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ORIGINAL ARTICLE

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Effects of a low carbohydrate diet and graded exercise during the follicular and luteal phases on the blood antioxidant status in healthy women

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Abstract The aim of this study was to examine the effect of a low-carbohydrate (L-CHO) diet and graded cycling exercise on the enzymatic and non-enzymatic blood antioxidant defence system in young eumenorrhoeic women. Seven healthy physical education students exercised incrementally until they were fatigued under four different phase-diet conditions of the menstrual cycle, i.e. twice either during the mid-follicular or the mid-luteal phase, in each case either after 3 days of eating a normal mixed diet (59% carbohydrate, 27% fat, 14% protein) or 3 days of eating an isoenergy L-CHO diet (5% carbohydrate, 52% fat, 43% protein). In venous blood samples obtained at rest, immediately post test and during recovery, the activity of antioxidant enzymes and concentrations of reduced glutathione and selenium were determined. Plasma samples were analysed for concentrations of malondialdehyde, vitamin E (α-tocopherol), uric acid and activity of creatine kinase. The 3 days of the L-CHO diet, which had been preceded by glycogen-depleting exercise, resulted in a stimulation of the blood antioxidant defence system in young eumenorrhoeic women both at rest and during the graded cycling exercise to maximal oxygen uptake. It seems justified to presume that higher daily doses of haem iron, selenium and α-tocopherol provided by the L-CHO diet contributed to the enhancement of catalase activity, the rise in plasma concentrations of α-tocopherol and selenium, which resulted in better protection of the cell membranes against damage from peroxides, as reflected by a limited release of creatine kinase into plasma. With the exception of the case of glutathione reductase, the phases of the menstrual cycle had only minor effects on the indices of the blood antioxidant defence system.

Keywords Menstrual cycle · Low carbohydrate diet · Antioxidant enzymes · Vitamin E · Exercise

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Introduction

There is a growing number of reports related to various aspects of exercise-induced oxidative stress in humans and animals, although most of them have concerned male subjects. The sex differences in response to oxidative stress have been considered to rely on the better antioxidant protection provided by oestrogens acting as antioxidants capable of scavenging the reactive oxygen species (Sugioka et al. 1987), modulating activities of antioxidant enzymes (Massafra et al. 2000) and decreasing oxidative modification of low-density lipoproteins (Tang et al. 1996). Oestrogens have also been recognized as being important factors in maintaining post-exercise membrane stability and limiting the leakage of cell enzymes into the blood in women (Tiidus 2000). There are numerous reports that have documented sex-based differences in the response of skeletal muscle to exercise and the role of oestrogens in the prevention of exercise-induced damage to the muscle membrane, both in humans and animals (Bar et al. 1988). Surprisingly, only a few studies have been aimed at the evaluation of the effect of exercise on selected indices of antioxidant status in eumenorrhoeic or amenorrhoeic women (Kanaley and Ji 1991; Hernandez et al. 1999), and in only one study (Chung et al. 1999) were observations made on the post-exercise changes in plasma markers of lipid peroxidation [malondialdehyde (MDA), thiobarbituric acid reactive substances (TBARS)] and glutathione status during the follicular and luteal phases of the menstrual cycle.

The antioxidant defence system of blood has been reported to consist of two additively and synergistically acting mechanisms (Hebbel 1986). The first involves scavenger enzymes, including superoxide dismutase [SOD, Enzyme Commission (EC) no. 1.15.1.1], catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GSH-Px, EC 1.11.1.9), preventing or limiting initiation and propagation of free radical chain reaction. The second system is involved in repairing the damage induced by oxidative stress, through regeneration of proteins modified by oxidation (e.g. haemoglobin by methaemoglobin reductase) or by recycling low molecular weight antioxidants [e.g. reduced glutathione (GSH) by glutathione reductase (GR, EC.1.6.4.2)]. Additionally, antioxidant defence has been shown to rely on a number of nonenzymatic free radical scavengers present in plasma, such as uric acid (UA; Ames et al. 1981), oestrogens (Sugioka et al. 1987) or those provided by the diet (Sen 2001). Among the antioxidant nutrients, the most important are α-tocopherol (vitamin E), ascorbic acid (vitamin C), carotenoids (especially β-carotene and lycopene) and retinol (vitamin A). Antioxidant properties have also been attributed to some amino acids, mainly tryptophan, cysteine and tyrosine (Meucci and Mele 1997). Several antioxidant enzymes, such as SOD, GSH-Px and CAT require metal cofactors (copper, zinc and manganese for SOD, selenium for GSH-Px and iron for CAT). It may be presumed that the efficiency of the antioxidant defence system depends on an adequate intake of foods rich in antioxidant vitamins and trace elements indispensable for the biosynthesis of antioxidant enzymes. The intake of antioxidant nutrients is strongly dependent on diet composition, the low-carbohydrate, high-fat, high-protein diet seeming to be a better source than the normal mixed (M) diet.

