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Monitoring exercise stress by changes in metabolic and hormonal responses over a 24-h period

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Summary. Metabolic and endocrine responses of 14 subjects of varying levels of fitness to an intensive anaerobic interval training session were assessed before exercise and at 2 h, 4 h, 8 h and 24 h postexercise. The endocrine response of the same subjects to a control day, where they were required not to exercise, was also assessed and compared with the values obtained on the interval training day. Uric acid, urea, and creatine phosphokinase concentrations still remained elevated above pre-exercise values 24 h postexercise. Lactate, creatinine, testosterone and cortisol concentrations were significantly elevated above pre-exercise values immediately postexercise but these had reversed by 2 h postexercise. Over the remainder of the recovery period testosterone concentrations remained significantly lower than values measured at similar times on the control day. This was shown to be due directly to a change in testosterone as sex hormone binding globulin concentration remained constant throughout the recovery period. The data indicate that when comparisons of data were made to control (rest) days, imbalances in homeostasis, due to intensive training, are not totally reversed within the next 24-h. The data also demonstrate that the parameters measured undergo the same variations in subjects with a wide range of physical fitness, indicating that these parameters could be used to monitor exercise stress and recovery in athletes of a wide range of abilities. The more acute responses to exercise could be mistaken for overtraining if insufficient recovery time were not permitted between the final exercise session and taking blood samples, further emphasising the need to be able to recognise the difference between the fatigue associated with acute exercise and a state of chronic fatigue that may result from too little regeneration time within the training programme.

Key words: Overtraining - Testosterone - Cortisol - Urea - Uric acid

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Introduction

It is well-established that the body must be put under substantial exercise stress to induce changes in homeostasis indicative of training, yet the adaptations to training appear to occur over a period of reduced training which is sufficient to allow the body to re-eastablish homeostasis. Failure to incorporate these recovery periods may lead to chronic fatigue and overtraining (Bompa 1983). One of the current problems in the training of athletes is to be able to distinguish between the normal fatigue associated with isolated training sessions and that associated with the long term fatigue which may lead to overtraining (Kuipers and Keizer 1988). Many exercise induced metabolic and endocrinological changes have now been measured in athletes (Keast et al. 1988; Kuipers and Keizer 1988). Yet while there is no doubt that acute exercise induces changes in a wide array of metabolic parameters, including hormones, the use of these for the prediction of the overtrained athlete is still not possible (Kuipers and Keizer 1988).

There is still a need to investigate comprehensively metabolic changes induced by the training regimes that are used by athletes in training. Intensive interval training is a major component of the training programmes of most sports from marathon runners to team sportsmen. We have chosen a series of biochemical parameters which have been implicated in the past as indicators of exercise stress and we have investigated their dynamics concurrently in 14 subjects immediately after an exhaustive interval training session and over the ensuing 24 h. The data provided an insight into the time course of recovery from intensive training of the type undertaken in this study.

Methods

Subjects

Fourteen active male subjects (aged 18-25) gave informed consent following clearance to conduct the study from the Committee of Human Rights of The University of Western Australia. The sub-

jects represented a range of fitness levels from recreational runners to international athletes. All of the subjects were engaged in physical activity involving running at least 2 days a week.

Experimental protocol

1st session. Subjects reported to the Human Physical Performance laboratory at The University of Western Australia. They were requested not to eat or drink fluids except water on the morning of the test. Subjects completed an incremental exercise test which began at 7.00 a.m. with 3 min exercise on a treadmill at 8 $km \cdot h^{-1}$ and 1% grade. Progression was accomplished by increasing the treadmill speed by 1 $km \cdot h^{-1}$ for each subsequent exercise intensity. Each exercise intensity was of 3-min duration and 2-min s rest were enforced between each. The test concluded when the subject could not complete a 3-min exercise period. Gas analysis was conducted throughout the test using a metabolic trolley consisting of an oxygen analyser (Applied Electrochemistry, Sunnyvale, Calif.), a carbon dioxide analyser (Applied Electrochemistry) and a ventilometer (Morgan, Gillingham, UK). Ventilation, oxygen consumption and respiratory exchange ratio (R) values were computed and displayed on a monitor every 30 s. Ear lobe blood samples were collected into capillary tubes for subsequent lactate analysis during the 1st min of each 2-min rest period. Lactic acid assays were performed on a lactic acid analyser (Analox, London, UK). Onset of blood lactic acid accumulation was determined as the treadmill speed which elicited a blood lactate concentration of 4 $mmol \cdot l^{-1}$ (Stegmann and Kindermann 1982). Maximal values for blood lactate, oxygen consumption, R, ventilation and heart rate (f_c) were determined.

