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ORIGINAL ARTICLE

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Exercise-induced oxidative stress and muscle performance in healthy women: role of vitamin E supplementation and endogenous oestradiol

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Abstract The purpose of this study was to investigate the individual and combined antioxidant effects of menstrual cycle phase-related alterations in blood serum oestradiol concentrations and of dietary vitamin E supplementation on exercise-induced oxidative stress and muscle performance. A group of 18 sedentary women, aged 19–35 years, were given supplements of 300 mg α-tocopherol ($n = 10$) or placebo ($n = 8$) daily during the course of two menstrual cycles. The subjects exercised the knee isokinetically to exhaustion after cycling submaximally at 50% maximal oxygen uptake during the menstrual and preovulatory phases of their menstrual cycles. Blood samples were taken before and after the exercise, to evaluate haematocrit, plasma lactic acid and malondialdehyde concentrations, erythrocyte antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and apolipoprotein B containing lipoprotein (non-high density lipoprotein, HDL, fraction) oxidation. Serum vitamin E, follicle stimulating hormone, luteinizing hormone and oestradiol concentrations were measured in pre-exercise blood samples. Neither vitamin E supplementation nor oestradiol concentrations influenced SOD and GPx activities or the susceptibility of the non-HDL fraction to oxidation while at rest. Plasma malondialdehyde concentration was unaffected by exercise, however significant reductions in erythrocyte SOD and GPx activities and increased susceptibility of the non-HDL fraction to oxidation were noted after exercise. Exercise-induced changes were reduced when oestradiol concentration was high in the preovulatory phase, independent of the serum vitamin E concentrations. In addition, both pre- ($r = 0.58$, $P < 0.05$) and post-exercise ($r = 0.73$, $P < 0.001$) GPx activities in placebo administered subjects were positively correlated with oestradiol concentrations. In conclusion, these findings suggest a better protective role of oestradiol against oxidative injury, compared to vitamin E. Exhausting muscle performance was, however, not influenced by vitamin E supplementation and/or cycle-phase related changes in oestradiol concentrations.

Key words Free radical · Menstrual cycle · Exhausting exercise · Antioxidants

Introduction

Strenuous physical exercise is known to induce oxidative stress and free radical generation in many biological tissues, and free radicals have been implicated in the peroxidation of lipids (Dekkers et al. 1996). Numerous investigations have shown increases in byproducts of lipid peroxidation following exercise, and malondialdehyde (MDA), a byproduct of lipid peroxide, has been very commonly studied as a marker of oxidative tissue damage during exercise (Duthie et al. 1990). Oxidation of plasma lipoproteins have been studied in various models and measuring their susceptibility to oxidation has attracted attention as a means of evaluating oxidative stress to lipoproteins (Arteaga et al. 1998; Chajés et al. 1996; Santanam et al. 1998; Wetzstein et al. 1998).

Given the potential role of the reactive species in mediating tissue damage, cells contain several naturally occurring antioxidant defence mechanisms to prevent oxidative injury. The enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx) are important components of this protective system. Favourable changes in antioxidant enzyme activities after exercise have been reported in several studies (Aslan et al. 1998; Dekkers et al. 1996; Goldfarb et al. 1994; Sürmen-Gür et al. 1999).