Numerous previous studies have been dedicated to the assessment of the benefits from antioxidant supplementation in physically active individuals and professional athletes, mostly men (Ji 1995; Sen 2001). Very little is known about the effects of altering the proportions of dietary fats and carbohydrate on the antioxidant status of physically active individuals, both men and women. To our knowledge, there are no published reports on the combined effect of exercise, phase of the menstrual cycle and macronutrient composition of the diet. Our study was therefore aimed at the evaluation of the effects of the phase of the menstrual cycle [mid-follicular, MF, (low oestrogen) compared to mid-luteal, ML, (high oestrogen)] and diet composition on the blood antioxidant status in healthy female subjects subjected to incremental cycling until they were exhausted.

Methods

Subjects

Seven nonsmoking eumenorrhoeic female physical education students volunteered to take part in this study. All subjects were informed of the purpose and the nature of the study before giving their written consent to participate in the experiment, which had been approved by the Ethics Committee at the Medical University of Silesia in Katowice. The basic characteristics of the subjects are presented in Table 1.

None of the women had taken oral contraceptives, vitamin supplements or medication for at least 6 months before and during the experiment. The phases of the menstrual cycle were monitored by measuring morning basal body temperature (BBT) and were confirmed by assaying oestradiol (E_2) and progesterone concentrations in serum taken at rest.

Experiment design

Each subject participated in four experiments under different conditions of the phase of the menstrual cycle and diet, i.e. either twice during the MF phase (between 6 to 8 days after the onset of menses) when the E_2 concentration was low or twice during the ML phase of the menstrual cycle (4–6 days after ovulation, as confirmed by a rise in BBT and high concentrations of E_2 and progesterone in the serum). In each case the subjects exercised after 3 days of eating either a ketogenic, low-carbohydrate (L-CHO) diet consisting of 5% of the energy intake from carbohydrate, 52% from fat, and 43% from protein or a normal M diet (59% carbohydrate, 27% fat, and 14% protein) (Table 2). Both diets had the same daily energy content (164 kJ·kg body mass^{-1}) and all foods were prepared at the students' canteen according to the instructions from the research team. Prior to each series of experiments the subjects ran on a treadmill for approximately 1.2 h (20 min at 6 km·h^{-1} then at 8 km·h^{-1}) until exhaustion to deplete the endogenous glycogen stores in the exercising limbs. After 3 days on the specific diet the subjects reported to the laboratory in the morning (8–10 a.m.) after an overnight fast to perform an incremental

Table 1. Mean (SEM) physical characteristics of the subjects ($n = 7$). *M* Mixed, *L-CHO* low carbohydrate, *MF* mid-follicular, *ML* mid-luteal phase of the menstrual cycle

Age (years)	Height (m)	Body mass (kg)			
		Normal M diet		L-CHO diet	
		(MF)	(ML)	(MF)	(ML)
21.0(1.1)	1.67 (0.04)	61.4 (4.9)	61.3 (4.3)	60.9 (4.3)	61.1 (4.6)

Table 2. Mean daily intake of macronutrients, antioxidant vitamins and iron as assessed from food records for a subject of 60 kg body mass. The data for recommended dietary allowances (RDA) have been derived from the National Academy of Sciences, Dietary Reference Intakes for Dietary Antioxidants and Related Compounds, 2000 (USA) (Sen 2001), and the Institute of Foods and Nutrition, Recommended Dietary Allowances for the population of Poland, 1998 (Poland). *CHO* carbohydrate

Variable	Mixed-diet	Low-CHO diet	RDA
Energy intake, [kcal (MJ)]	2,332 (9.8)	2,240 (9.4)	2,350 (9.9)
%Protein	14	43	15[b]
%Carbohydrate	59	5	55[b]
%Fat	27	52	<30[b]
Vitamin A (RE)[a]	1,331	1,059	800/800
Vitamin E (mg)	8.7	20.7	15/10
Vitamin C (mg)	53.3	1.1	75/70
Iron (mg)	12.7	22.7	15/18

[a]Retinol equivalents: 1 retinol equivalent = 1 μg retinol or 6 μg beta-carotene
[b]Suggested percentage in the diet

maximal oxygen uptake ($\dot{V}O_{2max}$) test on a cycle ergometer (Monark-829E). After a short warm-up cycling at 20 W, the subjects cycled in stages of 3 min duration starting at a power output of 30 W, and increasing progressively by 30 W. The exercise was continued until the subjects felt exhausted. The trials were performed about 1 month apart (one menstrual cycle length), in a randomized order of the combination of the phase of the menstrual cycle and diet. Blood samples were withdrawn from the antecubital vein into heparinized tubes before the exercise and then at 3, and 60 min, and 24 h following the cessation of the test. The eating of the specific diet by the subjects was, in each series of the experiment, extended into the recovery period.

