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Oral Contraceptives Improve Endothelial Function in Amenorrheic Athletes

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Athletic amenorrhea has been associated with endothelial dysfunction and unfavorable lipid profile. Estrogen substitution may reverse these metabolic consequences. The aim of this study was to evaluate the effects of oral contraceptives (OCs) on endothelial function measured as flow-mediated dilatation (FMD) of the brachial artery, the lipid profile, and blood markers of endothelial activation (inflammation) in amenorrheic athletes. Age- and body mass index-matched groups of young endurance athletes with amenorrhea (n = 11), regularly cycling athletes (n = 13), and sedentary controls (n = 12) were examined before and after 9 months of treatment with a low dose, monophasic, combined OC (30 μg ethinyl estradiol and 150 μg levonorgestrel). The amenorrheic athletes displayed the lowest FMD at baseline and the largest increase after OC treatment. FMD also increased in the control group, but not in the regularly menstruating athletes, who had the highest values of FMD before treatment. All three groups, particularly the controls, showed moderate unfavorable changes in the lipid profile in accordance with previous known effects of a second generation OC. Furthermore, there was an overall increase in some inflammatory markers (high sensitive C-reactive protein and TNF-α) and a decrease in one of the markers (vascular cell adhesion molecule-1). We conclude that amenorrheic athletes benefit from treatment with OC with respect to endothelial function. OC treatment is also associated with some modest alterations in the lipid profile and in markers of inflammation. **(*J Clin Endocrinol Metab* 90: 3162–3167, 2005)**

AMENORRHEA (AM) IN young endurance athletes is associated with endothelial dysfunction, assessed by flow-mediated vasodilatation (FMD) with a noninvasive ultrasound technique (1). In a recent study we also found an association between impaired FMD and unfavorable lipid profile, with increased levels of total cholesterol (Chol), low density lipoprotein (LDL), and apolipoprotein B (Apo B) in amenorrheic athletes (2). These recognized risk factors for atherosclerosis might be another burden to the amenorrheic athlete in addition to the three related medical conditions known as the female athlete triad (3, 4), including AM (5, 6), eating disorders, and low bone mass.

Estrogen has an important regulatory role in vascular function. The effects involve both direct rapid effects due to activation of endothelial-derived nitric oxide (NO), leading to vasodilatation (7–9), and long-term genomic effects, resulting in increased NO synthase activity (9). In addition, estrogen has mainly favorable effects on the lipid profile. Estrogen also decreases LDL oxidation and thereby positively affects endothelial function (7). The beneficial effects of estrogen on vascular function may to some extent be attenuated by its influence on thrombogenic proteins and inflammatory markers (7, 9).

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Abbreviations: AM, Amenorrhea; Apo B, apolipoprotein B; BA, brachial artery; Chol, cholesterol; FMD, flow-mediated dilatation; fT_4, free T_4; HDL, high-density lipoprotein; hsCRP, highly sensitive C-reactive protein; LDL, low-density lipoprotein; Lp(a), lipoprotein(a); NO, nitric oxide; NTG, nitroglycerin; OC, oral contraceptive; PRL, prolactin; RM, regularly menstruating; TG, triglycerides; VCAM, vascular cell adhesion molecule-1.

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FMD varies during the menstrual cycle in relation to estradiol levels (10–12). Decreased levels of endogenous estrogens lead to reduced bioavailability of NO and decreased endothelial function. There is evidence that estrogen replacement therapy is capable of restoring the ability of the endothelium to produce NO (7, 9). In premenopausal women, the impairment of endothelial function develops within one month after bilateral oophorectomy and is reversed after estradiol replacement therapy (13). Virdis *et al.* found that although treatment with oral contraceptives (OCs) in young healthy women was associated with an abnormal lipid profile, no adverse effects on endothelial function measured by strain-gauge plethysmography were detected (14).