2nd session. One week later subjects again reported to the laboratory at 6.30 a.m. after having abstained from strenuous physical activity for at least 36 h, and not having eaten or consumed fluid except water on the morning of the test. The subjects after providing a blood sample undertook an anaerobic exercise regime consisting of 25 1-min exercise periods on a treadmill separated by 2-min rest periods where the subjects were allowed to walk on the spot beside the treadmill. The exercise intensity used was the one prior to that which the subject failed to complete in the first exercise session. Except for the imposed training session, subjects were requested not to exercise except by walking. Throughout the 24-h data collection period they were also requested not to eat or drink fluids except water within 2 h of donating a blood sample. The f_c were recorded using an electrocardiograph during the final 10 s of each 1-min exercise period. Blood samples were taken 15 min pre- (PRE), 3–5 min post (POST), 2 h post (2H), 4 h post (4H), 8 h post (8H), and 24 h postexercise (24H). The PRE blood sample was provided at 7.00 a.m.

3rd session. This session was completed approximately 4 weeks following the anaerobic training session. Subjects reported to the laboratory four times throughout the day having observed the same exercise and dietary restrictions described for session 2. The four times were 7.00 a.m. (7C), 10.00 a.m. (10C), 1.00 p.m. (1C) and 4.00 p.m. (4C). During each of these sessions subjects donated a 30-m blood sample. Subjects were requested not to exercise until after the 4.00 p.m. blood sample.

Blood sampling

Prior to sampling each subject was required to rest quietly in an upright position for 10 min. A 30-ml blood sample was collected from the antecubital vein at each sample time, using a winged cannula attached to a vacutainer (Becton Dickinson Rutherford, N. J.) bleeding system. A fresh venous puncture was used for each sample. On the control day, subjects donated blood samples during each of their four visits. A 10-ml blood sample was collected into clotting tubes to obtain serum, 5 ml into fluoride/oxolate, and 5 ml into ethylenediaminetetraacetic acid (EDTA). All samples were immediately chilled on ice following collection, centrifugation was conducted in a refrigerated centrifuge, and serum and plasma samples were stored at $-70°$ C prior to assaying. Serum was obtained by allowing blood to stand on ice for 2 h and then centrifuging at 1000 g for 20 min. Lactic acid analyses were performed on fluoride/oxolate treated samples. The EDTA treated samples were protected form light and used for creatine phosphokinase (CPK) determinations and for determinations of packed cell volume and haemoglobin concentrations using a Coulter STKS (Coulter Electronics, Hialeah, Fla.) analyser. Packed cell volume and haemoglobin were used to determine whether or not the training session caused a haemoconcentration using the equation of Dill and Costill (1974). Commercially available radio-immunassay kits were used for determinations of cortisol (Amersham, UK), testosterone (Mallinckrodt Diagnostica, RIA-MAT testosterone, Dietzenbach, FRG) and sex hormone binding globulin (SHBG) (Farmos, Orlunsalo, Finland). The following parameters were assayed enzymatically according to previously published methods: uric acid (Kageyama 1971), urea (Kaltwasser and Schlegel 1966), lactic acid (method of Hohorst 1963 modified by Drews 1974), and CPK (method of Rosalki 1967). Creatinine was assayed by the colorimetric method of Haeckel (1981).

Statistical analysis

One-way analyses of variance with repeated measures were used with the Fisher post hoc comparison being applied to determine the significant mean differences. Significance was established at the $P<0.05$ level. Analysis of all data was conducted using the Statview 512+ statistical package (Statview, Calabasas, Calif.) on a Macintosh computer.

Results

Incremental exercise test

Table 1 presents the physical and physiological characteristics of subjects measured in the incremental exercise test.

