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Effects of oral contraceptives on diurnal profiles of insulin, insulin-like growth factor binding protein-1, growth hormone and cortisol in endurance athletes with menstrual disturbance

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BACKGROUND: Menstrual disturbances in female athletes are often explained as a consequence of energy deficiency. Oral contraceptive (OC) treatment may have favorable metabolic effects. We evaluated effects of OCs on diurnal secretions of insulin, insulin-like growth factor binding protein 1 (IGFBP-1), growth hormone (GH) and cortisol in relation to changes in body composition in athletes with menstrual disturbance compared with regularly menstruating athletes and controls.

METHODS: Age- and BMI-matched groups of endurance athletes with menstrual disturbance (OAM, $n = 9$) and regularly cycling athletes (RM, $n = 8$) and sedentary controls (CTRL, $n = 8$) were examined, and hormone levels measured, before and after 8 months of treatment with a low-dose combined OC (30 μg ethinyl estradiol + 150 μg levonorgestrel).

RESULTS: Before OC treatment, the diurnal profile of insulin was lower ($P < 0.01$) and levels of IGFBP-1 ($P < 0.05$) and cortisol ($P < 0.05$) were higher in OAM athletes than in CTRL, whereas GH secretion was higher than in RM athletes ($P < 0.05$). After treatment, diurnal secretions of these hormones were similar between groups with an increase of IGFBP-1 in the regularly menstruating subjects only ($P < 0.001$). OC treatment increased body fat mass in OAM athletes ($P < 0.01$ versus baseline). The change in total fat mass correlated positively with pretreatment diurnal levels of GH ($r_s = 0.67$, $P < 0.01$) and cortisol ($r_s = 0.64$, $P < 0.01$).

CONCLUSIONS: OC treatment in endurance athletes with menstrual disturbance increases body fat mass and results in diurnal levels of insulin, IGFBP-1, GH and cortisol that are comparable to those in regularly menstruating subjects. These results suggest that OCs improve metabolic balance in OAM athletes.

Key words: oral contraceptives / female athletes / menstrual disturbances / insulin-like growth factor binding protein 1 / body fat

Introduction

Menstrual disturbances in female athletes are often explained as a consequence of hypothalamic inhibition owing to energy deficiency. The characteristic hormonal consequences of energy deficiency include disruption of the growth hormone (GH)/insulin-like growth factor (IGF)-I axis with low levels of IGF-I and insulin, whereas the levels of IGF binding protein (IGFBP)-1, GH and cortisol are increased (Laughlin and Yen, 1996; Waters *et al*., 2001; Rickenlund *et al*., 2004a). These changes could be regarded as adaptations to a hypometabolic state and are also seen in patients with anorexia nervosa (Gianotti *et al*., 2002; Stoving *et al*., 2007). Low IGF-I activity, as well as elevated cortisol secretion, may lead to inhibition of the hypothalamic–pituitary– gonadal axis, which could explain the decreased LH pulsatility in amenorrheic athletes (Laughlin and Yen, 1996; De Souza *et al*., 2003; Loucks and Thuma, 2003; Rickenlund *et al*., 2004a).

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We have previously demonstrated that oral contraceptive (OC) treatment results in gain in weight and fat mass in athletes with menstrual disturbance but not in regularly menstruating athletes and sedentary controls (Rickenlund *et al*., 2004b). The largest increase in fat mass was seen in athletes with the lowest amount of body fat before treatment (Rickenlund *et al*., 2004b). On the basis of these findings, we hypothesized that in irregularly menstruating athletes, OC treatment shifts the energy balance toward anabolism. In comparison, only small changes in body composition were found in regularly menstruating athletes and sedentary controls. The mechanisms by which OC treatment increases fat mass in athletes with menstrual disturbance are not fully established.

Combined estrogen/progestagen OC treatment results in inhibition of the hypothalamic–pituitary–gonadal axis. OCs also up-regulate the liver synthesis of several binding proteins (Song *et al*., 1989; Wiegratz *et al*., 2003a). Total levels of cortisol and thyroid hormones will increase owing to increased levels of their binding proteins, whereas the free fractions of these hormones remain essentially unchanged (Wiegratz *et al*., 2003a, b). Effects of OC treatment on glucoregulatory hormones are less explored, and there is little knowledge about the effects of OCs on IGFBPs (Karlsson *et al*., 1990; Westwood *et al*., 1999). However, both second generation and third generation OCs have been demonstrated to decrease levels of IGF-I, whereas IGFBP-3 remains unchanged (Balogh *et al*., 2000). When investigating the effects of short-term transdermal estradiol (E_2) in weight-stable amenorrheic athletes, Waters *et al*. (2003) reported no change in the levels of GH, IGF-I and IGFBP-1, whereas IGFBP-3 decreased. However, effects of OC treatment on diurnal hormone levels in athletes have not been studied. We hypothesized that the shift in energy balance by OCs is reflected by changes in diurnal glucoregulatory hormones.