Previous studies have demonstrated that decreased levels of endogenous estrogen unfavorably modify the lipid profile and vascular function in young women (7, 9, 15). The effects of oral estrogen therapy on the lipid profile are well known and referred to the first passage metabolism in the liver resulting in increased levels of high density lipoprotein (HDL), decreased levels of Chol and LDL, but also increased levels of triglycerides (TG) (16). Androgens and orally administered synthetic gestagens with androgenic properties, such as levonorgestrel, counteract some of the effects of estrogens (17, 18). In comparison with an OC containing desogestrel (a third generation OC), OC containing levonorgestrel (a second generation OC) had less favorable effects on HDL, Apo A, and LDL; equivalent increases in Apo B; and no effects on lipoprotein(a) [Lp(a)] (19). Other studies have shown that treatment with a combined second generation OC did not markedly influence the lipids or markers of inflammation in young women (20, 21). There is no information about the effects of OCs on endothelial function, lipids, and inflammatory markers in amenorrheic athletes.

The purpose of this study was to evaluate whether endo-

thelial dysfunction and unfavorable lipid profile in amenorrheic endurance athletes can be improved by treatment with a low dose, monophasic, combined OC.

Subjects and Methods

Subjects

Female athletes in endurance sports, such as medium- and long-distance running, marathon, orienteering, cross-country skiing, and triathlon, were recruited from universities and high schools specializing in sports and at public sports events and championships all over Sweden. Detailed information about the subjects has previously been reported (22, 23). Briefly, they were healthy, nulliparous, nonsmoking women, aged 16–34 yr, with body mass index of 18–24 kg/m^2. Endurance training criteria were defined as a minimum of 6 h of aerobic weight-bearing training of the legs or a minimum of 70 km of running weekly for a period of at least 6 months. Detailed information about the pattern of menstrual periods during the last year was provided from the athletes' sport diaries. AM was defined as no bleeding for the last 3 months, and regular menstruation was defined as periods at intervals of 22–34 d. No medications, including OCs within 1 yr before the study and asthma medications during the study period, were allowed. Intake of minerals/vitamins or nutritional supplements was accepted. None of the supplements was reported to include anabolic steroids.

Sedentary women were recruited from universities and high schools and from the staff at Karolinska University Hospital. They were screened using the same criteria as those used for the athletes, except that the amount of training was restricted to 1 h of light aerobic training a week. The study subjects were divided on the basis of endurance training and menstrual status into three groups: 11 athletes with AM, 13 regularly menstruating athletes (RM), and 12 regularly menstruating sedentary controls (CTR). The local committee for medical ethics approved the study protocol, and all women gave their informed consent to participate.

Experimental design

The women were examined before and after a mean period of 9 months of treatment with a low dose, monophasic, combined OC (30 μg ethinyl estradiol and 150 μg levonorgestrel on d 1–21, followed by a hormone- and tablet-free interval on d 22–28). Before OC treatment, RM subjects were examined in the early follicular phase (menstrual cycle d 1–5), whereas AM athletes were investigated on an arbitrary day. After OC treatment, investigations were performed at the end of the OC treatment cycle. The examinations started at 0730 h at the Women's Health, Clinical Research Unit at Karolinska University Hospital. Body weight, height, and blood pressure were recorded, and general health condition was examined. Fasting blood samples were collected from a peripheral vein in a resting state. After centrifugation of blood samples, sera were stored at −20 C until assayed.

Serum levels of FSH, LH, estradiol, TSH, free T_4 (fT4), and prolactin (PRL) were determined by time-resolved fluorescence immunoassay, using commercial kits (Autodelfia) from Wallac OY (Turku, Finland). The hormone detection limits and within- and between-assay coefficients of variation were: for FSH, 0.05 U/liter, 2%, and 3%; for LH, 0.05 U/liter, 2%, and 2%; for estradiol, 13.6 pg/ml, 5%, and 8%; for TSH, 0.005 mU/liter, 3%, and 5%, for fT_4, 1.6 pg/ml, 5%, and 4%; and for PRL, 0.04 μg/liter, 2%, and 4%, respectively.

Fat mass and bone mineral density (grams per square centimeter) were determined by dual energy x-ray absorptiometry using Lunar model DPX-L equipment (Lunar Radiation Corp., Madison, WI). Maximal oxygen uptake was determined with the treadmill test (Cardionics AB, Stockholm, Sweden), using the leveling-off criterion (24).