Table 1. Physical and physiological characteristics of the subjects. Data was derived from the initial session which involved the incremental exercise protocol

Paramter	Mean	SEM	Minimum	Maximum	Range
Maximum ventilation ($l \cdot min^{-1}$)	153.9	8.7	95.1	213.7	118.57
$\dot{V}O_{2max}$ ($l \cdot min^{-1}$)	4.59	0.26	2.85	6.90	4.05
$\dot{V}O_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	61.02	2.6	42.61	75.36	32.75
Mass (kg)	75.26	3.3	61.37	112.45	51.08
Sum of eight skinfolds (mm)	74.68	4.88	40.23	101.04	61.04
OBLA ($km \cdot h^{-1}$)	13.4	0.8	9.0	16.9	7.9
Maximal treadmill speed ($km \cdot h^{-1}$)	15.86	0.74	10.0	19.0	9.0

$\dot{V}O_{2max}$, Maximal oxygen consumption; OBLA, onset of blood lactate accumulation

Table 2. Heart rate data (beats·min^{-1}) for each of the 25 1-min exercise periods comprising the interval training session

Exercise period	Mean and SEM		*n*	Exercise period	Mean and SEM		*n*	Exercise period	Mean and SEM		*n*
1	165.7	4.1	14	9	174.3	4.1	14	18	176.2	4.1	14
2	168.0	4.2	14	10	175.2	3.9	14	19	177.1	4.0	14
3	171.0	4.2	14	11	175.5	4.1	14	20	176.3	4.2	13
4	171.9	4.3	14	12	175.7	4.2	14	21	177.2	4.2	13
5	173.0	4.2	14	13	175.2	4.2	14	22	177.2	4.2	12
6	171.6	4.6	14	14	176.2	4.1	14	23	178.2	4.0	12
7	172.5	4.6	14	15	176.3	4.1	14	24	179.5	4.1	12
8	173.8	4.1	14	16	175.8	4.3	14	25	184.7	4.5	12
				17	176.2	4.2	14				

Training session

The f_c measured in the final 10 s of each 1-min exercise period increased progressively and significantly ($F=16.227$, $P<0.001$) during the training session. Twelve of the 14 subjects completed all of the 25 exercise periods. One subject said he was exhausted after the 19th exercise period and the other after the 21st. The f_c for all exercise intensities are presented in Table 2.

Figures 1 and 2 present the means and standard errors and locations of statistical significance for the values obtained for the metabolites and CPK measured at each of the data collection points during the training session. The training session did not elicit a haemoconcentration.

Plasma lactic acid concentration was elevated significantly ($P<0.001$) at POST and had returned to PRE levels by 2H. Creatinine concentration increased significantly ($P<0.001$) at POST and had decreased to PRE values by 2H. Creatinine concentration continued to decrease progressively over the remainder of the recovery period. Uric acid concentration was significantly elevated ($P<0.001$) at POST and continued to increase reaching maximal values at 2H. Uric acid concentration decreased progressively over the remainder of the 24-H recovery period but remained significantly elevated from PRE levels throughout. Urea concentration increased significantly ($P<0.001$) after exercise and continued to do so over the subsequent 8H. The urea concentration did not rise significantly until 2H; however, it was still significantly elevated by 24H. The CPK concentration increased significantly ($P<0.001$) and progressively after exercise and continued to do so over the first 8 h of recovery and remained elevated at 24 h after the training session.

Figure 3 presents the data and significant differences for endocrinological data measured at matched sample times during both the interval training session and control days. On the test day cortisol concentrations before exercise mean [776 (SEM 47) nmol·l^{-1}] were higher than normal and suggested an anticipatory response to exercise and/or having blood samples taken. The 24H value was significantly lower than both the PRE value and the 7C value. All three values were taken at approximately 7.00 a.m. On the day of exer-