The aim of the present study was to evaluate the effects of OCs on the diurnal profiles of insulin, IGFBP-1, GH and cortisol in relation to changes in body composition in endurance athletes with and without menstrual disturbance and sedentary controls.

Materials and Methods

Subjects

This study included subjects randomly selected from a larger cohort of athletes and sedentary controls (Rickenlund *et al*., 2003). 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 in Sweden. Inclusion criteria were healthy, nonsmoking nulliparous women aged 16–35 years with BMI (kg/m^2) 18–24. Endurance training criteria were defined as a minimum of either 6 h of aerobic weight-bearing training or a minimum of 70 km of running or 6 h of specific endurance training weekly. Medical history including eating behavior and detailed information about the pattern of menstrual periods during the last year was provided from the subjects and was based on the athletes' sport diaries. Amenorrhea was defined as no bleeding for the last 3 months and oligomenorrhea as periods with intervals exceeding 6 weeks and 5–9 periods the last year. No medications were allowed. However, intake of minerals/vitamins or nutritional supplements was accepted.

Inactive women were recruited from universities and high schools and from the staff at the Karolinska University Hospital. They were screened using the same criteria as for the athletes, except that the amount of training was restricted to 1 h of light aerobic training a week. Furthermore, only regularly menstruating controls were included. All subjects underwent a general health examination and body weight, height and blood pressure were recorded. Subjects with menstrual disturbances also underwent a gynecological examination including ultrasound. The local committee for medical ethics approved the study protocol and all women gave their informed consent to participate.

Three groups of women characterized on the basis of endurance training and menstrual status, and matched for age and BMI were recruited: athletes with amenorrhea (6) or oligomenorrhea (3) (OAM) athletes, regularly menstruating (RM) athletes and sedentary regularly menstruating controls (CTR). A subset of women from these three groups was randomly selected to participate in the present study.

Experimental design

The women were examined before and after a mean period of 8 months of treatment with a low-dose monophasic, combined OC (30 μg ethinyl estradiol + 150 μg levonorgestrel days 1–21 followed by a hormone- and tablet-free interval days 22–28). Before OC treatment, menstruating subjects (RM, CTR, oligomenorrheic athletes) were examined in the early follicular phase (menstrual cycle days 1–5), whereas amenorrheic athletes were investigated on an arbitrary day. After OC treatment, investigations were performed during the last week of the active OC treatment cycle. The examinations started at 7.30 a.m. at the Women's Health Clinical Research Unit at the Department of Obstetrics and Gynecology, Karolinska University Hospital, Stockholm, Sweden.

Investigation of diurnal profiles started at 8.00 a.m. after an over-night fast. Continuous sampling from an antecubital vein via heparin-coated PVS tubing (Durascan Medical products ApS, Odense, Denmark) was carried out by means of a portable, battery-charged excentric pump (Carmeda, Stockholm, Sweden). Venous blood samples were obtained every 20 min for 24 h. The withdrawal rate was 8–10 ml/h, and ~3 ml was collected in each 20 min period. Blood samples were centrifuged and sera were stored at −20°C until assayed.

The sampling technique allowed the participants to move freely within the hospital area. During daytime, the subjects were instructed to change the reservoir tubes every 20 min, whereas experienced staff changed the tubes during the night. The period of sleep was recorded and most subjects slept between 23.00 and 07.00 h. Subjects received standard meals composed by a nutritionist. Breakfast was served at 08.00, 600 kcal; light snack meal at 10.30, 200 kcal; lunch at 13.00, 750 kcal; light snack meal at 15.30, 200 kcal and dinner at 18.00, 700 kcal and evening meal at 21.00, 250 kcal, all together 2700 kcal with the nutrient composition of 52% carbohydrate, 17% protein and 32% fat. The amount of 2700 kcal was calculated based on a moderate amount of energy for women with a corresponding weight and energy expenditure. One of the sedentary controls was served vegetarian meals with equal caloric and nutrient composition. The meals were consumed within 30 min.

Endurance capacity was assessed at the Department of Physiology and Pharmacology, Karolinska Institutet. Maximal oxygen uptake (VO_2 max) was determined while the subjects ran on a motor-driven treadmill (Cardionics AB, Stockholm, Sweden), using the leveling-off criterion (Åstrand and Rodahl, 1986). VO_2 max was determined by sampling expired air in Douglas bags. The oxygen and carbon dioxide contents were measured with a Beckman analyzer.

Body composition [bone mineral areal density (g/cm^2), lean body mass (LBM) and fat mass] was determined by dual energy X-ray absorptiometry

using the Lunar Model DPX-L equipment (Lunar Radiation, Madison, WI, USA).

The eating disorder inventory-2 (EDI-2) test was used as screening for eating disorders (Garner, 1991). This test measures 11 parameters: drive for thinness, bulimia, body dissatisfaction, ineffectiveness, perfectionism, interpersonal distrust, interoceptive awareness, maturity fears, asceticism, impulse regulation and social insecurity. The defined EDI score at risk for eating disorders, i.e. a cut-off point of 14 on the drive for thinness subscale (EDI-DT), was used in the evaluation (Garner, 1991). Furthermore, a 24 h recall of food intake was delivered by all subjects at baseline. The intakes of energy and nutrients were computed using a food database (Dietist XP 3.0).

