 30 years ago (22), with the recognition that special attention needed to be given to glycemic control under these circumstances. Since then, there have been several controlled studies that have assessed the ability of persons with type 1 diabetes to successfully achieve extreme mountaineering altitudes

(above 5,000 m) in comparison with nondiabetic mountain climbers (23–25). The first “Kilimanjaro Ireland” expedition reported a lower proportion of diabetic subjects (6/15) achieving the summit compared to controls (16/22) over a rapid (5 days) climbing ascent protocol (23). Studies performed afterwards which have allowed sufficient altitude acclimatization to occur, however, have generally shown a similar summit success rate in diabetic and nondiabetic climbers (24, 25). Likewise, the rates of acute mountain sickness symptoms development (i.e., high altitude pulmonary edema, cerebral hemorrhage, or retinal hemorrhage) are no different in diabetic compared with nondiabetic climbers. Metabolic control during mountain climbing also appears to be dependent on the level of antecedent training and the pace of ascent the diabetic climber experiences. In the rapid climbing of Mount Kilimanjaro by the Ireland expedition (noted earlier) (23), there was an increased glucose utilization due to the intense effort and the low ambient temperatures. These events in turn mandated insulin dose reductions of approximately 50% to avoid hypoglycemia, especially during the first 2 days of trekking. The altitude-induced anorexia that accompanied climbing in this study was another factor in decreasing the insulin doses, a fact that resulted in ketonuria in four, and mild ketoacidosis in two of the diabetic climbers (23). However in a study with diabetic climbers involving a longer ascent (37 days) of the Himalayan Cho Oyu peak, there was no report of hypoglycemia. In fact blood glucose rose both in the diabetic and the nondiabetic control climbers resulting in an increase in glycated hemoglobin in both groups, and a gradual insulin dose increase being required in the diabetics (24). Of note, in all the published studies there was a preponderance of men; however, it should be kept in mind that glucose utilization at high altitude differs between genders, as women tend to have reduced utilization rates (26). Finally, with mountain climbing, the challenge of maintaining near normal blood glucose is compounded by the notorious inaccuracy of glucose meters at high altitude and, or at low ambient temperatures (27, 28).

In summary, despite some inconsistencies in the reported success rates and metabolic alterations with extreme mountaineering observed in type 1 diabetic climbers, there appears to be agreement such an activity is possible in adequately trained diabetics devoid of complications provided appropriate precautions are secured (29). In contrast to marathon running or similar endurance events, extreme mountaineering remains a fairly restricted activity practiced by limited numbers of people. Nevertheless, members of climbing expeditions that include type 1 diabetic individuals should be encouraged to refer to the literature and be familiar with precautions as they prepare for their climbing experience.

SCUBA DIVING IN TYPE 1 DIABETES: ALLOWED BUT SAFE?

With approximately four million active divers in the US alone (30), and roughly one million diving certificates awarded annually worldwide by the Professional Association of Diving Instructors (PADI) (31), recreational diving is not usually viewed as an extreme sports activity. Nonetheless, with the threat of underwater hypoglycemia, it is widely considered as extremely hazardous for insulin-treated diabetic individuals who were in the past banned from the activity for many years. However, several surveys of diabetics who defied the ban, helped shape the notion that, under rigorous diving fitness

selection criteria, diving could be allowed in people with insulin-treated diabetes (32, 33). This concept was supported at the same time by several investigations attempting to assess the effect of diving on glucose levels and other parameters in type 1 diabetic patients. In one of the first controlled studies, Edge and associates monitored and compared the physical and biochemical responses of a small group of type 1 diabetic divers to those of matched, healthy divers in a hyperbaric chamber. None of the diabetic divers experienced hypoglycemia (34). In a larger and more real-life study, 40 type 1 diabetic subjects were compared to 43 sex- and age-matched nondiabetic divers while completing 555 dives over 5 days (35). To participate, blood glucose prior to diving had to be maintained at or above 80 mg/dL in the diabetics. It was monitored at 60, 30, and 10 min before the dive and immediately after the dive (a now standard protocol procedure). Although there were no symptomatic hypoglycemic reactions during or immediately after the dive, in 7% of the dives the diabetic divers surfaced with a blood glucose less than 70 mg/dL, compared to only 1% of the control divers ($p < 0.05$). In one diabetic diver, the glucose levels reached values as low as 41 mg/dL, while the lowest value recorded in the controls divers was 56 mg/dL. The dives were generally accompanied by a decrease in blood glucose of approximately 50 mg/dL in the diabetic subjects, and only 10 mg/dL in the controls. Additionally, to avoid hypoglycemia, pre-dive carbohydrate loading was carried out leading at times to significant hyperglycemia. The authors acknowledged this latter strategy could be hazardous as it theoretically increases the risk of decompression sickness.

Technological improvements have allowed the continuous subcutaneous glucose monitoring technology to be adapted to underwater performance and harnessed to aid diabetic divers' participation. Two research studies have examined this methodology and shown it to be technically feasible and beneficial. In a Swedish study (36), 12 diabetic divers and 12 healthy controls performed five dives over 3 days while connected to a continuous glucose monitoring system (proven to resist pressure conditions up to a depth of 24 m). There were no symptomatic or serious hypoglycemic episodes noted. The few low blood glucose values (< 70 mg/dL) detected, immediately before or after a dive, were found to be related to the mean blood glucose documented within the subjects in the 2 weeks preceding the study, and to the duration of their diabetes. The authors concluded that such systems could provide useful information prior to and during dives, and could add an element of safety to diving by diabetics. An Italian study, the "Diabetes Sommerso" project, assessed the feasibility of a combination of intensified group training with emphasis on diabetes management, pre-dive acclimatization, and the use of a continuous blood glucose monitoring device during immersion (37). This study of 12 type 1 diabetic subjects confirmed previous observations that, when appropriate precautions are taken, the occurrence of underwater hypoglycemia could be almost totally eliminated. Specifically, in this study, there were no hypoglycemic episodes experienced during the dives, and only four mild episodes following the dives. Interestingly, in several subjects despite reaching hyperglycemia after the dive on a number of occasions, blood ketones did not rise. Finally there was also no evidence for greater blood gas bubble formation, suggesting that hyperglycemia was not associated with a greater risk for decompression sickness development.

Furthermore, it is important to note that there does not appear to be an overrepresentation of diabetic patients among diving accident victims (38). This latter finding along with those of the few observational and prospective studies conducted allow the emergence of an overall picture which provides reassurance that diving is compatible with type 1 diabetes if strict safety precautions are implemented. Such safety precautions are included in the proceedings of the 2005 joint UHMS/DAN (Underwater Hyperbaric Medicine Society/Divers Alert Network) meeting that provides a framework for diving professionals and potential divers with diabetes (38). Nevertheless, one should keep in mind these guidelines were derived from a limited number of scientific research studies and involved only a small number of highly motivated subjects. Finally, many open questions remain to be addressed such as the effect of diving depth, and particularly that of varying water temperatures on insulin kinetics and glucose absorption. Answers to these questions will require more clinical and scientific research. Also the development of more sophisticated technologies incorporating features such as continuous glucose monitoring should be one of the next steps in making diving safer and more acceptable to people with type 1 diabetes.

EXTREME SPORTS AND TYPE 2 DIABETES MELLITUS?