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Vitamin E is well accepted as an effective lipid-soluble, chain-breaking antioxidant. Oestrogens have been reported to have significant antioxidant potential as well, and various investigations have been carried out to elucidate the effects of female sex hormones on lipid peroxidation (Massafra et al. 1996; Nakano et al. 1987; Tranquilli et al. 1995). Of these, several animal studies have shown that oestrogen inhibits lipid peroxidation in serum as well as tissue preparations (Nakano et al. 1987). Studies on human subjects have consistently supported the notion that oestrogen has potent antioxidant properties. Suematsu et al. (1977) noted lower concentrations of serum lipid peroxidation byproducts in women compared to men. Tranquilli et al. (1995) indicated reduced lipid peroxidation with oestrogen replacement during the menopause. It has been demonstrated that oestrogens can significantly inhibit low density lipoprotein (LDL) oxidation in vitro (Arteaga et al. 1998). The preventative effect of oestrogens in post-exercise muscle damage has been also demonstrated in a variety of animal and human models (Nakano et al. 1987; Tiidus and Houston 1993, 1994). Despite the fact that both oestrogens and vitamin E are potent inhibitors of lipid peroxidation, few studies have been designed to compare their individual or combined effects (Tiidus and Houston 1993, 1994), and these works have been limited to animal studies. Human studies seeking to elucidate the antioxidant properties of oestrogens have been mostly carried out on hormone replaced amenorrhoeic or postmenopausal subjects (Massafra et al. 1996; Santanam et al. 1998). In some cases responses of female subjects have been compared with those of male subjects, without taking the phase of the menstrual cycle into consideration (Suematsu et al. 1977). In a recent work, Chung et al. (1999) have shown that menstrual cycle phase had no significant influence on exercise-induced changes in blood glutathione status and plasma lipid peroxidation indices.

In the present study we hypothesized that cycle phase related elevations in oestradiol concentration would be as effective as vitamin E supplementation in protecting against exercise-induced oxidative stress, and that the effects of these two antioxidants together could be stronger than individually. Since there is lack of data comparing the influence of menstrual cycle phases and vitamin E on exercise induced oxidative stress in humans, an investigation on human subjects would be preferable to test this hypothesis. Therefore, in this study we aimed to compare the possible protective effects of vitamin E supplementation and endogenous oestrogens on post-exercise oxidative stress in women. To evaluate oxidation and antioxidant status, we examined plasma MDA, erythrocyte SOD and GPx activities, serum vitamin E and oestradiol (E2) concentrations, and susceptibility of apolipoprotein B containing lipoproteins (non-high density lipoprotein, HDL, fraction) to oxidation, which include very LDL and lipoprotein (a) together with LDL. On the other hand, since the effects of menstrual cycle on muscle performance (Jurkowski et al. 1981) and the preventive effect of vitamin E supplementation on exercise-induced muscle damage have been previously documented (Dekkers et al. 1996), we also investigated the combined effects of endogenous E2 concentration and vitamin E supplementation on muscle performance in fatiguing exercise.

Methods

Subjects

A group of 18 healthy sedentary women, aged 19–35 years, having normal menstrual cycles, who had not taken oral contraceptive drugs for the last 6 months, volunteered for the study. The subjects completed a questionnaire on menstrual status to ascertain their cycle regularity and were included in the study if it had been regular for three consecutive cycles and the average period of the cycles was between 28–35 days. After being informed about the study and test procedures, and the possible risks and discomfort that might ensue, they gave their written informed consent to participate. The volunteers were randomly divided into two groups, a vitamin E group (E), which was given a supplement of 300 mg $\cdot$ day^{-1} vitamin E (D-L α-tocopherol, $n = 10$), and a placebo group (P), which took a placebo comprising glucose ($n = 8$), during the course of two consecutive menstrual cycles. The intervention or treatment started with either menses or the preovulatory phase, according to the test order (described below). The mean duration of supplementation was 42 (SD 3) days and there were no complications to warrant any interruption of the supplementation. Selected physical characteristics of the subjects are presented in Table 1. When the subjects were interviewed about their occupational and leisure-time physical activities, none of them reported having been regularly engaged in leisure-time physical activity such as walking, running, swimming or other exercise during the last 10 years. Therefore, all subjects were at Level I according to the activity levels (for both occupational and leisure-time activities) that have been defined by Saltin and Grimby (1968). The activity level for all subjects remained relatively constant during the period of the experiment.