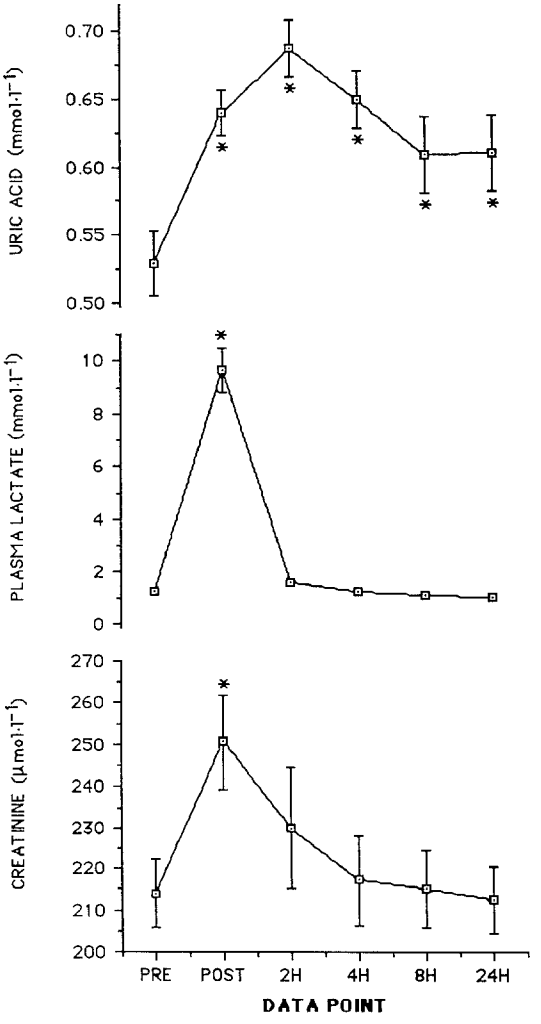

Fig. 1. Uric acid, creatinine and lactic acid response to the interval training session. Each of the data points is represented on the *x* axis and the metabolic parameters on the *y* axis. Values are presented with the mean and standard error. * Significant mean difference ($P<0.05$) between the pre-exercise (PRE) value on the training day and each of the other data points. *Error bars* are not visible when they lie within the size of the symbols. POST, 2H, 4H, 8H, 24H, data collection points immediately after and 2, 4, 8 and 24 h after exercise, respectively

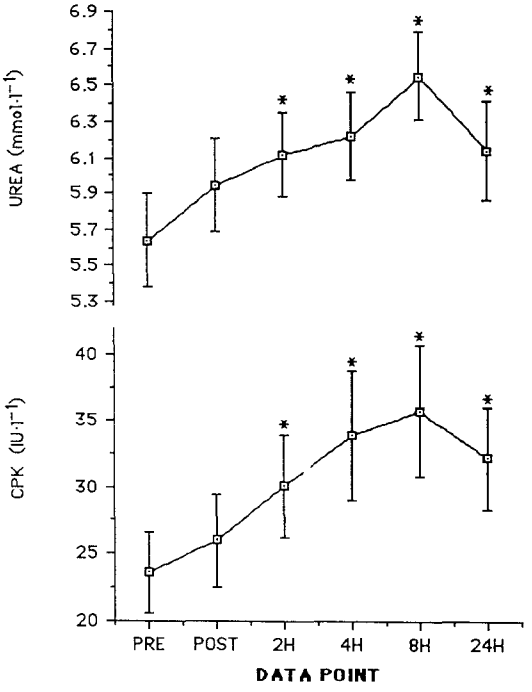

Fig. 2. Urea and creatine phosphokinase (*CPK*) response to the interval training session. Each of the data points is represented on the *x* axis and the values for *CPK* and urea on the *y* axis. Values are presented with the mean and standard error. * Significant mean differences ($P<0.05$) between the pre-exercise (PRE) value on the training day and each of the other data points. For definitions see Fig. 1

cise, the cortisol concentration after exercise was significantly elevated from the PRE value, however over the following 2 h cortisol concentrations dropped to the equivalent control day value (10C) (Fig. 3).

Testosterone concentrations prior to exercise could not be differentiated statistically from the 7C value on the control day however the 24H value was significantly lower ($P<0.001$) than the 7C value. The exercise session initially induced a significant increase in testosterone concentrations above both the PRE and 7C concentrations (see Fig. 3). This was reversed by 2H, and during the remainder of the recovery period the exercise day values were significantly lower than values for samples taken at equivalent times on the control day. The testosterone:cortisol ratio (T:C ratio) demonstrated a diurnal effect as demonstrated by the control day values. The PRE, POST and 24H values did not demonstrate statistical difference from 7C concentrations. The 2H value was significantly lower ($P<0.001$) than the 10C value, indicating that the exercise session delayed the diurnal effect of an increasing T:C ratio over the course of the day.