This chapter was written to specifically address the issue of the challenge extreme sports pose to athletes suffering from type 1 diabetes. But of the 350 million people affected by diabetes worldwide, about 90% of them suffer from type 2 diabetes. Thus comments on this more prevalent form of diabetes are warranted and seem especially needed since the proportion of people in the world with type 2 diabetes is increasing dramatically (a phenomenon which parallels rapidly spreading worldwide epidemic of obesity). Furthermore regrettably, one of the fastest growing segments of the population succumbing to the development of type 2 diabetes is children and youth. For example, in 1999–2000 approximately 9% of US adolescents between the ages of 12 and 19 years had prediabetes/diabetes; according to a recent survey the rate had increased to 23% by 2007–2008 (39). This obviously implies a greater proportion of the adult population will have diabetes in the future.

Regular physical activity is recommended as part of the therapeutic approach to type 2 diabetes in adults. Given the understandable reservation to resorting to pharmacological solutions, in children even more emphasis is placed on physical activity as a way to prevent or treat diabetes in this population. It is reasonable to expect that a fraction of these individuals might comply to the point of embracing moderate to vigorous physical activity as part of their lifestyle. While there is a moderate amount of literature showing that physical activity improves the metabolic control in youth with type 2 diabetes, there is essentially no research done on the challenges ahead for individuals with type 2 diabetes who might aim to go beyond recreational physical activity and pursue competitive sporting activities.

Conceptually, at the level of counterregulation and glucose homeostasis, the problem of not being able to reduce prevailing insulin levels at the time of exercise faced by type 1 diabetics is obviously less of a concern in the majority of patients with type 2 diabetes who do not use insulin (i.e., type 2 diabetes is defined as the non-insulin-dependent

form). Likewise, failure to mount a glucagon response to hypoglycemia, so common in type 1 diabetic patients, is also likely of minor concern in type 2 diabetic. On the other hand, the potential hyperglycemic effect of the hormonal response to strenuous exercise in patients with type 2 diabetes, who may augment their insulin secretion to cope with the increased demand, but also suffer from insulin resistance, has not been adequately studied. To date there have been a limited number of studies examining counterregulation and the glycemic response to exercise in subjects with type 2 diabetes (40). It has been suggested that in the post absorptive state, moderate exercise leads to an exaggerated glucose reduction, while vigorous exercise is accompanied by an increase in blood glucose in type 2 diabetics. In contrast, both moderate and vigorous exercise tended to decrease blood glucose in the postprandial state following either a mixed meal, or a high fat meal. These occurrences were accompanied by reduced insulin and C-peptide concentrations (evidence of improved insulin sensitivity) and amelioration in postprandial triglyceride and chylomicrons clearance. Recently, the continuous glucose monitoring technology was used to study the glycemic response of patients with type 2 diabetes who were performing a single bout of either short (10 min) high-intensity interval training (41), or 45 min of endurance or resistance exercise training (42), in the postprandial state. All these studies concurred in their findings showing reduced glucose excursions in the 24 h following the exercise challenge.

Other than occasional anecdotal reports (21), there appears to be essentially no systematic studies of the responses of type 2 diabetics to endurance-type extreme sporting events. In the absence of definitive scientific-medical guidance, type 2 diabetes patients training for such endurance events are now turning to forums on the Internet with a multitude of questions ranging from appropriate nutrition, medication adjustment, and precautions needed in the presence of their medical complications. At the same time, much like in the case of type 1 diabetes, the Internet is filling up with success stories of people who, with type 2 diabetes, are participating in triathlons and marathons (43) alongside with other less successful stories (44). Clearly this “mixed-bag” of commentary generates much confusion in the diabetic population seeking advice. Amidst this confusion, the lack of evidence-based professional guidance is all the more acute. Since continuous glucose monitoring technology is now possible (not only throughout endurance events, but also days after the completion of the event), this procedure could be utilized to systematically assess the glycemic profiles of type 2 diabetics who take part in such endurance events and related activities. Such studies would provide valuable information and shed light on this area. Until this information is available, health-care professionals will only be able to rely on very limited data and common sense when providing recommendations to their type 2 diabetic patients who wish to engage in intense physical activity.

CONCLUSIONS

Thanks to a better understanding of the challenges posed by intense physical activity on metabolism and glucose homeostasis, and with the help of currently available therapeutic and monitoring technologies, taking part in extreme sporting events is no longer beyond imagination for patients with type 1 diabetes. As the popularity of such events

is soaring, the number of amateur-recreational sports enthusiasts with type 1 diabetes who engage in such activities is expected to keep growing. Health-care practitioners will therefore be increasingly solicited to provide relevant, sports-specific advice to their patients. This chapter attempted to review the available scientific evidence on participation by such patients and to highlight the areas where data are lacking, with a special emphasis on type 1 as well as some insight to type 2 diabetes. As can be deduced from the material presented, the available scientific evidence is limited and there is a need for much further research work.

It will be a critical task for clinical investigators in years to come to fill in the information gap and provide clinicians the scientific basis needed to help their diabetic patients overcome the hurdles of extreme sports participation. In the mean time, it is suggested that sports organizations along with their health-care advisors, make recommendations based on the best currently available evidence (as well as the personal experience of the health-care provider and the diabetic patient).

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27 The Endocrine System in Overtraining

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INTRODUCTION

An athlete's main goal is to perform well and presumptively win competitions. As such, most athletes are never satisfied if they are not performing to their best and obtaining personal records. Because of this performance drive, athletes can train extensively hard in spite of everything else happening in their lives, to the point of overtraining. Overtraining has been defined as an imbalance between the training stress, including other life stresses, and the recovery or regeneration process (1–5). While scientists and sports medicine professionals know that overtraining occurs in athletes, most of the knowledge and/or information concerning it comes primarily from personal experience, case studies, and short-term experimental investigations, none of which because of methodological limitations have added substantially to the scientific literature as will be seen during this chapter. When an athlete becomes overtrained, it will usually take months if not years to recover (6–8); thus it would be highly unethical to deliberately overtrain an athlete just for science sake; therefore, scientists have very little long-term training information (8). In spite of this lack of direct scientific information, much work has been performed to examine, predict, and prevent overtraining in athletes, and this chapter will examine some of this work especially as it relates to the endocrine system and the current proposed mechanisms.

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In 1936, Selye (9) described the general adaptation syndrome (GAS) which proposes how individuals will adapt to stress if it is not too great. According to Selye (9) the GAS has three phases: an alarm phase (where the body recognizes a stressor and responds to it), the resistance phase (where physiological adaptations occur which results in an enhanced level of homeostasis), and the exhaustion phase (where adaptation is not possible because the body is overwhelmed, and if prolonged enough, death is the ultimate result). While Selye was not necessarily addressing exercise training in this stress model, sports medicine professionals have used the GAS to describe training and the resultant adaptations. Accordingly, when a short-term stressor is placed on the body, super-compensation occurs as the disturbance in cellular homeostasis leads to adaptations, and the athlete's exercise performance is enhanced (10, 11). However, if too great a stress or long-term stress occurs with insufficient recovery, then performance decrements occur and overtraining results (3, 8, 12–14). A small window probably exists as to when the stress and recovery are optimal, and a large window includes too little stress for the recovery (i.e., undertraining or detraining resulting in nonoptimal performance) and also too much stress and too little recovery (i.e., overtraining, also resulting in non-optimal performance).