Exercise tests

To evaluate maximal oxygen consumption ($\dot{V}O_{2max}$) and the exercise intensity equivalent to 50% of $\dot{V}O_{2max}$, the subjects exercised to maximal capacity following a 5 min warm-up by cycling (Monark 814E, Sweden) at 75 W. The exercise intensity during the test was changed every 3 min by 60 W from an initial 100 W until the subjects reported that they could not exercise any more. The subjects were instructed to maintain their pedalling rates as close to 60 rpm as possible and the mean pedalling rate recorded was 60 (SD 3) rpm for the tests. When the pedalling rate fell to 55 rpm, the subjects were verbally encouraged to pedal faster. If the subject

Table 1 Selected physical characteristics and basal vitamin E concentrations of the subjects in vitamin E (*E*) and placebo (*P*) groups. $\dot{V}O_{2max}$ Maximal oxygen uptake

	E (n = 10)		P (n = 8)	
	Mean	SD	Mean	SD
Age (years)	26	6	27	8
Height (cm)	165	6	158	8
$\dot{V}O_{2max}$ (ml $\cdot$ kg^{-1} $\cdot$ min^{-1})	29.7	6.7	28.9	4.3
Cycle period (days)	29	2	30	2
Basal vitamin E (mg $\cdot$ dl^{-1})	1.22	0.22	1.33	0.33

No significant differences between the groups by Student's-*t* test

could not return to the required rate, the test was terminated. During the maximal test, ventilatory parameters were continuously measured breath-by-breath using a metabolic analyser (SensorMedics 2900C system, USA). The criteria for having achieved $\dot{V}O_{2\,max}$ were set at reaching a maximal heart rate with respect to age (220-age), a ventilatory equivalent for O_2 (minute ventilation/oxygen uptake) of close to $30\ l \cdot min^{-1}$ and respiratory exchange ratio greater than 1.15. All the tests met these criteria.

To evaluate the maximal total work of the extensor muscles of the dominant knee, the subjects were familiarized with the test procedures and performed three consecutive submaximal warm-up trials They then performed four maximal concentric(CON)-eccentric(ECC) combined contractions at an angular velocity of 60° a second. The test was conducted on a Cybex 6000 (USA) computer controlled isokinetic dynamometer, which was calibrated before every test session. The subjects were positioned sitting with the backrest at a 90° angle and were instructed to grip the sides of the seat during the tests. The thigh, pelvis and trunk were stabilized with straps. An adjustable lever arm was attached to the leg by a padded cuff just proximal to the lateral malleolus. The axis of rotation of the dynamometer arm was positioned just lateral to the lateral femoral epicondyle.

Fatigue test

The subjects abstained from strenuous physical activity the day before and on the day of the test. Following a light breakfast 3 h before the tests, they arrived at the laboratory and skinfolds were measured at four sites, namely triceps, biceps, suprailiac and subscapular, using skinfold calipers (Holtain, England), and body fat percentages were calculated from the equation suggested by Womersly and Durnin (1974). After resting for 20 min lying down on a bed, blood samples (pre-exercise) were collected.

Following the rest, the subjects cycled for 15 min at 50% $\dot{V}O_{2\,max}$, rested for 3 min and then performed three periods of exhausting isokinetic exercise, which consisted of CON-ECC maximal contractions of the extensor muscles of the dominant knee until exhaustion, with 1 min rest between the period of exercise. During the fatigue test, CON and ECC total work, number of repetitions and duration until exhaustion were recorded as parameters of performance. A fall to 50% of the maximal CON total work, which had been determined in the earlier tests, in two consecutive contractions was accepted as the index of fatigue. The tests were carried out during the menstrual (M, day 1–3 of the cycle) and preovulatory (PO, day 12–16 of the cycle) phases, at the same time of day, allowing 12- to 16-day intervals between tests for recovery of the muscles. The order of the tests was randomized for subjects during the course of two consecutive menstrual cycles as PO-M (50% of the subjects in each group) and M-PO (the rest of the subjects in each group), to minimize the effects of fatigue and subjects' adaptations to procedures and device. The estimated cycle phases were subsequently verified by measurements of plasma concentrations of follicle stimulating hormone (FSH), luteinizing hormone (LH), and E2, evaluated as described below.