Analytical procedures

Fresh whole blood samples were immediately assayed for GSH by a colorimetric method (Beutler et al. 1963) with 5,5'-dithiobis-2-nitrobenzoic acid and the activity of GSH-Px by the method of Flohe and Gunzler (1984) with tert-butyl-hydroperoxide as substrate. One GSH-Px activity unit was defined as 1 μmol nicotinamide-adenine dinucleotide phosphate (NADPH) oxidized per minute by GR to regenerate the GSH used by GSH-Px for reduction of the substrate. The remaining blood was centrifuged for 10 min at 1,000 *g* at 4°C to separate plasma and erythrocytes. The erythrocytes were washed three-times with cold (4°C) saline and kept frozen at –20°C, for not longer than 2 weeks, until being assayed for activities of antioxidant enzymes, i.e. SOD using a commercially available RANSOD kit (No.RS505; Randox Laboratories Ltd., UK), CAT by the method of Aebi (1974) and GR according to Glatzle et al. (1970). The activity of CAT was expressed as the rate constant (k) of a first order reaction of hydrogen peroxide decomposition related to the haemoglobin (Hb) content ($k \cdot gHb^{-1}$). One GR activity unit was defined as the reduction of 1 μmol of oxidised glutathione (GSSG) per minute, monitored by a decrease in absorbance at 340 nm due to oxidation of NADPH consumed per each molecule of GSSG reduced. The activities of all antioxidant enzymes were measured at 37°C and expressed per 1 g of haemoglobin as assessed by a standard cyanmethaemoglobin method using a diagnostic kit (no. HG980; Randox Laboratories Ltd., UK).

Fresh plasma samples were assayed for activity of creatine kinase (CK, EC 2.7.3.2) at 37°C using a diagnostic kit (ANALCO) based on a quantitative kinetic method (Szasz et al. 1976) and for UA by using a reagent kit (ANALCO) with 3,5-dichloro-2-hydroxybenzene sulphonic acid/4-aminophenazone chromogenic system in the presence of horseradish peroxidase and uricase (Fossati et al. 1980). The remaining plasma aliquots were stored at –80°C until subsequent biochemical analyses. The concentrations of the lipid peroxides in plasma were assayed using the thiobarbituric acid (TBA) test (Buege and Aust 1978) with modification, i.e. the addition of 0.01% butylated hydroxytoluene to lower the metal-catalyzed auto-oxidation of lipids during heating with TBA reagent, the extraction of the chromogen with n-butanol and reading the absorbance of the organic layer at 532 nm (Rice-Evans et al. 1991). The concentrations of the lipid peroxides were expressed as micromoles of MDA per litre of plasma, which was calculated from the calibration curve prepared with 1,1,3,3-tetraethoxypropane as an external standard. Plasma α-tocopherol concentration was measured using reverse-phase-high-performance-liquid-chromatography (HPLC; Beckman System Gold; Analogue Interface module 406 and Programmable Solvent module 126). Lipid extraction was performed using a method elaborated by Sobczak et al. (1999). Samples, blanks and standards (α-tocopherol; SIGMA) were injected on to a reverse-phase Supelcosil LC-18 column (4.6 mm×15 cm), the fluorescence was read (Fluorescence HPLC Monitor Shimadzu RF535) at 295 nm (excitation) and 325 nm (emission). The selenium content was determined in whole blood samples, after digestion in perchloric acid, using a fluorometric method according to Danch and Dróżdż (1996) which employed 2,3-diaminonaphtalene as a complexing agent. The piazselenol formed was extracted into cyclohexane and the fluorescence was read (Perkin-Elmer LS-30) at 365 nm (excitation) and 520 nm (emission). For each series of determinations selenium methionine (SIGMA) was used as an external standard. Plasma concentration of 3-hydroxybutyrate (3-HB) was assessed using the enzymatic method based on the oxidation of 3-HB by 3-hydroxybutyrate dehydrogenase to acetoacetate according to Williamson et al. (1962). The concentrations of E_2 and progesterone in the serum samples were determined by radioimmunoassay using diagnostic kits supplied by the Institute of the Atomic Energy, Swierk, Poland.

Statistics

Mean and standard error of the mean (SEM) were calculated for all the variables. All data were tested for homogeneity of variances using the Levene's test and then analysed using a 3-way or 2-way analysis of variance (ANOVA) for repeated measures, followed by the Newman-Keuls post-hoc comparisons where appropriate. For the post-hoc analyses, significance levels were adjusted using a Bonferroni correction. The adjusted *P* value was determined by dividing α (0.05) by the number of repeated comparisons (i.e. 4), so $P < 0.0125$ was the minimal accepted level of significance. In addition, Spearman rank order correlation coefficients were computed and a multiple regression analysis was applied to reveal relationships between the variables. All statistical analyses were computed using STATISTICA 5.0 (StatSoft, Inc., 1995) software.