Short-term overtraining, often called overreaching, is generally considered to be an appropriate component of training, typically lasts a few days to a couple of weeks (8, 15–18), and results in fatigue probably due to insufficient metabolic recovery and a temporary reduction in exercise performance (13, 18, 19). However, as the typical response of an athlete and coach to reduced performance is that the training program was not sufficient enough (i.e., that undertraining and/or detraining has occurred) and greater training load must occur to enhance exercise performance, overreaching can be expanded to overtraining due to lack of recovery and too much work. As very small differences in exercise performance (i.e., speed of running or skating) generally leads to great differences in exercise performance, athletes are always trying to get that last little edge to enhance their performance time. To illustrate this, Snyder and Foster (20) examined speed skater velocity at the 1988 Olympics and observed that there was only a 0.3% difference in average velocity between all gold and silver medal performances and the mean difference between all first and fourth place finishers was only 1.3%. This difference in velocity covered races ranging from 0.5 to 15 min in length. The overtraining syndrome has been shown to result in performance decrements of 0.7–15% (21) and to be similar to the exhaustion stage of Selye's GAS model (16, 22). The incidence of overtraining has ranged from approximately two-thirds of elite distance runners (3, 16, 23, 24) to 20–50% of swimmers, basketball, soccer, individual and team sport players (3, 21, 25–28), and military personnel (29).

Unfortunately, little is known about the adaptation process and the recovery needed to maximize physiological adaptations and thus exercise performance (19). What may work for one athlete may not work for another, as everyone responds to stress in a different manner (9): One athlete may adapt to a training program, while another may have performance decrements due to overtraining on the same program (3, 13, 30). The border between training adaptation and performance enhancements and overtraining and performance decrements is not concrete, and probably involves other factors (or stresses) than just exercise training (3, 13). In spite of this scientists have tried to identify markers of performance or physiological function that would enhance our ability

Table 1
Typical symptoms of overtraining as shown by their prevalence in the reviewed literature

Impaired exercise performance	Decreased reproductive hormone levels	Decreased maximal oxygen uptake
Increased ratings of perceived exertion (RPE)	Normal submaximal and decreased maximal lactate levels	Enzymatic markers in blood
Increased incidence of infections	Elevated basal metabolic rate	Decreased maximal power output
Change in appetite	Prolonged recovery	Increased respiratory rate
Decreased muscular strength	Heart rate recovery	Changes in body weight
Blood glucose levels during exercise	Easily and chronically fatigued	Decreased efficiency of movement
Mineral depletion	Loss of competitive desire	Resting heart rate
Feelings of depression	Altered blood pressure	Increased irritability
Disturbances in mood	Exercising heart rate	Changes in sleep patterns
Lack of motivation	Muscle soreness	Loss of coordination

to train athletes (19). As such scientists have identified performance decrements, exhaustion, changes in mood state, reduction in lactate threshold and workload at 4 mM lactate, maximal exercise reductions, and competitive incompetence among others (see Table 1) as signs and/or symptoms of the overtraining syndrome.

However, many problems became apparent with the research and the literature concerning overtraining of athletes. Initially, all excessive exercise training was termed overtraining, staleness, or burnout; however, with some of these, recovery occurred quickly (days), while with others recovery took much longer (weeks to months, possibly years). Thus overreaching, an appropriate form of training with sufficient recovery, was defined as excessive exercise training from which an athlete could recover in days to a few weeks, while overtraining was excessive exercise training which required a much longer period of recovery (31). However, unless one gives an athlete sufficient time to recover, one does not know whether the athlete is overreached (probably a positive training response) or overtrained (probably a negative training response)—as distinguishing between the two is very difficult (30). Therefore, in 2000 Budgett et al. (23) proposed that the syndrome be called the “unexplained underperformance syndrome” (UPS) as the term overtraining syndrome implied causation. Then in 2006 the European College of Sports Science published a position statement which defined overreaching and overtraining (8). As such, overtraining is the performance of strenuous exercise training (thus a verb) that leads to overreaching or the overtraining syndrome. Overreaching then can be short-term or functional overreaching as would be used in a periodized training cycle or nonfunctional (or extreme) overreaching, that is moving in the direction of the overtraining syndrome and requiring more recovery time (8, 32, 33). The definitions bring to light that the physiological adaptations from training through overreaching (functional to nonfunctional) to the overtraining syndrome occur on a continuum, which is fluid and affected not just by exercise training but by other stresses

as well. Overreaching is generally perceived to be peripheral fatigue, while overtraining syndrome is more of a central fatigue (3, 19, 34), and some have suggested that the symptoms can be greater in athletes who are overreached than in those who have the overtraining syndrome (8). Due to the time necessary to recover from the overtraining syndrome and the ethical nature of the research area, most studies that have examined the area of overtraining more than likely have only functionally or at the most nonfunctionally overreached their subjects (8, 32).

The realization that overtraining probably has not been examined experimentally led Halson and Jeukendrup (32) to ask "Does Overtraining Exist?" and in fact they state very compelling arguments as to why it may not. Their arguments include that most studies that have examined overtraining have not shown performance decrements, and while it would seem intuitive that if a continuum occurs from training to overtraining, that characteristics would change proportionally to training, this does not always happen. In addition, most studies do not have any baseline measurements for comparative purposes, and great differences in the methods were used when performing the research. From this, Halson and Jeukendrup (32) concluded that not much could be stated conclusively about overreaching and the overtraining syndrome, due to a lack of true experimental studies in the area. These points and difficulties must be kept in mind when examining the overtraining literature. Also, since few truly experimental studies concerning overtraining and the overtraining syndrome are available in the literature, this chapter uses more review articles than might be a normal practice to examine the current state of the body of knowledge on the topic.

ENDOCRINE FUNCTION AND THE OVERTRAINING SYNDROME

As the endocrine system is very involved in physiological adaptations and recovery to stress, much effort has been placed in examining hormone levels during different periods of training. The results of this work are two hypothesized mechanisms by which endocrine function affects exercise performance and may lead to the overtraining syndrome: sympathetic/parasympathetic imbalance and neuroendocrine dysfunction.

Since 1959, Israel (34) and later others (3, 13, 16, 19, 35, 36) have discussed two forms of overtraining: the basedowoid and the addisonoid forms. While strict interpretation of these terms would indicate that thyroid hyperfunction (morbus Basedow) and adrenal hypofunction (morbus Addison) would occur, neither the thyroid nor the adrenal glands have been solely shown to be directly involved in the overtraining syndrome. Rather, it is hypothesized that the sympathetic system (basedowoid) is activated during the early stages of overtraining, while in later stages of overtraining, the sympathetic system is inhibited and the parasympathetic system (addisonoid) predominates (13, 22). The sympathetic form has also been associated more with high-intensity explosive type sports such as sprinting, jumping, and throwing, while the parasympathetic form has been thought to be more prevalent in distance athletes (4, 6, 13, 16, 19, 21, 36). While initially presented as two separate syndromes, current thinking is that they are both part of a continuum which extends from training to overreaching to the overtraining syndrome (2, 13, 16, 19, 21, 35), with the sympathetic form occurring initially, then the sympathetic system is inhibited, leading to the parasympathetic form

(6, 19). As such, catecholamine levels have been examined in overtrained athletes with decreases (16, 37, 38) and increases (3, 6, 21, 25, 39) or no change (40) observed. Methodological problems (capillary vs. venous blood, half-life time, time of day, relationship to exercise, etc.) and intra-individual variability seem to cause this conflict in the results and leave the use of catecholamine levels, and quite possibly other hormone levels as well, problematic for the determination of the overtraining syndrome (8, 38, 41, 42). Similarly, as free catecholamine levels have been used as an indicator of overtraining, especially the sympathetic nervous system activity, plasma levels typically only reflect an acute level (e.g., response to exercise), while 24-h urine levels would give an indication of average activity for the day (4); thus the collection period greatly affects the results and their interpretation. Finally, the type of exercise training may affect the analysis as it has been suggested that volume-related overtraining may affect hormone levels more than intensity-related overtraining (40, 43).