Collection and treatment of blood samples

Blood samples were taken from an antecubital vein at rest and immediately after exercise. Pre- and post-exercise blood samples were kept on ice and protected from light during sample preparation. Haematocrit (Hct) as well as lactic acid (LA) concentrations were measured on the day of the experiment. Whole blood samples, separated for SOD and GPx measurements, were kept at +4 °C and studied within 48 h. Plasma separated for MDA measurements and sera separated for vitamin E measurements were kept at −40 °C until studied within 1 week. The remaining sera were used to determine FSH, LH, and E2 concentrations. Plasma processed for non-HDL fraction oxidation was studied on the day of the experiment. All parameters were evaluated in both pre- and post-exercise blood samples, while vitamin E and hormone concentrations were assayed only in pre-exercise blood samples.

Biochemical analyses

The Hct were determined using Abbott Cell-Dyn (USA). As an index of plasma lipid peroxidation, plasma MDA concentrations were determined by measuring the thiobarbituric acid reactive substances according to the spectrophotometric method of Kamal et al. (1989), using 1,1,3,3-tetraethoxypropane (Fluka, Switzerland) as the external standard. Susceptibility of the non-HDL fraction to oxidation was determined by using the method described by Zhang et al. (1994) and expressed in terms of lag time (minutes).

To evaluate blood antioxidant status, erythrocyte SOD and GPx activities were measured using test kits (Randox, UK), and expressed as units per millilitre Hct. Serum vitamin E concentrations were measured using the method described by Varley et al. (1976), and expressed as milligrams per decilitre. Plasma LA concentrations were determined using commercial kits (BioMerieux, France) and expressed as millimoles per litre. The FSH, LH and E2 concentrations were determined using commercial kits of ACS 180 (USA), in ACS 180 autoanalyser (Chiron/ Diagnostics, USA). All plasma or serum post-exercise values were adjusted for haemoconcentration using the following equation, derived according to the suggestion of Van Beaumont (1981) and used in evaluating the results:

$$\text{post-exercise}_{\text{adjusted}} = \text{post-exercise}[\text{Hct}_{\text{pre}}(100\text{-Hct}_{\text{post}})]/[\text{Hct}_{\text{post}}(100\text{-Hct}_{\text{pre}})]$$

Statistics

Statistical differences were investigated using Student's *t*-test for comparison in selected physical characteristics. Two-way factorial analysis of variance (ANOVA) for repeated observations was used for multiple comparison between the groups and occasions. When a significant *F*-value was found Scheffé post hoc tests were performed. To estimate whether changes in the parameters were clinically meaningful, the standardized response means (mean change divided by SD of the change) were calculated to measure the effect size. An effect size of 0.20–0.49 was considered as small, 0.50–0.79 as moderate and more than 0.80 as large (Thomas and Nelson 1990).

Data gathered during the PO and M phases were pooled for E ($n = 20$) and P ($n = 16$) groups, separately, and Pearson product moment correlation was then performed to test the relationships between E2 concentrations and the remaining parameters, and between vitamin E concentrations and the remaining parameters. Statistical significance was accepted for $P < 0.05$.

Results

The physical characteristics of the subjects in E and P groups were comparable (Table 1). Body mass and body fat percentages were similar during two phases without any statistical difference between E and P groups (Table 2). The basal total vitamin E concentrations in serum were no different between the E and P groups before supplementation began, however significant elevations were recorded in the E group after supplementation ($P < 0.001$) independent of the phase of the menstrual cycle. The FSH, LH and E2 concentrations of E and P groups at PO and M are presented in Table 3. The E2 concentrations in both groups were significantly

Table 2 Vitamin E concentrations after supplementation (*Vit E suppl.*), body mass and percentage of body fat of the subjects at preovulatory (*PO*) and menstrual (*M*) phases of the menstrual cycle in vitamin E supplemented (*E*) and placebo (*P*) groups

	E (n = 10)				P (n = 8)			
	PO		M		PO		M	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Vit E suppl. (mg · dl^{-1})	2.11[a]	0.40[a]	2.09[a]	0.33[a]	1.24	0.21	1.41	0.19
Body mass (kg)	60.2	7.1	60.3	6.6	58.4	5.8	58.1	5.7
Body fat (%)	19.8	3.9	19.1	3.7	21.7	5.1	21.7	4.9