Results

No significant changes in either the body mass of the subjects (Table 1) or the body mass index [21.7 (1.6) to 21.9 (1.7) $kg \cdot m^{-2}$] between the exercise trials were found. The detailed dietary records revealed considerable differences in daily intakes of antioxidant vitamins and iron provided by the M and L-CHO diets. The results are presented in Table 2. The L-CHO diet caused a considerable increase in concentrations of noradrenaline at rest. Adrenaline concentration tended to increase as well, although the difference with respect to the level recorded after the M diet was significant only in the MF phase (data presented in Manowska et al. 1999). After 3 days of carbohydrate restriction a ketosis developed in all subjects, as shown by a substantial rise in plasma 3-HB, which increased from 0.10 (0.02) and 0.13 (0.02) $mmol \cdot l^{-1}$ as recorded after the M diet for the MF and ML phases, respectively, to 0.53 (0.10) and 0.50 (0.13) $mmol \cdot l^{-1}$ after the L-CHO diet. A significant effect of the diet on 3-HB level was revealed by 2-way ANOVA ($F = 21.57$; $P < 0.0005$). Serum E_2 concentrations at rest recorded after 3 days on the M or L-CHO diet, in the MF phase were 186.6 (51.3) or 248.4 (57.6) $pmol \cdot l^{-1}$, respectively, and they were significantly higher ($P < 0.01$) in the ML phase [388.7 (43.4) or 432.4 (98.1) $pmol \cdot l^{-1}$, respectively]. Similarly, serum progesterone concentrations at rest after the M or the L-CHO diet in the MF phase were 2.03 (0.16) or 1.74 (0.19) $nmol \cdot l^{-1}$, respectively, and they were significantly higher ($p < 0.0005$) in the ML phase [37.17 (2.51) or 32.82 (5.16) $nmol \cdot l^{-1}$, respectively]. These results confirmed that the subjects were in the desired phase of their menstrual cycles for each of the exercise trials.

The main goal of our study was to investigate whether a substantial alteration in the macronutrient composition of the diet and changes in oestrogen concentrations during the menstrual cycle would affect rest and post-exercise activities of the antioxidant enzymes present in blood. The results are presented in Table 3. Ingestion of the L-CHO diet had no significant effect on the activity of SOD, which did not depend on the phase of the menstrual cycle either. There was a tendency for slightly lower SOD activities to occur immediately post-exercise with a further return, during the recovery, to the levels close to or slightly higher than those recorded pre-exercise. Despite the lack of significant differences between the pre- and post exercise values a repeated-measures 3-way ANOVA showed that there was a significant ($F = 4.35$; $P < 0.01$) time-effect. There was a tendency towards higher CAT activities while the subjects were on the L-CHO diet; the effect of the diet appeared to be significant ($F = 20.27$; $P < 0.0005$ by 3-way ANOVA). The activity of CAT tended to increase in response to exercise and, depending on the diet consumed, it reached its maximal value immediately post-test (after the M diet) or after 1 h of recovery (after the L-CHO diet). There was a significant time-effect ($F = 4.39$; $P < 0.01$ by 3-way ANOVA).

Irrespective of either the diet consumed or the phase of the menstrual cycle the post-exercise changes in GSH-Px activities were small and insignificant. After the L-CHO diet the rest enzyme activities tended towards slightly higher values. Whole blood selenium concentration, as assessed at rest, was higher after the L-CHO diet [63.3 (7.8) and 69.9 (10.8) μg selenium·l^{-1} during MF and ML, respectively] compared with the M diet [53.9 (9.9) and 55.7 (7.7) μg selenium·l^{-1}], and, as shown by 2-way ANOVA, the effect of the diet appeared to be significant ($F = 11.36$; $P < 0.005$). Daily dietary intakes of selenium were not evaluated as no data concerning selenium content in Polish foods were available. However, it seems justified to presume that a high daily consumption of eggs, meat and white cheese with the L-CHO meals would guarantee a higher, compared with the M diet, selenium intake.

After both diets, there was a trend towards higher GR during the follicular phase. The effect of the phase of the menstrual cycle on GR activity was very close to the level of significance ($F = 7.66$; $P = 0.0107$ by 3-way ANOVA). The GR activity at rest appeared to be negatively correlated with E_2 (Spearman rank test $r = -0.45$; $P = 0.016$) and progesterone ($r = -0.57$; $P < 0.005$).