Under appropriate conditions, exercise training leads to adaptations which stabilize the pituitary-adrenocortical system by lowering resting levels of the stress hormones (2, 13). However, excessive exercise training has been hypothesized to lead to neuroendocrine overload, which may result in any number of the overtraining syndrome symptoms (2). Thus, hypothalamic-pituitary dysfunction has been hypothesized with overtraining to disrupt the balance between anabolic (i.e., testosterone) and catabolic (i.e., cortisol) hormones, and therefore may affect/prolong recovery (13, 19, 44).

Adlercreutz et al. (45) suggested monitoring the ratio of testosterone to cortisol as a means of examining this anabolic/catabolic balance in the athlete. Levels used to indicate an overtrained state were a decrease of 30% in the testosterone/cortisol ratio or values <0.035 (45). While some athletes have been identified with this technique (29, 45–48), many others have been observed to be overtrained by having met the specified criteria, yet with no change in the testosterone/cortisol ratio (12, 18, 38, 44, 49–52). Banfi and Dolci (53) observed that the ratio of free testosterone/cortisol was very useful in monitoring professional soccer players, but the criterion values used, while decreased from the control period, were much greater than the 0.035 proposed by Adlercreutz et al. (45). Likewise, Hoogeveen and Zonderland (41) showed decreased levels of the testosterone/cortisol ratio in heavily trained cyclists, but not to the levels proposed by Adlercreutz et al. (45). Interestingly, Lane et al. (48) showed in cyclists during heavy training that low daily dietary carbohydrate intake can induce a significant and substantial ($>40\%$) reduction in the free testosterone/cortisol ratio in as little as 4 days.

As such, cortisol levels in relation to the overtraining syndrome have been shown to be both variable (54) and equivocal, with investigations showing no change (12, 15, 38, 44, 48–52, 55, 56), increases (14, 41, 46–48, 57), and decreases (14, 42, 54, 58) in the values, and thus cortisol by itself seems to be a poor marker of overtraining (54).

Finally, while many studies of heavily trained athletes have shown decreased levels of testosterone (29, 35, 41, 46–48, 50, 55, 59), not all studies have reported similar results (12, 35, 38, 44, 49, 51, 52, 57). Even though regulation of testosterone levels is primarily controlled by the hypothalamic-pituitary axis, low levels of testosterone in overtrained males could be due to central (hypothalamic, pituitary) and/or peripheral (i.e., reduced gonadal blood flow and/or adrenal overload) mechanisms.

For years, altered reproductive function has been known to occur in female athletes (60–64), affecting anywhere from 6 to 79% of the athletes (63). Hypothalamic-pituitary dysfunction has been one of the mechanisms that has received support for the development of amenorrhea in female athletes (60–62, 64). While the overtraining syndrome has not necessarily been associated with amenorrhea in female athletes (14), excessive training, especially in distance runners, has been discussed as well as and in combination with chronic restricted and/or inadequate caloric intake (62–64). Reproductive function and/or dysfunction in males is much more difficult to observe than that in females as the most overt symptoms would include decreased libido and sperm count (59, 65).

Many other hormones have been examined in the hopes of providing a diagnostic tool for determining the overtraining syndrome. As many of the symptoms of the overtraining syndrome are similar to central fatigue, 5-hydroxytryptamine (5-HT) has been examined and/or indirectly assessed through a related hormone—prolactin (66). Hackney et al. (65, 67, 68) and Bedgett et al. (66) observed that plasma prolactin release was higher in overtrained athletes than in well-trained athletes, and thus these athletes may have greater 5-HT receptor sensitivity. Resting and acute post-high-intensity resistance exercise overtraining shows no changes in growth hormone levels (35). The hormone leptin is known to regulate energy balance and suppress appetite (43, 69, 70); thus it could also be a component of the overtraining syndrome. However, plasma leptin levels were not changed with short term (8 days) strenuous training nor were changes in leptin related to changes in plasma cortisol (which increased) and changes in the testosterone to cortisol ratio (which decreased) (47). Changes in plasma leptin, however, were significantly related ($r=0.596$) to decreases in serum testosterone (47). Baylor and Hackney (71) did report reduced leptin levels in some female rowers undergoing functional overreaching, and this change was related to reductions in triiodothyronine (T_3). Since the testosterone/cortisol ratio has not been shown to be extremely reliable, Atlaoui et al. (54) examined the 24-h urinary cortisol/cortisone ratio in elite swimmers and observed that the ratio was related to exercise performance and tracked changes in the training program. More research with this ratio may be warranted.

The exercise performance implications of the endocrine system changes that occur with overtraining are incompletely understood, as discussed above. Some of the problems presented could be methodological. Others could be that the hormone levels of each athlete are so individualized as is the athlete's response/adaptations to their exercise program that consensus information is not likely to occur. Food intake and composition can also alter the resting concentrations of a number of hormones as do stress and diurnal and seasonal variations. The resultant conclusion from examining the literature relative to the overtraining syndrome is that no one hormone can be tested to confirm or refute the occurrence of the overtraining syndrome; thus single blood tests are not of value in determining if the overtraining syndrome has occurred or not.

While single blood tests for hormone levels have not been found to be beneficial, Meeusen and colleagues (5, 7) have tested a two-bout exercise protocol that has shown significant differences between control, overreached, and athletes with the overtraining syndrome. As one of the symptoms of overreaching and the overtraining syndrome is fatigue and affected athletes can typically start a performance or practice at normal pace but then have to reduce the pace due to the fatigue, Meeusen and colleagues (5, 7)

hypothesized that having athletes perform two maximal exercise tests would distinguish between the different groups of athletes. To this end, a testing protocol was devised which involved the subjects performing two incremental exercise tests to exhaustion 4 h apart as might occur when subjects perform two workouts in a day. Blood samples were then collected before and after each exercise bout and analyzed for hormone levels. During the first exercise test the appropriately training athletes and the overreached athletes had similar results both in respect to exercise performance and hormonal responses (5). However, exercise performance during the second exercise bout was decreased 3% in the appropriately trained subjects, 6% in the overreached subjects (following a training camp where training volume was increased 58%), and 11% in the subject who reported to the laboratory with the overtraining syndrome (5). Hypothalamic-pituitary hormone levels during the second test were likewise different between all three groups, with the overtrained athletes' hormone levels not increasing (5). During a second study ten athletes who had decreased exercise ability performed the two-bout exercise protocol (7). Retrospectively, five of the athletes were classified as nonfunctional overreaching, and five of the athletes were classified with the overtraining syndrome (7). While exercise performance was not different between the two groups of subjects, following the second exercise bout, ACTH and prolactin levels were much higher for the nonfunctional overreaching athletes than for the athletes with overtraining syndrome (who showed small or no increases in these hormone levels) (7). No appropriately trained athletes were included in this second investigation. Collectively, the results suggest that due to the fatigue associated with overreaching and the overtraining syndrome, the two-bout exercise protocol may be useful as a monitor of the two. Unfortunately, the group differences were only distinguishable after the second exercise bout to exhaustion, and performance in just one bout of exercise was not beneficial in classifying the athletes. Thus, both exercise bouts seem to be needed for the protocol to be diagnostic. However, since the two tests to exhaustion were performed within a very short period of time (i.e., 4 h), repeated performance of this test throughout a training season would need to be built into the training program regime, or if not it could add excessively to the exercise training performed.