Endothelial function

Nine AM athletes, nine RM athletes, and 10 CTR underwent investigation of endothelial function both at baseline and after OC treatment. Eight subjects (two AM, four RM, and two CTR) did not participate on both occasions, which corresponds to 22% of all subjects. The dropouts were living outside the Stockholm region and were not able to complete the schedule. Endothelial function was determined in the afternoon, 3–5 h after a light meal. Brachial artery (BA) flow velocity, FMD, and endothelium-independent nitroglycerin (NTG)-induced dilatation were examined according to the method described by Celermajer *et al.* (25). The measurements were made noninvasively using a high resolution scanner (128 XP/10 c, Acuson, Mountain View, CA) with a 7-MHz linear array transducer. The left BA was scanned longitudinally 1–10 cm above the elbow, where a clear image was found with the artery placed horizontally across the screen. Baseline measurements of blood flow and the inner diameter of BA were performed at rest. Reactive hyperemia was obtained by distal forearm artery occlusion with a 12.5-cm blood pressure cuff inflated to 300 mm Hg for 4.5 min. Blood velocity was measured immediately after cuff release, and the diameter of the artery was measured 50–60 sec after deflation. The BA diameter was measured again after a 10-min rest, followed by administration of 0.4 mg sublingual NTG. Four minutes after NTG treatment, blood velocity and diameter measurements were repeated. To minimize variability, one experienced operator performed all investigations.

All analyses of BA diameters were performed off-line by one investigator, who was unaware of the subject's group and the sequence of the ultrasound scan. Three consecutive late diastolic frames taken coincidentally with the R-wave on the electrocardiogram were analyzed at rest (baseline) and subsequent to different stimulations. The average diameter of the three frames was calculated. Blood flow was calculated from Doppler velocity, vessel diameter, and heart rate. The increase in blood flow after reactive hyperemia is presented as a percentage of the basal flow values. The within-individual variations between two determinations of FMD performed during the same day and between the determinations made on separate days in the laboratory are 0.88 ± 0.82% and 3.3 ± 2.7%, respectively, as previously reported (26).

Serum lipids and inflammatory markers

Serum lipids (TG, Chol, HDL, Apo A, and Apo B) were determined by enzymatic methods using kits from Beckman Coulter, Inc. (SYNCHRON LX Systems, Fullerton, CA). LDL was determined using the Friedewald formula (27). Lp(a) was immunochemically determined with kinetic nefelometry (Beckman Coulter, Inc.). Highly sensitive C-reactive peptide (hsCRP) was immunonefelometrically measured with a kit from Dade Behring (Deerfield, IL). IL-6, TNF-α, and soluble vascular cell adhesion molecule-1 (VCAM) were measured by immunoassays from R&D Systems, Inc. (Minneapolis, MN). Detection limits and within- and between-assay coefficients of variation were: for TG, 10 mg/dl, 2.3%, and 3.1%; for Chol, 5 mg/dl, 1.1%, and 1.6%; for HDL, 5 mg/dl, 3%, and 4.5%; for Apo A, 25 mg/dl, 5.0%, and 6.3%; for Apo B, 35 mg/dl, 2.0%, and 3.9%; for Lp(a), 0.02 g/liter, 2%, and 4%, (undetectable values were set at 0.01); for hsCRP, 0.16 mg/liter (undetectable values were set at 0.09), 3.4%, and 2.1%; for IL-6, 0.04 pg/ml, 7%, and 7%; for TNF-α, 0.12 pg/ml, 6%, and 13%; and for VCAM, 2.0 ng/ml, 4.3%, and 8.5%.