The next goal of the present study was to examine whether the macronutrient composition of the diet, menstrual cycle phase-related changes in E_2 concentrations and graded cycling exercise would affect low molecular weight antioxidants, such as concentrations of GSH, α-tocopherol and UA as well as MDA as a lipid peroxidation marker – the results are summarized in Table 4. No significant effect of the diet, phase of the menstrual cycle, or exercise on blood GSH concentrations was found. Plasma α-tocopherol concentrations, which were within the reference range for subjects with an adequate α-tocopherol status (i.e. more than 16.2 μmol·l^{-1} or 6.5 mg·l^{-1}; Morrissey and Sheehy 1999), tended to increase immediately post-test, and then to decline after 24 h of recovery, to a level lower than that recorded at rest (Table 4). Plasma concentrations of α-tocopherol were slightly higher in subjects consuming L-CHO meals but the effect of the diet did not reach significance ($F = 4.50$; $P = 0.044$ by 3-way ANOVA). The

Table 3. Mean (SEM) changes in activities of the antioxidant enzymes: superoxide dismutase (*SOD*), catalase (*CAT*), glutathione peroxidase (*GSH-Px*) and glutathione reductase (*GR*) in response to diet composition, phase of the menstrual cycle and graded exercise. *U* Units (for definitions of units see Methods), *k* rate constant of a first order reaction of hydrogen peroxide decomposition related to the haemoglobin content. *Rest* pre-exercise

Parameter	Time of sample	Mixed diet		Low-carbohydrate diet	
		Mid-follicular phase	Mid-luteal phase	Mid-follicular phase	Mid-luteal phase
SOD (U·gHb^{-1})	Rest	883.5 (63.3)	838.4 (38.9)	875.4 (93.3)	892.0 (71.5)
	3 min post	870.3 (50.1)	748.4 (50.1)	785.2 (42.5)	779.4 (53.6)
	1 h post	888.8 (85.1)	912.9 (85.9)	910.1 (55.8)	849.8 (60.7)
	24 h post	931.4 (72.5)	902.4 (93.7)	871.7 (26.4)	1,034.4 (74.5)
CAT (k·g Hb^{-1})	Rest	152.0 (10.2)	207.8[c] (17.5)	208.0[b] (12.2)	221.7 (15.4)
	3 min post	192.4 (14.0)	228.0 (18.7)	241.2 (10.9)	240.5 (9.7)
	1 h post	178.3 (18.9)	206.4 (17.5)	254.4[b] (5.1)	265.3 (8.4)
	24 h post	209.1[a] (16.0)	213.5 (20.7)	233.7 (13.4)	232.6 (9.3)
GSH-Px (U·gHb^{-1})	Rest	19.3 (2.3)	20.3 (2.5)	22.9 (1.4)	24.2 (1.2)
	3 min post	20.4 (3.5)	18.8 (2.7)	25.3 (1.7)	23.0 (1.1)
	1 h post	18.4 (2.4)	19.6 (2.3)	25.6[b] (2.7)	20.3 (1.6)
	24 h post	20.0 (3.9)	22.2 (3.4)	24.6 (1.4)	21.0 (1.3)
GR (U·gHb^{-1})	Rest	21.8 (1.4)	15.3[c] (2.2)	21.8 (1.9)	17.8 (1.2)
	3 min post	21.8 (1.4)	16.1[c] (2.4)	20.8 (2.8)	16.9 (1.3)
	1 h post	23.0 (2.2)	18.5 (3.4)	23.2 (1.5)	17.7[c] (1.7)
	24 h post	21.4 (0.8)	16.6 (2.4)	20.7 (1.6)	17.7 (1.6)

[a]Significantly different ($P < 0.05$) from the rest value
[b]Significantly different ($P < 0.05$) from the corresponding value after the mixed diet
[c]Significantly different ($P < 0.05$) from the corresponding value in the mid-follicular phase

Table 4. Mean (SEM) changes in blood glutathione (*GSH*), plasma α-tocopherol (vitamin E), uric acid and malondialdehyde (*MDA*) concentrations in response to diet composition, phase of the menstrual cycle and graded exercise. *Rest* pre-exercise