The SHBG concentrations did not vary significantly over either the control or exercise day nor were the control and exercise day values different. Because the SHBG values demonstrated little variation, the fluctuations in the T:SHBG ratio to a large extent mirrored those of the testosterone concentration values. The 2H T:SHBG ratio was significantly lower ($P<0.05$) than the ratio at the equivalent time on the control day (10C)

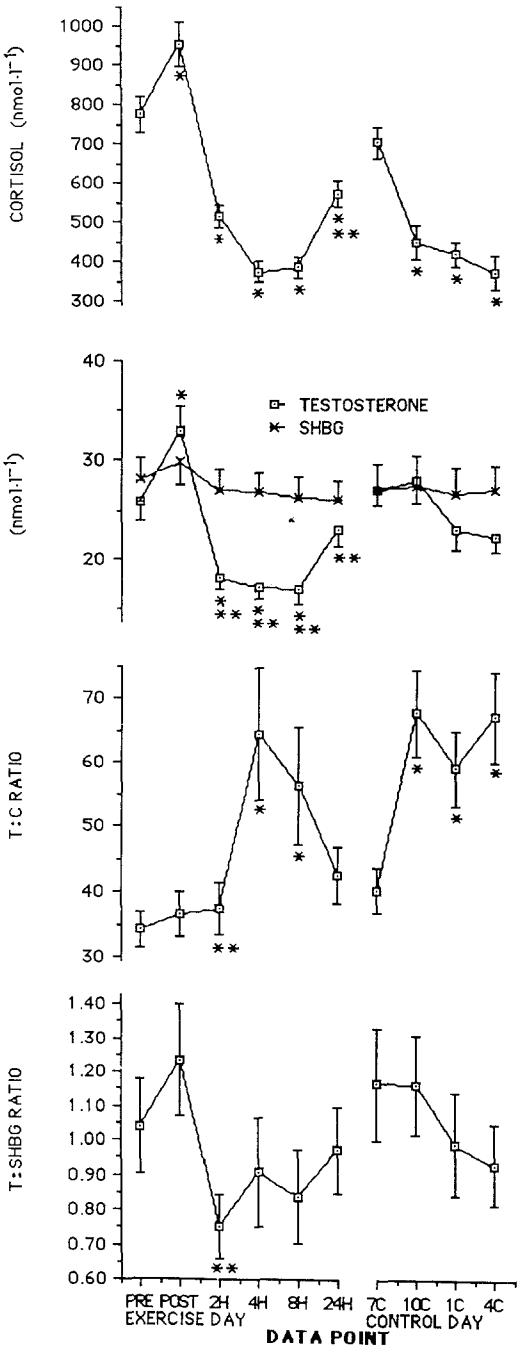

Fig. 3. The cortisol, testosterone and sex hormone binding globulin (SHBG) responses to an interval training session and a control day. Each of the data points is represented on the *x* axis and the endocrine parameters on the *y* axis. Values are presented with the mean and standard error. * Indicate significant mean differences ($P<0.05$) between the pre-exercise (PRE) value on the training day and each of the other data points; ** indicate significant mean differences ($P<0.05$) when the training session data points are compared with control day data points where values were derived from samples taken at a similar time of day. For this comparison, the 2 h post (2H) value was compared with the 10.00 a.m. (10C) value, the 4 h post (4H) value with the 1.00 p.m. (1C) value, the 8 h post (8H) value with the 4.00 p.m. (4C) value and the 24 h postexercise (24H) value with the 7.00 a.m. (7C) value. T:C ratio, testosterone:cortisol ratio

and reflected less free testosterone in the blood stream at that point in the day, on the training day, compared with the control day.

Discussion

Elevated serum creatinine concentrations after exercise have been implicated in compromised glomerular filtration which can lead to elevated serum concentrations of urea and uric acid (Allen and Keenan 1988). Creatinine concentration was elevated significantly immediately at POST (Fig. 1); however, it had returned towards PRE concentration by 2H suggesting that glomerular filtration may have returned to normal by this time. It has also been suggested that increases in uric acid concentrations in the blood postexercise is the result of lactic acid induced blockage of renal clearance (Nichols et al. 1951). Our data does not support this concept, as uric acid concentrations continued to increase for several hours after lactic acid concentrations had returned to baseline values (see Fig. 1). However, the effects of lactate acidosis on renal clearance can take a longer time than the time to clear the increased concentration in blood (Knochel et al. 1974). Changes in the serum concentrations of urea and uric acid postexercise were therefore most likely to be the result of increased production, rather than compromised excretion rates due to reduced filtration, beyond 2H. Increased uric acid concentration following exercise may also result from increased purine base degradation (Allen and Keenan 1988; Harkness et al. 1983) and this may be enhanced by the formation of inosine-5-monophosphate in muscle during intensive exercise followed by degradation to purine precursors and conversion to uric acid in the liver (Knochel et al. 1974). With severe intensive exercise of the type used in our studies, significant decreases in intracellular adenosine 5′-triphosphate (ATP) concentration can be expected (Harkness et al. 1983). This may to some extent be rapidly reversed by the rephosphorylation of adenosine 5′-diphosphate (ADP) (Porter and Whalen 1981), but it is clear that there was an associated catabolism of purines to uric acid which might be associated with tissue damage (Harkness et al. 1980, 1983; Jenkins 1988; Banister et al. 1985). Uric acid concentrations remained elevated 24 h after the training session possibly as a result of continued low concentrations of ATP following the exhausting exercise involving a large muscle mass (Harkness et al. 1983). These findings suggested that recovery of the cells' energy reserves may require longer than 24 h following intensive exercise and, if this is so, programming of more than one high intensity training session per day may effectively place a cumulatively greater stress on the cells to produce the same degree of exercise in later training sessions. Performance levels could then eventually fall due to a continuing energy crisis within the stressed cells.