[a] Difference ($P < 0.05$) in comparison with *PO* (*P*) and *M* (*P*), respectively, by ANOVA

Table 3 Follicle stimulating hormone (*FSH*), luteinizing hormone (*LH*), and oestradiol concentrations of the subjects during the preovulatory (*PO*) and menstrual (*M*) phases of the menstrual cycle in vitamin E supplemented (*E*) and placebo (*P*) groups

	E (n = 10)				P (n = 8)			
	PO		M		PO		M	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
FSH (mIU · ml^{-1})	4.9	2.3	4.7	2.6	3.6	1.5	5.0	2.6
LH (mIU · ml^{-1})	12.6	6.8	5.6*	2.6*	8.4	4.9	4.8**	3.9**
Oestradiol (pg · ml^{-1})	175[a]	77[a]	79***	23***	145[a]	48[a]	65***	33***

*,**,***$P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively, between *PO* and *M*, by ANOVA
[a] Differences ($P < 0.05$) in comparison with *M* (*E*) and *M* (*P*), by ANOVA

greater in PO compared to M ($P < 0.001$), and were not significantly different between the two groups in identical phases.

Pre-exercise lactate concentrations were not significantly different between groups and phases (mean range from 1.2 to 1.5 mmol · l^{-1}), by ANOVA. Post-exercise lactate concentrations were significantly elevated (mean range from 6.6 to 8.2 mmol · l^{-1}, $P < 0.001$) in all groups and phases, and the elevations were not significantly different between groups or phases, when tested by ANOVA. Pre- and post-exercise SOD and GPx activities, MDA concentrations and lag times in non-HDL fraction oxidation are presented in Fig. 1. None of these parameters were affected by vitamin E supplementation or by the phase of the menstrual cycle whilst at rest. When post-exercise SOD and GPx activities, MDA concentrations and lag times in non-HDL fraction oxidation of the two groups in the two different phases of the menstrual cycle were compared to their pre-exercise values by ANOVA, post-exercise SOD activity was significantly decreased in all comparisons and the decrements were more pronounced during menses in both groups, with greater effect sizes. The GPx activity, on the other hand, was significantly decreased only during the menstrual periods of the two groups, displaying greater effect sizes. Plasma MDA concentrations did not show any significant change after exercise, yet the effect sizes were greater in the P group compared to that in the E group. Post-exercise lag times in non-HDL fraction oxidation were significantly reduced in the M phase of both the E and P groups, with greater effect sizes, and were not changed in the PO phase. Effect sizes calculated for these parameters are presented in Fig. 2.

Correlation coefficients were statistically significant between E2 and post-exercise GPx ($r = 0.73$, $P < 0.001$) and pre-exercise GPx ($r = 0.58$, $P < 0.05$) for the P group (Fig. 3).

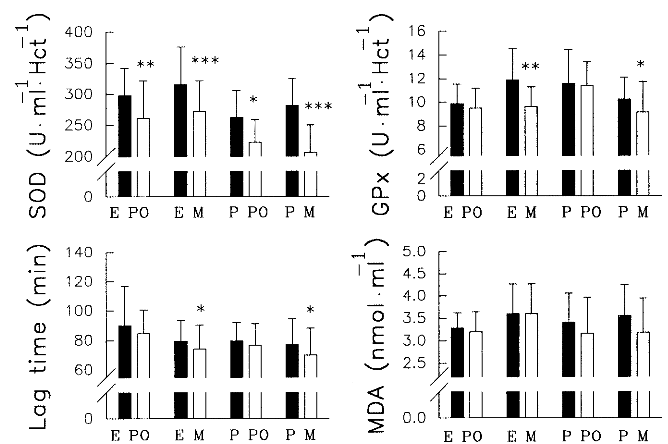

Fig. 1 Pre- (*filled bar*) and post-exercise (*unfilled bar*) values of superoxide dismutase (*SOD*), glutathione peroxidase (*GPx*), malondialdehyde (*MDA*) and lag time in non-high density lipoprotein fraction oxidation (*Lag time*) of the subjects at preovulatory (*PO*) and menstrual (*M*) phases of the menstrual cycle in vitamin E supplemented (*E*, $n = 10$) and placebo (*P*, $n = 8$) groups. *Hct* Haematocrit. *Figures* represent mean and SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to pre-exercise value by ANOVA