Parameter	Time of sample	Mixed diet		Low-carbohydrate diet	
		Mid-follicular phase	Mid-luteal phase	Mid-follicular phase	Mid-luteal phase
GSH ($\mu g \cdot mgHb^{-1}$)	Rest	2.4 (0.3)	2.2 (0.3)	2.4 (0.3)	2.3 (0.3)
	3 min post	2.9 (0.3)	2.4 (0.3)	2.7 (0.3)	2.5 (0.4)
	1 h post	2.6 (0.2)	2.0 (0.3)	3.0 (0.3)	2.2 (0.3)
	24 h post	2.6 (0.2)	2.2 (0.3)	2.6 (0.3)	2.2 (0.3)
α-tocopherol ($mg \cdot l^{-1}$)	Rest	11.4 (0.5)	12.1 (1.1)	12.4 (0.7)	12.3 (0.4)
	3 min post	11.5 (0.5)	12.3 (0.7)	14.2[b] (0.8)	13.0 (0.4)
	24 h post	11.2 (0.7)	10.8 (0.9)	12.1 (0.4)	11.7 (0.6)
Uric acid ($mg \cdot dl^{-1}$)	Rest	3.3 (0.4)	3.6 (0.2)	4.2 (0.3)	3.7 (0.2)
	3 min post	3.7 (0.4)	3.6 (0.3)	3.6 (0.6)	3.2 (0.2)
	1 h	5.6[a] (0.5)	4.5 (0.4)	4.7 (0.5)	3.4 (0.3)
	24 h	4.1 (0.5)	5.3[a] (0.4)	5.1 (0.3)	4.5 (0.4)
MDA ($\mu mol \cdot l^{-1}$)	Rest	4.1 (0.6)	4.3 (0.6)	3.6 (0.5)	4.0 (0.8
	3 min post	5.4 (0.8)	5.3 (0.9)	4.5 (0.7)	4.2 (0.7)
	1 h post	3.8 (0.5)	5.6 (0.9)	4.6 (0.7)	4.3 (0.6)
	24 h post	4.6 (0.9)	4.1 (0.6)	3.7 (0.6)	3.3 (0.6)

[a]Significantly ($P < 0.05$) different from the rest value
[b]Significantly ($P < 0.05$) different from the corresponding value after the mixed diet

UA concentration in plasma was affected neither by the diet consumed nor the phase of the menstrual cycle. There was a trend for a delayed increase in UA during the recovery from acute graded cycling exercise, which was reflected by a significant time effect ($F = 11.05$; $P < 0.001$ by 3-way ANOVA). Irrespective of the diet consumed and the phase of the menstrual cycle there were no significant differences between the pre- and post-exercise plasma MDA concentrations, though they tended to be slightly lower after the L-CHO diet.

Lipid peroxidation is considered to be one of the major factors responsible for an increase in the permeability of cell membranes, which results in a *leakage* of intracellular proteins into the circulation. The process is known to be enhanced during strenuous exercise and seems to be indicative of damage to the muscle membrane. Therefore, an attempt was made to evaluate exercise-induced changes in the activity of CK in the plasma; the results are presented in Table 5. There was a marked tendency towards a decrease in both pre- and post-exercise CK activities after eating L-CHO meals for 3 days; the effect of the diet appeared to be significant ($F = 26.43$; $P < 0.0001$ by 3-way ANOVA). Irrespective of the diet consumed, CK activity tended to increase immediately after the cessation of exercise and grew further to a peak value attained after 1 h of recovery after the M diet or tended to decline to the baseline in subjects eating the L-CHO diet. All CK activity data were within the reference range for women (24–175 $U \cdot l^{-1}$; Szasz et al. 1976). With the aim of revealing an eventual relationship between activity of creatine kinase at rest (CK_0) and the remaining variables (the oestrogens included) to characterize the blood antioxidant system a multiple stepwise regression analysis was applied. A significant negative relationship was found between CK_0 and catalase (CAT_0) activity at rest ($P < 0.01$) and concentration of selenium ([*Selenium*]) ($P < 0.05$) as formulated in the following regression equation:

$$CK_0 = -0.48 \times CAT_0 - 0.34 \times [\text{selenium}] + 112.79$$

(coefficient of determination $r^2 = 0.43$, $P = 0.00091$).

Discussion

The most important finding of this investigation was that the L-CHO diet appeared to have a favourable effect on the blood antioxidant defence system in healthy and physically fit female subjects, both at rest and during graded cycling exercise to $\dot{V}O_{2max}$.

Table 5. Mean (SEM) changes in activities of plasma creatine kinase (*CK*, EC 2.7.3.2) in response to diet composition, phase of the menstrual cycle and graded exercise. *U* Units (for definitions of units see Methods), *Rest* pre-exercise

Parameter	Time of sample	Mixed diet		Low-carbohydrate diet	
		Mid-follicular phase	Mid-luteal phase	Mid-follicular phase	Mid-luteal phase
CK ($U \cdot l^{-1}$)	Rest	73.8 (4.8)	70.6 (4.2)	56.9 (1.3)	54.2 (2.3)
	3 min post	76.2 (5.0)	76.3 (4.1)	76.0 (6.8)	63.9 (3.7)
	1 h post	79.1 (5.1)	79.9 (7.0)	62.5 (4.8)	60.6[a] (4.2)
	24 h post	78.5 (4.1)	74.4 (5.5)	61.2 (6.0)	56.9 (4.4)

[a]Significantly ($P < 0.05$) different from the corresponding value after the mixed diet