Persistently elevated urea concentrations after exercise can reflect an imbalance in protein metabolic homeostasis and may indicate delayed recovery from training stress (Viru 1987). Return of urea concentrations to baseline levels may reflect a return of protein metabolism to a state of balanced homeostasis and hence full recovery from a training session. Our results demonstrated that urea concentrations can remain elevated for at least 24 h following intensive interval exercise, as has been previously demonstrated in endurance exercise (Viru 1987). It has been suggested that urea concentrations of greater than 8.3 $mmol \cdot l^{-1}$ are indicative of an overtrained state (Kindermann 1986) indicating that there may be a critical urea concentration which should not be exceeded and, if reached, a period of regeneration may be necessary. The values recorded in this study did not approach the 8.3 $mmol \cdot l^{-1}$ limit. It is possible that cumulative exercise effects are required to elevate urea concentrations towards the suggested overtraining threshold.

Urea concentrations may be elevated by factors other than protein turnover including an increased consumption of dietary protein and dehydration; provision for the control for these factors must therefore be made when using urea as an indicator of protein turnover.

The elevated CPK concentration throughout the recovery period of this study (Fig. 2) suggested that some muscle damage occurred as a result of the training session (Ebbeling and Clarkson 1989). The fact that this was not reversed within 24-h suggested that including too many training sessions of the nature used in this study within a too short period may result in a progressively increasing state of muscle damage as the result of insufficient regeneration time.

The initial increase and then decrease in testosterone concentrations observed in this study may account for many of the inconsistencies evident in the literature, some of which report elevations and some decreases in testosterone concentrations following exercise (Cumming et al. 1989; Hackney 1989). The initial increase in testosterone concentrations following short duration exercise has been reported previously (Cumming et al. 1989) and may be largely due to a decreased metabolic clearance as the result of depressed hepatic blood flow and a possible haemoconcentration (Kindermann et al. 1982; Cumming et al. 1989). In this study there was no significant haemoconcentration induced by exercise, with haemoglobin and packed cell volume remaining unaltered from PRE values immediately following the training session. Suppressions in testosterone concentrations which may last several days have been reported following long duration submaximal activity (Cumming et al. 1989). Our data demonstrated that shorter duration anaerobic exercise can also elicit reductions in circulating testosterone concentrations which persists for at least 24 h. It is possible that repeated intensive exercise of this nature may result in persisently depressed testosterone concentrations which may contribute to oligospermia, decreased libido, decreased skeletal and cardiac muscle hypertrophy, anaemia, osteoporosis, artherosclerosis (Cumming et al. 1989), and suppressions in immune function (Keast et al. 1988; Cumming et al. 1989). Decreased testosterone concentrations in recovery may also result in a lower rate of muscle glycogen

restoration, as animal studies have shown that testosterone increases the activity of muscle glycogen synthetase (Gillespie and Edgerton 1970). Normal testosterone concentrations may be associated with a more rapid restoration of the stored phosphate pool through enhanced rate of creatine phosphate restoration (Sutton et al. 1973).