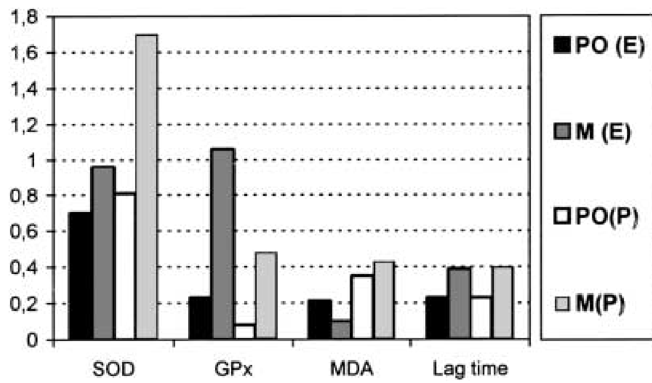

Fig. 2 The effect size (mean change following exercise divided by SD of the change following the exercise) of superoxide dismutase (*SOD*), glutathione peroxidase (*GPx*), malondialdehyde (*MDA*) and lag time in non-high density lipoprotein fraction oxidation (*Lag time*) of the subjects during the preovulatory (*PO*) and menstrual (*M*) phases of the menstrual cycle in vitamin E supplemented (*E*) and placebo (*P*) groups

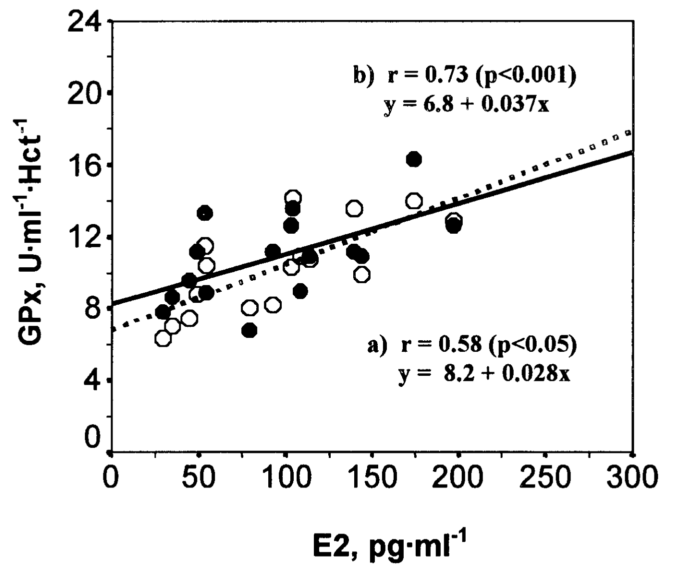

Fig. 3 Relationships: (*a*) between oestradiol (*E2*) and pre-exercise (*solid line* and *filled circle*) glutathione peroxidase (*GPx*); and (*b*) between *E2* and post-exercise (*dashed line* and *empty circle*) *GPx* in placebo group (n = 16). *Hct* Haematocrit

Three periods of CON and ECC total work, the number of maximal contractions and the duration of exercise until exhaustion in the knee extensor muscles were not significantly different between the groups or occasions (Table 4). In addition, these parameters for each of the three periods of exercise were not significantly different between the groups or occasions either (data not presented).

Discussion

Several human and animal studies have investigated the protection given by vitamin E and oestradiol against the pro-oxidative effects of exercise. The limited amount of work in the literature comparing the individual or combined effects of these antioxidants in human subjects led us to design the present study with eumenorrhoeic women, to compare the effects of changing amounts of endogenous oestradiol and a dietary supplement of vitamin E. Exhausting exercise has been reported to lead to oxidative damage and favourable changes in antioxidant status (Dekkers et al. 1996). Therefore, an incremental exercise model consisting of both ECC and CON contractions until exhaustion, following submaximal cycling exercise, was designed to be performed by sedentary women in two different, namely M and PO phases of their menstrual cycles.