The composition of the diet is critical for adequate provision of antioxidant nutrients such as vitamins, minerals essential for antioxidant enzymes and amino acids with antioxidant capacity. In this respect, one may presume that L-CHO meals would provide higher intakes of lipophylic nutrients such as α-tocopherol and vitamin A. It is well known that all antioxidant enzymes require trace elements such as selenium, zinc, copper, iron and manganese. A high protein diet based on a large intake of red meat will also provide greater quantities of readily available haem iron, which is indispensable for synthesis of CAT. The present study demonstrated that α-tocopherol and iron (mainly as haem iron) intakes with the L-CHO diet, as assessed from the food records, were about twice as high as those supplied with the normal M diet. It should be stressed that, with respect to the recommended dietary allowances, daily intakes of iron and α-tocopherol with the M diet were insufficient. Daily intake of vitamin C with the L-CHO meals was significantly lower than that provided with the normal (M) diet, however, even in the latter case, it was inadequate. Despite substantially higher daily α-tocopherol intakes with the L-CHO diet, there was only a tendency towards slightly higher plasma α-tocopherol concentrations in subjects consuming the high-fat-high-protein meals. In this respect our results are consistent with those reported by Sacheck et al. (2000) who also observed only a slight increase in plasma α-tocopherol concentrations in female athletes after 3 days of eating a high-fat (more than 60 g fat·day^{-1}) diet despite significant differences in daily α-tocopherol intakes. Our finding of a post-exercise rise in plasma α-tocopherol concentration supports observations made by other authors who explained this phenomenon by an enhanced mobilization of tocopherol from its body pool, mainly due to very low density lipoprotein breakdown during lipolysis (Pincemail et al. 1988). Such a response could be beneficial to the attenuation of an exercise-induced oxidative stress.

The results of the present study revealed that neither the diet, the exercise nor the phase of the menstrual cycle had any influence on erythrocyte SOD activity, which was maintained within the physiological range for healthy adults (750–1,200 U·gHb^{-1}; Fitzgerald et al. 1992). Our findings support observations made by other authors who have also reported that menstrual cycle-related changes in oestrogen concentrations had no influence on the erythrocyte SOD activity at rest (Massafra et al. 2000; Akova et al. 2001). Similarly, GSH-Px activities were not significantly altered by either the diet composition, phase of the menstrual cycle or exercise, although they were slightly higher after the L-CHO diet, which might have been related to a higher ($P < 0.005$), compared to the M diet, concentration of blood selenium. It should be stressed, however, that blood selenium concentrations in the majority of our subjects, after both diets, were relatively low and indicative of marginal selenium deficiency, as it did not reach the lower limit of the reference range set at 80–250 μg·l^{-1} (Koller and Exon 1986). These findings support our previous report that documented an inadequate blood selenium status in the Polish population of young adults (Kłapcińska et al. 1998).

Dietary status was found to have influence on CAT, as the activity of this enzyme was enhanced following a 3 day consumption of L-CHO meals. It seems likely that the positive effect of the L-CHO diet on CAT may have relied on a higher (almost double) dietary intake of iron provided by the large portion of red bovine meat in the L-CHO meals. The CAT is known to be a haem protein containing four ferriprotoporphyrin groups per molecule, corresponding to an iron content of 0.09% (Aebi 1974). Therefore, it seems likely that higher haem iron intake with the L-CHO diet might have resulted in an increase of erythrocyte CAT content. Acute graded cycling exercise, as well as the oestrogen status, had only minor effect on CAT.

The GR was the sole antioxidant enzyme having an activity affected by the menstrual cycle-related changes in oestrogens concentration, as higher pre- and post-exercise activities of the enzyme were recorded in the MF phase. In this respect, our findings are in agreement with those reported by Kanaley and Ji (1991) who found that GR activity during exercise was significantly higher ($P < 0.01$) in amenorrhoeic (low E_2) compared to eumenorrhoeic women. The significant negative correlation, as found in the present study, between GR activity and E_2 and progesterone concentrations, additionally supports the hypothesis that the hormone status in healthy women may affect the antioxidant enzyme system in the blood. The GR plays a vital role in preserving a stable intracellular redox state and antioxidant defence by maintaining an adequate ratio of GSH to GSSG. The GSSG concentration should be kept low (50–100-fold less than GSH) as GSSG is toxic to the cell because of its capacity to trigger disulphide cross-linkage of proteins, enzymes and DNA (Ji 1995). The GSH is used by GSH-Px for reduction of H_2O_2 and peroxyl radicals. It also serves as a scavenger of hydroxyl radicals or singlet oxygen, and is involved in the regeneration of α-tocopherol and ascorbate. Liver is the major organ for de novo GSH synthesis from where it can be mobilized to other tissues such as skeletal muscles. Hepatic GSH efflux into the plasma was found to be under hormonal control exerted by cyclic adenosine monophosphate-dependent factors, glucagon (Lu et al. 1990) or adrenaline (Sies and Graf 1985). This may, most likely, explain the slight post-exercise increases in GSH concentration seen in our subjects as both glucagon and plasma catecholamines (adrenaline and noradrenaline) concentrations were higher at the cessation of the $\dot{V}O_{2max}$ test (Manowska et al. 1999). Additional factors that might have contributed to the post-exercise increase in GSH concentrations could have been a decreased blood flow through kidney and a lower GSH uptake by this organ during exercise (Meister and Anderson 1983). Our observations on exercise-induced alterations in GSH status are in contrast to those of Chung et al.