OTHER HYPOTHESIZED MECHANISMS OF THE OVERTRAINING SYNDROME

Recently, two other mechanisms have been proposed related to the overtraining syndrome; one of these is the tissue trauma theory proposed by Smith (72, 73). Smith (72, 73) reviewed the current hypothesized mechanisms of the overtraining syndrome (such as was done above) and then proposed that since none of these mechanisms totally explained the overtraining syndrome, possibly these mechanisms function in response to trauma to the tissue. Hence, trauma to the muscle, skeletal, and/or joint systems could be the initiator of the overtraining syndrome. Exercise training and competition are known to result in stress/injury of the tissue as part of the adaptive process (72, 73) as put forth by Selye (9) many years ago. This trauma could be due to the eccentric nature of the exercise, the increased energy needs, and/or ischemia, etc., which would result in mild inflammation as adaptation occurred. With insufficient recovery any of these initial adaptive occurrences

could lead to a decrement in performance, the single most recognized sign of overtraining. Thus, Smith (72, 73) hypothesized that exercise-induced microtrauma leads to local acute inflammation which results in the release of cytokines. With increased training (high volume and/or high intensity) and insufficient recovery, the local acute inflammation is perpetuated to local chronic inflammation and the enhanced level of cytokines released results in activation of the circulating myocytes. The activated myocytes then produce large quantities of proinflammatory cytokines, which leads to a systemic immune/inflammatory response. The systemic inflammation is then proposed as the central cause of the overtraining syndrome. The primary cytokines proposed to be involved with the overtraining syndrome are IL-1b, TNF-a, and IL-6, though little research has been performed with the overtraining syndrome and the cytokine levels (72, 73). The results of the functions of these cytokines (e.g., mood change, loss of appetite, altered hormone levels—decreased testosterone and increased cortisol, fatigue, decreased muscle mass, and increased infection) are for the most part very similar to the symptoms of overreaching and the overtraining syndrome, thus the proposed mechanism. More work needs to be performed in this area to solidify this hypothesis, but it is interesting to note that elevated prolactin is associated with proinflammatory mediators, and that previously mentioned studies have reported increased prolactin in overtraining athletes (40, 46, 66, 68, 74).

Morgan et al. (24) initially drew the parallel between altered mood state and overreaching/the overtraining syndrome; however Armstrong and VanHeest (75) recently connected the two more thoroughly. Armstrong and VanHeest (75), in examining both the overtraining syndrome and major depressive disorder literature, observed that both share common brain structures, immune responses, and endocrine pathways. Hence, they proposed that the two shared a very similar mechanism. Morgan et al. (24) previously had observed that up to 80% of athletes with the overtraining syndrome had significant mood changes and elevated levels of psychological depression. Part of this mechanism could be that both depression and the overtraining syndrome have a dose-response relationship with stressful events. Likewise, hypothalamic-pituitary dysfunction and an enhanced parasympathetic activity have been observed in depressed patients (75). While many similarities exist, Armstrong and VanHeest (75) strongly recommend that further research be performed in this area before antidepressant medications are used with athletes who have the overtraining syndrome.

TRAINING CONSIDERATIONS TO PREVENT THE OVERTRAINING SYNDROME

A decrement in exercise performance seems to be the only diagnostic standard of overreaching and the overtraining syndrome (1, 8, 18, 32, 66, 76). Reduced performance ability is likely related to increased fatigue, but what causes the fatigue is not known (40) though it may be related to tissue trauma and the activity of the cytokines in response to inflammation as described by Smith (72, 73). Therefore, performance testing is probably necessary to monitor fatigue and reduced performance, though the type of test and its duration is currently not known (8, 32, 75).

Proper exercise training planning is critical in the prevention of overreaching and the overtraining syndrome, as no one marker or measure has been observed to be a direct indicator (13, 14, 16, 21, 22, 75–78). Acute overtraining/overreaching has been studied

by increasing training load (either volume or intensity) for 2–4 weeks, but this is not nor should it be typical of normal training (78). Similarly, the risk of overtraining increases after 3 weeks of intensified training or prolonged monotonous training (4). Therefore, appropriately planned training programs using periodized training techniques (13, 30, 52) and varied training loads to avoid monotony in training (4, 16, 35, 77) are recommended. With periodized training progressive increases in training intensity and/or volume occur for a given period (usually weeks), then a recovery period occurs. The pattern then continues with progressive increases and recovery planned so that stress and adaptation occur without overreaching or the overtraining syndrome (79, 80). While periodized training is highly recommended to insure that adequate recovery occurs, very little experimental research has been conducted on the results of periodized training. With horses progressive increases in training loads were tolerated as long as sufficient recovery time (i.e., moderate intensity endurance runs) occurred following the high-intensity training days. When the high-intensity training days were not followed by recovery days, the horses were observed to have symptoms of overtraining (76). In humans, as full recovery from an intense exercise bout may require at least 48 h (2, 18), planned recovery is necessary in a training program to insure that excessive training does not occur.

One method that might be useful in monitoring training is examination of the blood lactate/rating of perceived exertion (RPE) ratio (81). Fatigue associated with overreaching and the overtraining syndrome has resulted in higher submaximal RPE scores (40). Conversely, while overreaching and the overtraining syndrome have not been shown to alter submaximal blood lactate levels, maximal exercise blood lactate levels have decreased (7, 20, 58, 81). Thus, decreases in the blood lactate/RPE ratio have been shown to occur during periods of overtraining (40) and may be a simple way for coaches and athletes to monitor training and recovery needs. However, further work is necessary on this potentially useful biomarker.

Monitoring hormone levels of athletes during training is expensive, impractical, and probably of questionable benefit (13, 18, 21, 38, 53, 66, 78, 82). Also, as hormone values generally are different between athletes, obtaining a single diagnostic value is very difficult (82). At present, accessing the physiological responses to repeated maximal bouts of exercise, either on 1 day or over multiple days, is probably the most helpful in diagnosing overtraining syndrome (5, 7, 82) but also potentially adds to the excessive stress of the exercise training program.

As suggested from the literature on amenorrheic female athletes, future work on the causes of the overtraining syndrome may necessitate examining food intake (30), specifically the amount of carbohydrates (48, 58, 83, 84) and protein (84–86) consumed. Work on inflammation and the role of the cytokines as proposed in the theory of Smith (72, 73) also would seem to be an appropriate future course of research.