Cortisol has catabolic and testosterone anabolic effects on protein metabolism (Schmitt et al. 1981; Adlercreutz et al. 1986; Urhausen and Kindermann 1987). Testosterone serves to offset the catabolic functions of cortisol and depressed concentrations of testosterone may therefore hinder the repair of muscle damage associated with high intensity activity (Cumming et al. 1989; Ebbeling and Clarkson 1989). Elevated cortisol concentrations immediately after exercise, as demonstrated by our data, and elevated basal cortisol concentrations reproted in overtrained athletes (Barron et al. 1985) may therefore contribute to muscle catabolism, especially when this coincides with depressed testosterone concentrations. Cortisol mediated catabolism may be accelerated when repeating high intensity training sessions prior to full recovery of testosterone concentrations and, as indicated by our data, this may occur when more than one intensive training session is conducted on one day (Dressendorfer et al. 1987). This may be one mechanism leading to overtraining, as muscles are provided with little opportunity to regenerate and are under constant catabolic stress.

Elevated cortisol concentrations suppress testicular steroidogenic processes therefore reducing the concentrations of serum testosterone (Cumming et al. 1983, 1989). Our data demonstrated that cortisol was elevated significantly from PRE concentrations following exercise (Fig. 3); however, concentrations had returned towards control day values at 2H as reported previously (Bunt 1986). Testosterone concentrations remained significantly depressed from control day values over the 24-h recovery period. The data does not therefore support the concept that elevated cortisol concentrations were the sole cause of the depressed testosterone concentrations in recovery. Mechanisms for the exercise induced changes in circulating testosterone concentrations are yet to be fully elucidated (Cumming et al. 1989).

No change in SHBG concentrations were observed, indicating that fluctuations in free testosterone were not the result of alterations in serum binding capacity, although testosterone does bind to albumin with low affinity and altered albumin binding cannot be excluded by this study (Cumming et al. 1989). The SHBG has a half-life of several days (Urhausen and Kindermann 1987) and therefore changes were not to be expected in this study. Elevations in SHBG concentration may be a delayed response to physical stress of several days duration (Hakkinen et al. 1988).

It has been proposed that the balance between catabolic and anabolic processes may be expressed by the T:C ratio (Aldercreutz et al. 1986; Urhausen and Kindermann 1987). We have found that the T:C ratio demonstrated considerable variation over the course of a control day, varying from 40.4 (SEM 3.5) in the morning to 67.2 (SEM 7.0) in the afternoon. Time of sampling is clearly a factor to be considered if attempting to use the T:C ratio as a tool for diagnosing overtraining and should be standardised for routine sampling.

Measuring testosterone concentrations immediately after exercise would be misleading in terms of the T:C ratio as testosterone concentrations were significantly elevated above PRE and control day values immediately after exercise. The reason for this was not clear nor were the mechanisms for the decrease in free testosterone during the regeneration period (Urhausen and Kindermann 1987). Alterations in hypothalamic-pituitary-gonadal axis regulation have been hypothesised as the underlying mechanism (Hackney 1989). The literature has implicated this system with exercise stress related conditions including hypothalamic-pituitary dysfunction [associated with elevated basal corticol concentrations (Barron et al.1985)], decreased sperm count and libido (associated with depressed testosterone concentrations) following periods of heavy training (Griffith et al. 1990; Ayres et al. 1985; Cumming et al. 1989) and exercise induced amenorrhoea (Cumming 1987; Highet 1989).

An aim of this study was to determine whether the trends observed were evident in athletes over a wide range of performance levels. The data in Table 1 reflects that such a range of subjects was obtained as indicated by the large variation in parameters indicative of fitness levels. The results of the time course experiment demonstrated that the parameters measured undergo the same variations in subjects over a wide range of physical fitness, indicating that they could be used to monitor exercise stress and recovery in athletes of a wide range of abilities. This is reflected by the significant trends and small errors and is important as it is likely that athletes at all levels of performance, provided they are highly motivated to train, may be susceptible to changes in metabolic and hormonal parameters that have been previously associated with overtraining (Kindermann 1986; Kuipers and Keizer 1988). These observations may be useful to investigators interested in research in the overtraining area, as it suggests that active but not necessarily elite athletes can be used as subjects for studies.

It is clear from the uric acid, urea, CPK and testosterone results from this study, that recovery from intensive interval training may require longer than 24 h. Clearly, if the regeneration period following a single intensive training session is greater than 24 h, the regeneration time for a series of intensive sessions designed to create a strong adaptational stimulus may require several days. An understanding of any natural diurnal cycles for measured variables will be required as significant diurnal variation was evident for cortisol and testosterone on the control day of this study. It is therefore necessary to standardise the time at which blood samples are collected.

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