Results of hormone concentrations reveal that the cycle phases were accurately estimated (Table 3). As expected, E2 concentrations were significantly elevated in PO compared to that in M and the parameters measured in these two phases were compared to indicate the effects of E2. The results of the present work also show that the dose of vitamin E used in the present study to supplement the diet significantly increased the plasma

Table 4 The results of concentric and eccentric total work (CON_{TW} and ECC_{TW} respectively), number of contractions (n_{contr}) and duration (time) until fatigue during the preovulatory (*PO*) and menstrual (*M*) phases of the menstrual cycle in vitamin E supplemented (*E*) and placebo (*P*) groups

		E (n = 10)				P (n = 8)			
		PO		M		PO		M	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Totals	CON_{TW} (J)	2294	615	2354	641	2326	953	2296	909
	ECC_{TW} (J)	2786	760	2750	765	2645	962	2751	1093
	n_{contr}.	35.9	5.4	35.6	6.1	35.1	6.2	35.6	6.7
	Time (s)	97.4	17.0	99.3	17.9	97.0	17.4	96.6	23.0
Ratios	CON_{TW}:n_{contr}.	64.7	17.3	66.6	16.4	65.0	19.0	63.4	17.3
	CON_{TW}:time	23.8	5.8	24.2	6.6	23.6	7.4	23.6	6.6
	ECC_{TW}:n_{contr}.	78.5	21.3	78.0	20.6	74.3	19.4	76.2	21.6
	ECC_{TW}:time	29.1	8.0	28.4	8.1	26.9	7.3	28.6	8.6

No significant differences between groups and occasions by ANOVA

vitamin E concentrations. The elevations in vitamin E concentrations were however not affected by the E2 oscillations throughout the menstrual cycle (Table 2).

There have been conflicting reports on the effect of vitamin E supplementation on plasma antioxidant enzyme activities (Goldfarb et al. 1994; Monget et al. 1996). Our results indicate that vitamin E, given in a dose of 300 mg $\cdot$ day^{-1} for approximately 6 weeks, had no influence on erythrocyte SOD and GPx activities at rest. Other studies which have examined the effects of E2 supplementation or menstrual cycle phase-related E2 concentrations on erythrocyte antioxidant enzymes, have indicated discrepant results (Massafra et al. 1996, 1998). Of these, Massafra et al. (1998) have indicated significant menstrual cycle phase-related changes in GPx with a moderate positive correlation between E2 and GPx changes, as observed in group P in the present study ($r = 0.58$). Gürdöl et al. (1997) have shown that SOD did not exhibit any significant difference between premenopause and early menopause subjects, suggesting that activity of this enzyme was not influenced by circulating E2 concentrations. Similarly, our results indicated that cycle related changes in E2 concentrations did not influence erythrocyte SOD activity or plasma MDA concentrations at rest.

According to the results of the present work, neither vitamin E supplementation nor high concentrations of E2 had a significant protective effect on susceptibility of the non-HDL fraction to oxidation in plasma samples at rest. The study of Chajès et al. (1996), reporting that vitamin E contents had no influence on LDL oxidation at rest, and the findings of Santanam et al. (1998) indicating no significant effect of E2 on LDL oxidation, support these results.

After the exercise protocol, significant elevations in Hct were observed and this was interpreted as the effect of haemoconcentration, and accordingly all plasma post-exercise values were adjusted according to the Hct values, as described in the methods section. It has been previously reported that the more intense the exercise, the more is lactate produced (Stanley et al. 1985). The present model of exhausting exercise caused significant elevations in LA content as expected.