(1999) who reported that blood GSH concentrations in women subjected to submaximal treadmill running exercise (30 min at 75%–80% $\dot{V}O_{2max}$) decreased significantly post-test. It should be stressed, however, that similarly conflicting results on exercise-induced alterations in GSH status were obtained from research with men in whom an increase (Sahlin et al. 1991), no change (Gohil et al. 1988) or a decrease (Laaksonen et al. 1999) have been reported. As was shown by Gohil et al. (1988), the most likely reason for these discrepancies may be the protocol of the exercise, which was found to modify the response, as prolonged submaximal exercise (90 min at 65% $\dot{V}O_{2max}$) resulted in a substantial GSH decrease, whereas no significant changes in blood GSH concentrations were found after exercise to $\dot{V}O_{2max}$. On the other hand, our results are in agreement with those reported by Chung et al. (1999) that menstrual cycle phase had only a minor influence on blood GSH concentration, though there was a trend towards slightly higher values during the MF phase whilst GR activities were higher.

During strenuous exercise, when the rate of ATP hydrolysis exceeds that of its resynthesis, purine nucleotide catabolism is activated, which results in hypoxanthine and UA accumulation in plasma (Sahlin et al.1991). The UA is a powerful antioxidant present in plasma, capable of scavenging singlet oxygen and radicals (Ames et al. 1981), thus protecting erythrocytes against peroxidative damage. Non-strenuous exercise performed below the anaerobic threshold was found to have no influence on purine nucleotide degradation (Yamanaka et al. 1992). In the present study neither the diet nor the phase of the menstrual cycle affected plasma UA concentration, but there was a significant time-effect with a marked trend to a delayed rise in UA level during recovery, which may imply an involvement of adenylate kinase reaction in ATP resynthesis during exhausting graded cycling exercise. In this respect our findings are consistent with those reported by other authors (Sahlin et al. 1991).

Exercise-induced oxidative stress is considered to be the main cause of lipid peroxidation which leads to a loss of fluidity and an increase in the permeability of cell membranes, followed by a release of cytosolic proteins and enzymes into the blood. An attack by free radicals on polyunsaturated fatty acids in cell membranes results in formation of hydroperoxides that are subsequently decomposed to various aldehyde compounds, MDA being the major product (Rice-Evans et al. 1991). In the present study none of the factors tested, neither the diet nor the cycle-phase related changes in oestrogen status nor exercise had a significant influence on plasma MDA concentration. Similar results were obtained by Sacheck et al. (2000) who also observed only a slight trend towards lower pre- and post-exercise plasma MDA concentrations in female athletes habitually consuming a high-fat diet and subjected, during the early follicular phase, to a submaximal (45 min at 75% $\dot{V}O_{2max}$) downhill (-10°) treadmill run. Our findings are also consistent with those reported by Chung et al. (1999) and Akova et al. (2001), who found that submaximal exhausting exercise performed by healthy women had only a minor effect on plasma MDA concentration.

One of the most interesting findings of the present study was that an increase in dietary fat and protein intake while ingesting the L-CHO diet (main effect) resulted in a significant decrease in CK activity. Surprisingly, CK activity at rest did not significantly depend on oestrogen concentrations, despite the evidence given by many authors who have pointed to the antioxidant properties of E_2 and the important role of female sex-hormones in maintaining the integrity of the cell membrane post-exercise and limiting CK release from skeletal muscle (Tiidus 2000; Bar et al. 1988). Using multivariate regression analysis, both the activity of CAT at rest and whole blood selenium concentration were found to be strong determinants of the activity of CK at rest, thus suggesting that the short-term consumption of the L-CHO diet may have resulted in a better, compared to the normal M diet, protection of skeletal muscle membranes against peroxidative damage.

In conclusion, this study showed that eating a L-CHO diet for 3 days , when preceded by glycogen-depleting exercise, resulted in a stimulation of the blood antioxidant defence system in healthy eumenorrhoeic women, both at rest and during graded cycling exercise to $\dot{V}O_{2max}$. It seems justified to presume that the higher daily intakes of haem iron, selenium and α-tocopherol provided with the L-CHO diet contributed to the enhancement of CAT activity, the rise in plasma concentrations of selenium and α-tocopherol, which resulted in a better protection of the cell membranes against exercise-induced peroxidative damage, as reflected by the limited release of CK into the plasma. With the exception of GR, the phases of the menstrual cycle had only minor effects on indices of the blood antioxidant defence system.

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