As the difference in performance time between a gold medal and not medaling is very small in most cases, most athletes are willing to train excessively to the point of overtraining in order to prepare for competition, as most would rather become overtrained and fail in their competition due to that than go into the competition not sufficiently prepared. Thus coaches and athletes have to learn to adjust training when increased physical and/or emotional stress is apparent to maximize the training adaptations while reducing the probability of overreaching and/or the overtraining syndrome occurring.

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28 Hormones as Performance-Enhancing Agents

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INTRODUCTION

The word doping is probably derived from the Dutch word *dop*, the name of an alcoholic beverage made of grape skins used by Zulu warriors to enhance their prowess in battle. The term became current around the turn of the twentieth century, originally referring to the illegal drugging of racehorses. The practice of enhancing performance through foreign substances or other artificial means, however, is as old as competitive sport itself.

Today, the use of doping agents is no longer restricted to competing athletes; young adolescents in schools and noncompeting amateurs also use them. Recent studies show that approximately 1% of the entire population in the United States of America (USA) and Sweden use androgens (2). Bodybuilders and non-athletes use androgens to increase muscle mass and “to look better.” One study about androgen users in the USA demonstrated that approximately 80% were recreational athletes and bodybuilders (3).

It is apparent that many athletes take a “cocktail” of drugs making it virtually impossible to denote any single agent as causing a specific outcome or adverse event.

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Despite very little evidence of beneficial effect of most doping agents and significant potential adverse consequences, many athletes and coaches continue to search for ways to mask their use to avoid detection or to start using new agents that might be more difficult to detect or not tested currently.

INSULIN

Physiology

Insulin is a 51-amino acid peptide hormone synthesized and secreted in a pulsatile fashion from the beta cells of the islets of Langerhans in the pancreas into the portal vein. Pancreatic β -cells secrete 0.25–1.5 units of insulin per hour during the fasting state, accounting for over 50% of total daily insulin secretion, with the remainder being meal-related.

Glucose is the principal stimulus for insulin secretion, although other macronutrients and hormonal and neuronal factors also may alter this response.

Insulin stimulates the uptake of glucose into muscle and fat by making available an increased number of glucose transporters (Glut-4) at the cell membrane, thus increasing the flux of glucose to the interior of the cell. However, its main effect is inhibitory to lipolysis, glycolysis, gluconeogenesis, ketogenesis, and proteolysis (4, 5).

Insulin regulates hepatic glucose output by inhibiting gluconeogenesis and promoting glycogen storage. Similarly, in muscle cells, insulin-mediated glucose uptake enables glycogen to be synthesized and stored, and for carbohydrates, rather than fatty acids or amino acids, to be utilized as the immediately available energy source for muscle contraction. The use of insulin following a bout of exercise may replenish glycogen and ATP stores more quickly than rest or feeding alone.

Although insulin stimulates the uptake of amino acid into cells and promotes protein synthesis in a range of tissues at high insulin concentrations, the major action of insulin is to inhibit the breakdown of proteins (proteolysis), which occurs at lower insulin concentrations.

Rationale

The primary source of carbohydrate during exercise derives from muscle glycogen stores. The greater the amount of glycogen stored, the longer one should be able to exercise. In addition, insulin leads to the accumulation of amino acids in muscle and theoretically additional substrate for protein synthesis and increase in muscle mass.

Performance Enhancement

The theoretical performance benefits of insulin are mediated by an increase in muscle glycogen storage and the inhibition of proteolysis but have not been demonstrated in clinical or scientific trials. That does not deter athletes from injecting insulin and its analogues, likely because insulin is but one of a “cocktail” or drugs along with training to enhance anabolic activity. In addition one must consider more rapid recovery from training and competition, but again there are no data to show this effect.

Adverse Events

The most common adverse effect of insulin use is hypoglycemia. Most athletes who abuse insulin are likely adept at balancing the ingestion of carbohydrate when injecting rapidly acting insulin analogues.

Another problem associated with insulin is weight gain, although most competitive athletes are accustomed to diet and follow training regimens that allow them to have a strict control over weight gain.

Detection

Regular and some short-acting insulins are very difficult to detect, because of the very short time one can note a high concentration of insulin (half-life approximately 4 min) or a high ratio of insulin to C-peptide, as has been used to distinguish subcutaneous regular insulin use from insulin-stimulating medications such as the sulfonylureas. These agents drive the endogenous secretion of proinsulin, an equimolar compound composed of insulin and its connecting C-peptide. Both of these analytes may be detected with commercially available immunoassays from blood samples (6).

Insulin analogues may be detected by chromatography followed by mass spectrometry given their sensitivity and specificity to determine precise molecular weights and amino acid “tags” of peptides and proteins. These have proved useful to detect and identify synthetic insulins or their degradation products (7).

HUMAN GROWTH HORMONE

Physiology

The main isoform of hGH is a 191-amino acid, 22-kDa peptide with a significant amount of a 20-kDa splice variant form. GH functions as a major metabolic hormone in the adult by optimizing body composition and physical function and regulating energy and substrate metabolism. Metabolic actions of GH also closely interact with those of insulin in the control of fat, glucose, and protein metabolism during the fasted and fed states. GH promotes fat metabolism by enhancing lipolysis and fatty acid oxidation (8, 9). This function is particularly important during the fasted state, when GH secretion is enhanced, resulting in the partitioning of fuel utilization toward fat and the sparing of protein. GH exerts profound effects on glucose metabolism both directly and indirectly by antagonizing insulin action. GH enhances glucose uptake and utilization in cells: this is referred to as its insulin-like effects. At the whole-body level, GH suppresses glucose oxidation and utilization while enhancing hepatic glucose production, potentially for increased use of glucose that is non-oxidative in nature (10). Protein anabolism is a signature property of GH that reduces urea synthesis, blood urea concentration, and urinary urea excretion. GH also acutely stimulates amino acid uptake and incorporation into protein in vivo (11).

hGH is secreted in a pulsatile fashion every hour or two and has significant peaks approximately 90 min after the onset of deep sleep and within minutes of completing a bout of exercise. Different stimuli affect the frequency and magnitude of the GH pulses.

Exercise and physical stress increase GH levels (12). Emotional deprivation is associated with suppressed GH secretion, and attenuated GH responses to provocative stimuli occur in endogenous depression (13). Nutrition plays a major role in GH regulation. Chronic malnutrition and prolonged fasting increase GH pulse frequency and amplitude (14). Obesity decreases basal and stimulated GH secretion (15).

Rationale

rhGH is a popular drug of abuse in athletes because of its anabolic and lipolytic properties. Its detection is difficult, and it is prevalent in the sports environment. Abusers believe that rhGH will increase their muscle mass, muscle strength, and aerobic and anaerobic exercise capacity and also may help to recover faster after connective tissue sports injuries. All are considered by the athlete to enhance his/her performance.

Performance Enhancement

rhGH is quite actively being abused by athletes, up to 5% of US high-school students have tried growth hormone as an anabolic agent (16).

Despite its popularity, there is no conclusive evidence that rhGH improves athletic performance (17).

A recent study in recreational athletes demonstrated that in the short term, growth hormone significantly increased lean body mass, reduced fat mass, and improved sprint capacity, but not strength, power, or endurance (18).