Post-exercise plasma MDA concentrations, were not altered in either of the groups in this study. Various results have been reported concerning changes in plasma lipid peroxidation due to exercise. In a previous study we found similar results to the present study with unchanged MDA concentrations in exercising subjects when MDA values were adjusted for haemoconcentration (Sürmen-Gür et al. 1999). We suggest that discrepancies in results concerning post-exercise plasma MDA concentrations are due to differences in the exercise protocols used, subject groups, and the methods used in calculating the post-exercise concentrations.

To our knowledge only very few investigations have shown decreases in post-exercise erythrocyte SOD and GPx activities (Aslan et al. 1998; Sürmen-Gür et al. 1999), supporting the findings of lower antioxidant enzyme activities after exercise in the present study. The loss in enzyme activities may be explained by the modifications in enzyme protein as a consequence of oxidative damage caused by exercise. Ji et al. (1988) have proposed that free radical oxidation of enzyme protein residues plays an important role in enzyme inactivation and protein degradation with strenuous exercise. Although significantly reduced in all groups, decreases in SOD activities were more pronounced in groups that had lower E2 concentrations (M) in the present study, and the effect size for the M phase was greater in P compared to that in E (Fig. 3). The decreases in GPx activities were significant only in the above mentioned groups that had lower E2 concentrations (Fig. 1). In addition, post-exercise GPx was positively correlated with E2 in P. Whether vitamin E supplemented or not, subjects with higher E2 concentrations maintained their pre-exercise GPx activities after the exercise. The protein molecule of the antioxidant enzymes might have been exposed to less free radical attack in groups with higher E2 concentrations, indicating a better protective effect of this female hormone compared to vitamin E. However, since protein oxidation has not been evaluated in this study, this statement needs further investigations to be supported. In an earlier study, Nakano et al. (1987) reported that 2-hydroxy oestradiol had significantly higher antioxidant properties, even exceeding the antioxidant effects of vitamin E. Later, Tiidus and Houston (1993, 1994) have suggested that female rats may be relatively well protected against exercise induced oxidative stress even in vitamin E deficient conditions, possibly due to higher oestrogen concentrations. The results of the present work call further attention to the antioxidant role of E2 in eumenorrhoeic women, as it supplies a better protection against exercise-induced oxidative stress, even in elevations of physiological amounts.

Our results with post-exercise susceptibility of non-HDL fraction to oxidation show a parallel pattern with those changes observed with antioxidant enzymes. Decreases in lag time of LDL oxidation due to exercise have been reported previously (Wetzstein et al. 1998). In the present study, decreases were seen only in the groups with lower E2 concentrations, and these decreases were not protected by vitamin E supplementation. On the other hand, whether vitamin E supplemented or not, the non-HDL fraction in groups with higher E2 concentrations were protected against the oxidative damage of exercise, supporting the hypothesis that E2 has a better antioxidant effect than vitamin E in the present model.

Although E2 and vitamin E concentrations were significantly different on the two test occasions and between groups respectively, these antioxidants had no influence on muscle performance, either individually or cumulatively. This has been previously reported for sex hormones (Gür 1997), and vitamin E (Jakeman and Maxwell 1993). However the combined effect of vitamin E and E2 on muscle performance has not been reported before. The results of the present study indicated for the first time that there was no combined effect of the

periodic fluctuations in E2 through the normal menstrual cycle and vitamin E supplementation on muscle performance to fatigue.

In conclusion, results of the present work suggest that, neither vitamin E supplementation nor cycle-phase related higher E2 concentrations have any influence on erythrocyte antioxidant enzyme activities and the susceptibility of non-HDL fraction to oxidation at rest. Although the present model of exhausting exercise did not alter plasma MDA concentrations, it caused significant reduction in erythrocyte SOD and GPx activities and increased the susceptibility of the non-HDL fraction to oxidation. This exercise-induced damage was more attenuated by E2 in physiologically higher concentrations than by vitamin E. However, E2 and vitamin E did not have any cumulative antioxidant potential. Also, results of the present study showed that neither vitamin E supplementation nor menstrual cycle phase-related higher E2 concentrations, individually or cumulatively had any influence on exhausting muscle performance.

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