Statistical analysis

Normally distributed values are given as the mean ± SD; other values are given as the median and quartile range (P_{25}–P_{75}). Differences within and between groups were analyzed using a two-way ANOVA with one within factor (OC treatment) and one between factor (groups). In the case of a significant interaction, simple effects were examined, *i.e.* effects of one factor holding the other factor fixed. If no interaction was present, Fisher's least significant difference test was performed to make all pairwise comparisons among group means. If the assumption of equality of population variances was not tenable, an ANOVA model with separate variance estimates was used, Proc Mixed in SAS (SAS Institute, Inc., Cary, NC). For some positively skewed distributed variables, log transformation was performed before ANOVA. If the pretreatment differences between the groups were statistically significant, an analysis of covariance was performed to adjust for these differences. Correlations were assessed using Pearson product-moment correlation. A value of $P < 0.05$ was considered statistically significant. Power analysis for observed results showed sufficient power to make the correct conclusions about the differences in OC effect within and between groups for the primary variable FMD and the other variables, with the possible exception of TG. The software used was Statistica 6.1 (StatSoft, Inc., Tulsa, OK) and SAS System 8.2 (SAS Institute, Inc.).

Results

Baseline characteristics for the study groups are presented in Table 1. The AM athletes had the lowest percentage of total fat mass of all groups. Age of menarche was higher in the AM group compared with RM athletes. There were no differences in the onset of training, amount of specific endurance training, and maximal oxygen uptake between the athlete groups. Levels of FSH and LH were significantly lower in RM compared with CTR subjects. Both athlete groups had significantly lower estradiol values than the CTR group. The AM group had the lowest levels of fT_4 and PRL of all groups.

Endothelial function

FMD in athletes with AM was decreased at baseline and increased significantly during OC treatment (Fig. 1 and Table 2). FMD also increased in the CTR group, but not in the RM athletes, who had the highest values of FMD before OC treatment. There were no significant differences in FMD between groups after OC treatment.

Serum lipids and markers of inflammation

Levels of lipids and inflammatory markers are presented in Table 3. There were no significant differences between groups before OC treatment. However, before treatment, the AM group had the highest levels of Chol, LDL, and Apo B of all groups, and LDL and Apo B tended to be increased compared with levels in RM athletes ($P = 0.07$ and $P = 0.06$, respectively). During OC treatment, HDL levels decreased in all three groups, whereas levels of Apo A only decreased in the CTR group. There was an overall increase in levels of Apo B [$F(1,33) = 27.5$; $P < 0.001$]. OC treatment also increased levels of hsCRP and TNF-α in all groups [$F(1,33) = 40.4$; $P < 0.001$ for hsCRP and $F(1,33) = 7.1$; $P < 0.05$ for TNF-α], with no differences between groups. Furthermore, there was an overall decrease in levels of VCAM [$F(1,33) = 58.4$; $P < 0.001$].

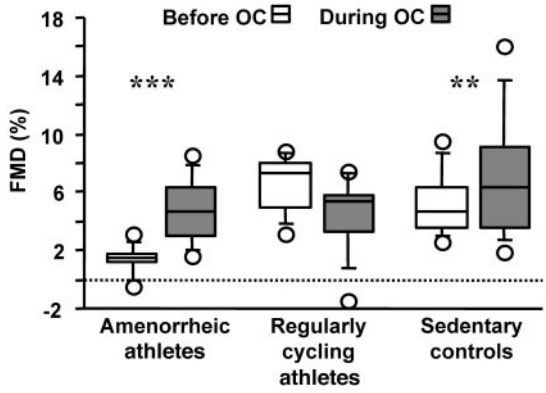

FIG. 1. Effects of combined OC on endothelial function measured as FMD, presented as the median and quartile range (P_{25}-P_{75}) in AM athletes, RM athletes, and CTR subjects. FMD was significantly increased in the AM athletes ($P < 0.001$) and the CTR group ($P < 0.01$), whereas FMD remained unchanged in the RM athletes.

Correlations

The change in FMD after OC treatment correlated inversely to FMD at baseline in all subjects ($r = -0.54$; $P < 0.05$). There were also negative correlations between the changes in HDL and VCAM from the respective baseline values (for HDL: $r = -0.74$; $P < 0.001$; for VCAM: $r = -0.37$; $P < 0.05$) in all subjects. However, the change in FMD did not correlate to the change in any of the lipid variables or markers of inflammation.