The mechanisms through which GH acts on exercise performance are more complex than the simple increase in lean body mass. For instance, GH stimulates erythropoiesis under various conditions and exerts significant cardiovascular effects, increasing plasma volume and peripheral blood flow and enhancing left ventricular stroke volume and cardiac output (19, 20). All these factors may contribute to improved aerobic capacity. Evidence suggests that GH therapy alone, in the absence of some form of exercise program, may increase lean body mass, but not functional capacity, indicating that training may have to be combined with GH replacement in these patients to increase physical performance.

It is unlikely that an athlete uses rhGH in isolation, thus making it quite difficult to define its exact role to alter athletic performance. There is however one very well-controlled study that shows an effect on strength in a group of well-defined abstinent steroid abusers using rhGH (21). Other studies also show a small increase in muscle strength (22, 23).

Adverse Events

The most common side effects of rhGH replacement include edema, arthralgias, and myalgias. Although GH antagonizes insulin action, the risk of developing hyperglycemia is very low. Other adverse effects including sweating, fatigue, and dizziness have been reported after rhGH administration in healthy individuals. The severity of these side effects may be augmented in those athletes who use "cocktails" combining rhGH with anabolic steroids, which could have synergistic effects, such as fluid retention and interaction with cardiac function (24, 25).

Detection

Detection of rhGH may be accomplished in blood samples by two different strategies—the isoform and the marker methods. The former is capable of detecting the use of rhGH for approximately 24–36 h and the latter approach for up to approximately 2 weeks (26).

- (a) The GH-isoform method is based on the ability of certain assay antibodies (monoclonal) to detect only the most common isoform (22 kDa) which is identical to the recombinant molecule (also noted as “rec”) and others to detect most of the common isoforms of pituitary hGH which is composed of 45–55% 22 kDa and the rest multiple different isoforms (antibodies also noted as “pit”). Each burst of hGH secretion from the pituitary contains multiple isoforms but administered rhGH contains only the 22-kDa form. The pharmacologic precept is that a large dose of rhGH will dampen or shut down pituitary hGH release and what will be measured is virtually all 22 kDa. One submits samples to both the rec and pit assays and forms a ratio between the two. Any ratio of the 22-kDa form to the pit forms above 1 indicates administration of rhGH within the recent past (27).
- (b) The GH-marker method is based on the precept that the administration of rhGH will produce increases in certain circulating analytes (markers) (GH-responsive proteins—IGF-I axis) and those of bone and connective tissue turnover (anabolism and catabolism). Markers of the IGF-I system remain elevated for several days following cessation of rhGH administration (although IGF-I itself may remain raised for more than 1 week); however, markers of collagen synthesis and/or breakdown may remain elevated for up to 8 weeks. The test for rhGH, and perhaps IGF-I, based on this marker approach relies on a combination of markers leading to the derivation of specific algorithms which differ for the various combination of biomarkers and for men and women (28).

INSULIN-LIKE GROWTH FACTOR I

Physiology

IGF-I is the main effector for the action of hGH. Systemic IGF-I is synthesized primarily in the liver, where its production is GH-dependent; IGF-I is also produced in multiple extrahepatic tissues, where it acts locally as an autocrine/paracrine growth factor under the control of multiple hormones, including hGH (29). Most biological actions of IGF-I are mediated through the type I IGF-I receptor (IGF1R), which is structurally and functionally related to the insulin receptor.

Whereas hGH is insulin antagonistic several hours after ingesting a meal, the main effect of hIGF-I is to reduce glucose levels (insulin-like). It is strongly anabolic in muscle, but has a very much diminished effect on lipids, in comparison to hGH. In fact, children with virtually no IGF-I (growth hormone receptor deficiency, Laron type) gain a disproportionate amount of fat when treated for many years with rhIGF-I (30).

Rationale

The rationale for using rhIGF-I as an ergogenic aid differs little from that of rhGH. The potential benefits include increased muscle protein synthesis and the sparing of glycogenolysis. It stimulates glycogen synthesis and increased fatty acid availability.

There are many sites in the Internet advertising the sale of IGF-I as a more powerful drug than rhGH, and its purported benefits include improvements in energy and endurance, tissue repair, muscle growth, rebuilding of cartilage, and ligament repair.

Performance Enhancement

The prevalence of IGF-I abuse is probably much lower than that for GH because, unlike GH, there is no readily available natural source, and therefore all IGF-I is obtained through recombinant DNA technology. At present there are no clinical or scientific trials that have shown a performance benefit for rhIGF-I use.

rhIGF-I has been used as a growth-promoting agent in children with both primary IGF-I deficiency and in a few genetic conditions which are associated with short stature (31).

Adverse Events

The most common adverse effect of rhIGF-I is hypoglycemia. Some unique side effects include jaw pain, headache, fluid retention, and myalgia. As with rhGH there has been an incidence of isolated intracranial hypertension.

In the longer term, there is the theoretical aspect of tumorigenesis, although not enough data exist. There are some associative data on patients with certain cancers (e.g., breast, prostate, and colon) who have had higher IGF-I levels in the years before their cancers became detected (32).

Patients with GH insensitivity treated with rhIGF-I have also experienced lymphoid tissue hypertrophy, encompassing tonsillar/adenoidal growth and associated snoring and sleep apnea, and thymic and splenic enlargement (33).

Detection

rhIGF-I is available as a commercial product and may have a similar rationale to rhGH for its use as an ergogenic agent to improve sport performance. It is clear that the hGH isoform test would not detect doping with rhIGF-I. Theoretically, the GH-responsive marker approach should work given that many, but not all, of the metabolic effects or hGH are mediated by IGF-I (formerly called somatomedin-C).

ERYTHROPOIETIN

Physiology

Erythropoietin (EPO) is a 30.4-kDa glycoprotein hormone that is mainly produced by the kidney and is a key regulator of red blood cell production (34). EPO stimulates the proliferation and differentiation of bone marrow erythroid precursors (35). Its production is inversely related to the partial pressure of O₂ in the blood. Following administration, there is a direct relationship between hemoglobin level and increased performance following administration of rHuEPO in rats and humans (36).

Successful cloning of the human EPO gene (37) allowed for production of recombinant human erythropoietin (rHuEPO) and later the approval to treat patients with anemia. More recently several newer generations of EPO analogues have been produced (38).

Rationale

EPO leads to the production of red blood cells. Since these carry oxygen to active muscles, one should expect enhanced endurance performance because of the additional flux of oxygen.

Performance Enhancement

Due to its effect of increasing hemoglobin (Hgb)-bearing erythrocytes responsible for the oxygen-carrying capacity of the blood, EPO has been used extensively as a performance-enhancing aid in sports, particularly in endurance disciplines requiring an adequate supply of oxygen to the heart and the muscles.

An improvement of up to 5–10% was estimated in humans due to increased maximum capacity to transport and utilize oxygen ($VO_{2\max}$), velocity at $VO_{2\max}$, and maximal aerobic power (39, 40).

Adverse Events

Serious side effects may occur with EPO abuse, including hypertension, headaches, and an increased rate of thrombotic events as a result of an EPO-induced rise in the hematocrit and thickening (increased viscosity) of the blood (41). In addition, EPO withdrawal may be implicated in neocytolysis, that is, the hemolysis of young red blood cells in the presence of increased hematocrit (42). Ultimately, EPO abuse may cause death (43).