Discussion

In this study we found that OC treatment clearly improves endothelial function in AM athletes, especially those with the lowest FMD before OC treatment. In CTR subjects, FMD also increased, but to a lesser extent than in the AM group, whereas FMD remained unchanged in RM athletes, who had the highest values at baseline. Our results are in agreement

TABLE 1. Baseline characteristics in AM athletes, RM athletes, and CTR subjects

	AM (n = 11)	RM (n = 13)	CTR (n = 12)	Significance
Age (yr)	19.5 ± 5.1	21.4 ± 4.0	20.9 ± 4.2	NS
Age at menarche (yr)	14.0 ± 1.4	12.2 ± 1.2	13.1 ± 1.4	<0.01[a]
Weight (kg)	54.2 ± 5.5	56.4 ± 4.3	56.4 ± 6.2	NS
Height (cm)	170 ± 5	169 ± 4	169 ± 5	NS
Body mass index (kg/m^2)	18.6 ± 1.1	19.7 ± 1.3	19.6 ± 1.6	NS
Fat mass, total (%)	15.4 ± 3.0	21.2 ± 4.3	24.2 ± 4.9	<0.01,[a] <0.001[b]
Training				
Age at onset of training (yr)	9.3 ± 3.0	10.8 ± 3.1		NS
Amount of training (h/wk)	7.5 ± 1.0	7.7 ± 1.8		NS
VO_2 max (ml/kg·min)	58.7 ± 3.0	55.3 ± 4.4	42.3 ± 3.5	<0.001[b,c]
Hormone levels				
FSH (IU/liter)	5.9 (4.7–6.6)	5.3 (4.8–5.7)	6.8 (5.0–7.5)	<0.05[c]
LH (IU/liter)	1.8 (1.3–6.2)	3.8 (2.5–4.5)	5.6 (3.9–6.5)	<0.05[c]
Estradiol (pg/ml)	29 (18–35)	30 (25–42)	38 (32–58)	<0.05[b,c]
TSH (mIU/liter)	2.16 ± 0.78	2.41 ± 0.78	2.52 ± 0.83	NS
fT_4 (pg/ml)	8.8 ± 1.2	9.4 ± 1.4	10.8 ± 1.5	<0.05,[b] <0.001[c]
PRL (μg/liter)	5.5 (5.2–5.9)	13.0 (9.9–25.6)	11.5 (7.5–13.0)	<0.01,[a] <0.05[b]

Values are expressed as the mean ± SD or the median (P_{25}–P_{75}). Significant differences between groups are indicated. Conversion factors to Systeme International units: estradiol, 3.7 (pmol/liter), fT_4, 1.28 (pmol/liter). NS, Not significant.

[a] AM *vs.* RM.

[b] AM *vs.* CTR.

[c] RM *vs.* CTR.

TABLE 2. Effects of combined OC on FMD in AM athletes, RM athletes, and CTR subjects

	AM (n = 9)		RM (n = 9)		CTR (n = 10)	
	Before	During OC	Before	During OC	Before	During OC
FMD (%)	1.42 ± 0.98[a]	4.88 ± 2.20[b]	6.59 ± 1.90	5.25 ± 2.13	4.59 ± 2.57	7.01 ± 4.28[c]
NTGD (%)	15.4 ± 5.0	15.0 ± 3.4	18.1 ± 5.0	16.0 ± 4.1	20.6 ± 7.7	22.0 ± 8.5
Rest						
Systolic BP (mm Hg)	103 ± 8	105 ± 10	106 ± 10	111 ± 12	110 ± 11	113 ± 9
Heart rate (beats/min)	57 ± 9[d]	56 ± 7	55 ± 4	55 ± 8	74 ± 12	71 ± 11
BA diameter (mm)	3.5 ± 0.5[e]	3.4 ± 0.3[f]	3.7 ± 0.4	3.8 ± 0.4	3.2 ± 0.3	3.2 ± 0.3
Reactive hyperemia						
Heart rate (beats/min)	55 ± 9[d]	57 ± 7	55 ± 8	57 ± 10	72 ± 13	71 ± 11
BA diameter (mm)	3.6 ± 0.5[g]	3.5 ± 0.3	3.9 ± 0.4	4.0 ± 0.4	3.3 ± 0.5	3.5 ± 0.3
NTG						
Heart rate (beats/min)	58 ± 12[d]	59 ± 6	63 ± 4	64 ± 7	76 ± 11	74 ± 11
BA diameter (mm)	4.2 ± 0.5[h]	3.9 ± 0.4[c]	4.4 ± 0.4	4.4 ± 0.5	3.8 ± 0.3	3.9 ± 0.2

Values are expressed as the mean ± SD or the median (P_{25}–P_{75}). Significant interaction and differences within groups are indicated in the OC treatment column. Significant differences in baseline levels between groups are indicated in the first AM column. BP, Blood pressure; NTGD, nitroglycerin-induced vasodilation.

[a] $P < 0.001$, AM *vs.* RM. $P < 0.01$, AM *vs.* CTR. $P < 0.05$, RM *vs.* CTR.
[b] $P < 0.001$.
[c] $P < 0.01$.
[d] $P < 0.001$, AM *vs.* CTR and RM *vs.* CTR.
[e] $P < 0.05$, RM *vs.* CTR.
[f] $P < 0.05$.
[g] $P < 0.05$, AM *vs.* RM. $P < 0.01$, RM *vs.* CTR.
[h] $P < 0.01$, RM *vs.* CTR.

with a study by Virdis *et al.* (28), which demonstrated that estrogen substitution restores endothelial function after oophorectomy in premenopausal women. Furthermore, the positive effects of estrogen substitution on the endothelium in postmenopausal women are well known (7–9, 29). However, earlier studies of OC treatment in healthy young women have not shown any effect on endothelial function (14, 30). To our knowledge, there are no previous data on how OCs influence endothelial function in AM sports women. It has been shown that athletic AM is associated with endothelial dysfunction (1, 2). In the present study we found that OC treatment increased FMD in AM athletes to levels similar to those in menstruating subjects, showing that endothelial dysfunction in athletic AM is a reversible state.

The mechanism of improvement of endothelial function by OC treatment in athletic AM is most likely attributed to the estrogenic component, because estrogen is known to have beneficial effects on endothelium mainly by increasing NO bioavailability (7, 9, 29). In contrast, there is no conclusive evidence regarding the influence of gestagens on endothelial function. Progesterone administration alone does not adversely affect vascular function in postmenopausal women (31). Depending on the substance and the manner of administration, gestagens may indirectly affect endothelial function through liver effects on the lipid profile. Limited data suggest that gestagens also have some direct effects on endothelial function that might include inhibition of endothelial cell migration and smooth muscle proliferation (8). Long-term use of contraceptive depot medroxyprogesterone acetate in young women was associated with impairment in FMD, as assessed by cardiovascular magnetic resonance (32). Furthermore, medroxyprogesterone acetate administration to postmenopausal women offsets favorable effects of estrogen on endothelial function measured with ultrasound (33).

TABLE 3. Effects of combined OC on lipids and markers of inflammation in AM atheletes, RM athletes, and CTR subjects

Groups	AM (n = 11)		RM (n = 13)		CTR (n = 12)	
	Before	During OC	Before	During OC	Before	During OC
TG (mg/dl)	79 ± 30	96 ± 27	77 ± 32	93 ± 33	78 ± 39	77 ± 17
Chol (mg/dl)	185 ± 19	181 ± 39	165 ± 23	169 ± 27	177 ± 27	173 ± 27
HDL (mg/dl)	51 ± 8	47 ± 6[a]	51 ± 4	47 ± 5[a]	55 ± 9	43 ± 5[b]
LDL (mg/dl)	115 ± 19	115 ± 27	100 ± 19	108 ± 23	108 ± 23	112 ± 23
Apo A (mg/dl)	154 ± 15	152 ± 17	155 ± 12	153 ± 10	157 ± 21	142 ± 14[b]
Apo B (mg/dl)	89 ± 12	103 ± 28	77 ± 15	95 ± 21	81 ± 19	99 ± 20
Lp(a) (g/liter)	0.09 (0.02–0.12)	0.06 (0.02–0.10)	0.19 (0.08–0.26)	0.21 (0.07–0.28)	0.08 (0.02–0.23)	0.06 (0.02–0.21)
hsCRP (mg/liter)	0.19 (0.09–0.88)	0.75 (0.41–1.16)	0.45 (0.34–0.88)	1.39 (0.81–1.57)	0.40 (0.14–1.07)	1.14 (0.80–3.60)
IL-6 (pg/ml)	0.55 (0.48–0.77)	0.84 (0.46–1.23)	0.97 (0.69–1.98)	0.71 (0.53–1.14)	1.25 (0.76–1.68)	1.16 (0.71–1.48)
TNF-α (pg/ml)	1.4 (1.1–7.5)	1.4 (1.2–11.1)	1.4 (1.1–2.3)	1.6 (1.4–2.1)	1.5 (0.9–2.7)	1.5 (0.8–3.1)
VCAM (ng/ml)	500 ± 95	428 ± 91	483 ± 66	390 ± 53	526 ± 92	422 ± 112