Detection

EPO may be detected in urine by electrophoretic methods. All forms have a common protein backbone, but different carbohydrate moieties because they are engineered in Chinese hamster ovary (CHO) or baby hamster kidney (BHK) cells. The carbohydrate linkages differ significantly in structure and in electric charge, producing easily detected differences in electrophoretic mobility (38).

More recently other forms of rHuEPO have become available—darbepoetin- α or novel erythropoiesis-stimulating protein (NESP). It is a glycoprotein that has five amino acids that differ from the natural human protein, permitting additional carbohydrate to be attached and conferring a different electrical charge from the physiological molecule. A pegylated EPO called continuous erythropoiesis receptor activator (CERA) is the newest generation of rEPO whose plasma half-life is extended considerably by the attached polyethylene glycol molecule. The rationale for a direct isoelectric focusing test is that the recombinant molecules carry a different charge compared to the endogenous molecules. An electrophoretic separation is accomplished in an agarose gel and followed by a double-blotting (for protein) process. Acute use of one of the recombinant

molecules can be detected for 3–7 days only (except for CERA whose detection window is longer), although it is probable that the pharmacodynamic effect on red cells lasts longer. The newer iterations of the test can detect use of any of the first three generations of recombinant products (38).

Tests in the process of development include those with immunoaffinity purification using several different antibodies. Other, still investigational, products denoted as “synthetic erythropoietin-stimulating agents,” for example, peginesatide (Hematide) are EPO-receptor agonists. They are able to stimulate erythropoiesis but do not have the amino acid backbone of EPO. Thus they would not be detected in the usual doping assays. However, novel tests are being developed to detect potential abuse, using a screening assay (ELISA) followed by a confirmation assay consisting of immune purification followed by a separation step on SDS PAGE and double western blotting to detect the protein (44).

ANABOLIC STEROIDS

Physiology

Androgens are sex hormones that promote the development and maintenance of the male sex characteristics. Testosterone is the principal secreted androgen in men. Androgens have both virilizing and anabolic effects. For decades, pharmaceutical companies have attempted to develop androgens that have preferential anabolic activity and reduced or no androgenic activity; these compounds have been referred to as anabolic steroids. In males, more than 95% of testosterone is secreted by the Leydig cells under the control of luteinizing hormone (LH). The remainder is produced via conversion of weakly androgenic precursors in the adrenal cortex.

The principal androgenic testosterone metabolite, dihydrotestosterone, mainly induces development of the primary male sex organs, while testosterone (along with dihydrotestosterone) is mainly responsible for the secondary sexual (male) characteristics. Testosterone enhances muscle hypertrophy, strength, endurance, and power (45). In addition, testosterone has been suggested to lead to muscle anabolism via anti-glucocorticoid actions, potentiation of muscle IGF-I activity, and attenuation of myostatin action and signaling (46, 47). This metabolic action follows from the binding of testosterone to the androgen receptor.

Rationale

Testosterone is the drug of abuse most often detected in sports doping control in recent years. Athletes continue to use it because of its anabolic effects and cosmetic purposes.

Performance Enhancement

The positive effects of steroids on body composition include increased fat-free mass, muscle size, strength, and power are highly dose dependent and correlated with serum testosterone concentrations (48). The anabolic effect of testosterone is dose dependent,

and significant increases in muscle size and strength occur with doses of 300 mg per week or higher (49). Anabolic androgens have improved exercise tolerance and the adaptability of muscle to overload by protecting against muscle fiber damage and increasing the rate of protein synthesis during recovery (50).

Adverse Events

Many side effects associated with anabolic androgens use involve multiple organ systems (51), including acne, gynecomastia (in men), mood and psychiatric disorders (52), increased risk of suicidal or homicidal death (53), dyslipidemia (54), suppression of the hypothalamic-pituitary-testicular axis and spermatogenesis resulting in infertility, testicular atrophy, increase in liver enzymes, cutaneous striae, and injection-site pain (55). In adolescents with growth potential, there will be an acceleration of epiphyseal maturation of the long bones and shorter than predicted adult stature. In girls and women, hirsutism and disordered ovarian and menstrual cycles can occur with quite small doses of anabolic steroids.

Detection

Analytical techniques that have been applied to anabolic-androgenic steroids (screening and confirmation) have included sample preparations based on liquid-liquid extraction of urine samples, concentration of the extracts, and separation of the analytes by gas-liquid or thin layer chromatography (56).

Over the years there have been improvements and innovation in analytical techniques permitting improved sensitivity and specificity in the compounds (including multiple metabolites) and fragments detected. Most modern techniques include derivatization, followed by a chromatographic step, and one or more mass spectrographic analyses to detect ions and fragments. For anabolic-androgenic steroids (and many other small molecules), the identification is noted by its retention time for the chromatographic step and its relative abundance of characteristic ions compared to a stored library of such fragments and ions (56).

Further analysis of suspected exogenous anabolic steroid administration involves a stable isotope ($^{13}\text{C}/^{12}\text{C}$) ratio (57).

EPILOGUE

Gene Doping

Somatic gene therapy involves the manipulation of expression of specific genes or specific tissues. When done for the purpose of enhancing athletic performance (gene doping), it is considered a threat to sports and competition. World Anti-Doping Agency's (WADA) definition of gene doping is "1-The transfer of cells or genetic elements (e.g., DNA, RNA); 2-The use of pharmacologic or biologic agents that alter gene expression... with the potential to enhance athletic performance" (1).

Studies in humans have shown that it is possible to introduce new genetic functions in forms sufficient and stable enough to modify traits that produce serious disease and

thus to ameliorate life-threatening illness and ease suffering (58). However, it is a short theoretical leap of logic, but likely a very high practical hurdle, to alter athletic performance in individual athletes.

Prime examples are IGF-I with studies in mice showing muscle hypertrophy especially in response to resistance training (59) and myostatin based on a single boy with remarkable muscle hypertrophy caused by an inactivating mutation of the myostatin gene (60). There are a number of others (e.g., EPO) that are of great interest that have been part of laboratory experimentation (61).

More recently techniques to detect gene transfer, whether systemic or local (e.g., a single muscle), are effective under laboratory conditions. These methods are based on the polymerase chain reaction (62), using a direct-detection approach based on the presence or absence of transgenic DNA in peripheral blood (63), a mass spectrometric detection of small inhibitory RNAs (siRNAs) (64), and identification of exon/exon junctions which do not exist in the natural gene (65).

SUMMARY AND FUTURE

Of the agents noted above, EPO (and other agents in this family) and the anabolic-androgenic steroids are unequivocally performance enhancing. rhGH under unusual circumstances can be performance enhancing, but the preponderance of data do not show athletic enhancement. Data simply do not exist for the performance-enhancing qualities of insulin or IGF-I, although athletes have used both, but especially insulin itself.

What about the future? Part of that is outlined in the Epilogue (above) and likely with a myriad of additional genes. Those trying to enhance performance pharmacologically (laboratories and athletes) are always ahead of those trying to detect new compounds that may have ergogenic qualities. It is analogous to an “arms race.”

There are anecdotes concerning the use of growth hormone releasers—ghrelin, growth hormone-releasing hormone, and formulations of amino acids. However, data do not exist concerning their effect on athletic performance, and the physiologic precept is that if growth hormone is released endogenously or administered exogenously, the hypothalamus and pituitary become sub-responsive to natural stimuli.

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