Values are expressed as the mean ± SD or the median (P_{25}–P_{75}). Significant interaction and differences within groups are indicated in the OC treatment column. For overall effects of OC, see text. Conversion factors to Systeme International units; TG, 0.0113 (mmol/liter); Chol, HDL, and LDL, 0.0260 (mmol/liter); Apo A and Apo B, 0.01 (g/liter).

[a] $P < 0.05$.
[b] $P < 0.001$.

Vaginal administration of micronized progesterone combined with transdermal estradiol in postmenopausal women does not counteract the beneficial effects of estradiol treatment on FMD (34).

Because the lipid profile can modify endothelial function, OCs may affect endothelial function indirectly by changing the lipid profile. Estrogen has mainly beneficial effects on the lipid profile (7, 9). In contrast, different gestagens have diverse effects on the lipid profile and may counteract some of the effects of estrogen (16, 19, 35). In our study the subjects were treated with a second generation OC containing levonorgestrel with some androgenic properties. Although this treatment was associated with some unfavorable changes in the lipid profile, endothelial function was improved in all subjects, with the exception of the RM athletes. These results suggest that the estrogen component in OCs exerts a protective action on endothelial function.

Levels of HDL decreased modestly during OC treatment in the athletes; this was more pronounced in the CTR group. This change in the lipid profile is not unexpected in treatment with a second generation OC (18). There was no significant change in TG; however, the total sample size might have been too small (not enough power) to detect an effect. A low level of HDL is a well known, independent risk marker of cardiovascular disease. The long-term consequences of treatment with an OC leading to decreased HDL, however, are not known. It has been suggested that apolipoproteins are even better risk indicators of coronary disease than lipoproteins alone (36–38) and therefore should be included in the lipid analysis. In our study we found a decrease in levels of Apo A in the sedentary controls and an increase in Apo B levels in all groups. In summary, all three groups, but particularly the CTR group, showed modest unfavorable changes in the lipid profile after OC treatment. It may be speculated that physical activity to some extent counteracts the adverse effects of OC on serum lipids (39).

We found several overall changes due to OC treatment in inflammatory markers, including increases in hsCRP and TNF-α. These results are largely in agreement with those of previous studies showing that oral estrogen may increase proinflammatory cytokines (40, 41). The CRP increase during OC treatment is well known and is considered to be a result of the estrogenic proinflammatory effect, which is also associated with other changes in acute phase proteins originated from the liver (42). This proinflammatory effect of estrogen causes activation of endothelial cells, resulting in the increased expression of endothelial adhesion molecules with a potential proatherogenic action (7, 9). However, estrogen also acts in an antiatherogenic manner through an increased release of NO mediating vasodilatation and a decrease in the expression of endothelial adhesion molecules (7, 9). In support of this, we found an overall decrease in levels of VCAM. These results are in agreement with those reported by Seeger *et al.* (43), who showed that treatment with OCs decreases VCAM levels in young healthy women. The clinical implications of these changes remain to be elucidated.

In conclusion, we have demonstrated that endothelial dysfunction in athletic AM can be restored by OC treatment, suggesting a protective action of the estrogen component in OC on endothelial function. Treatment with OC is also associated with some modest alterations in the lipid profile and changes in markers of endothelial activation. However, the clinical importance of our findings needs to be determined in larger, long-term studies.

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