Assays

Fasting serum levels of FSH, LH, E_2, thyroid-stimulating hormone (TSH), free thyroxin (fT4) and prolactin were determined by commercial time-resolved immunofluorometric assays (TR-IFMAs) from PerkinElmer Life-Sciences, Turku, Finland (Autodelfia®). Serum concentrations of testosterone and sex hormone-binding globulin (SHBG) were measured by radioimmunoassay (RIA) in untreated serum, using commercial kits obtained from Diagnostic Products Corp., Los Angeles, CA, USA (Coat-a-Count® Testosterone) and Eurodiagnostics AB, MALMÖ, Sweden (SHBG).

Fasting levels of total IGF-I were determined by RIA after acid ethanol extraction (Nichols Products Corp.). The levels were expressed in μg/l of the World Health Organization (WHO) first International Reference Reagent IGF-I 87/518. Free IGF-I was determined using ultrafiltration by centrifugation as described previously (Frystyk *et al*., 1994). The dimeric complex of IGF-I and IGFBP-1 (binary complex) and IGFBP-2 were determined by specific TR-IFMAs as described previously (Frystyk *et al*., 1995, 2002; Krassas *et al*., 2003). Fasting levels of corticosteroid-binding globulin (CBG) were determined using a commercial RIA (Medgenix Diagnostics SA, Fleurus, Belgium).

Diurnal serum levels of insulin were determined by RIA, using a commercial kit obtained from Pharmacia Diagnostics, Uppsala, Sweden, and expressed as mIU/l of the WHO International Reference Preparation 66/304. Serum concentrations of IGFBP-1 were determined by RIA as described by Povoa *et al*. (1984). The IGFBP-2 and IGFBP-3 cross-reactivity was less than 0.5% and 0.05%, respectively. Diurnal serum concentrations of GH and cortisol were determined by commercial TR-IFMAs obtained from PerkinElmer LifeSciences (AutoDELFIA™). The concentrations of GH were expressed as μg/l of WHO First International GH Reference Preparation 80/505.

Detection limits and within and between assay coefficients of variation were for FSH 0.05 U/l, 2% and 3%; for LH 0.05 U/l, 2% and 2%; for E2 13.6 pg/ml, 5% and 8%; for TSH 0.005 mU/l, 3% and 5%; for fT4 1.6 pg/ml, 5% and 4%; for prolactin 0.04 μg/l, 2% and 4%; for testosterone 2.8 ng/dl, 6% and 10%; for SHBG 0.005 mg/l, 4% and 8%; for total IGF-I 6 μg/l, 5% and 7%; for free IGF-I 0.050 μg/l, 15% and 20%; for IGFBP-2 10 μg/l, 5% and 12%; for the dimeric complex 0.5 μg/l, 5% and 15%; for CBG 0.3 μg/l, 4% and 6%; for insulin 2 mIU/l, 6% and 6%; for IGFBP-1 3 μg/l, 3.0% and 10.0%; for GH 0.012 μg/l, 2.0% and 3.3%; and for cortisol 0.6 μg/dl, 1.1% and 2.9%, respectively.

Analysis of 24 h profiles

Analysis of the diurnal profiles of hormones and IGFBP-1 was made using a computerized pulsatile profile and smoothed baseline diurnal pattern analysis, applying the Pulsar program developed by Merriam and Watchter (1982). The program identifies the peaks and the smoothed baseline using the assay SD as a scale factor. The cut-off parameters G1–G5 for detecting the peaks were set to 2.5, 1.5, 1, 0.75 and 0.5 times the intra-assay SD for accepting peaks of 1, 2, 3, 4 and 5 points wideness, respectively. Peak splitting period was set to 1.5. The following four Pulsar parameters were used for further statistical analysis: area under curve (AUC), 24 h baseline mean, the number of peaks (peaks/24 h) and mean peak amplitude for 24 h.

Statistical analysis

Normally distributed values are given as the arithmetic mean and SD, whereas others are given as the median and quartile range (P_{25}–P_{75}). Differences between groups at baseline were analyzed using a one-way analysis of variance (ANOVA) followed by Fisher's least significant-difference *post hoc* analysis. A two-way ANOVA was used to evaluate effects of OC treatment within and between groups. In the case of a significant interaction (significant treatment effect between groups), *post hoc* analysis was performed using paired *t*-test. The distribution for some of the variables was skewed and therefore these data were log-transformed. Correlations were assessed using Spearman's rank-order correlation. A P-value of <0.05 was considered statistically significant. Software used was Statistica 6.1 (StatSoft® Inc., Tulsa, OK, USA).

Results

Baseline characteristics

Baseline characteristics for the study groups are presented in Table I. Athletes and sedentary controls were comparable as regards age, menarcheal age, weight and height. 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 were lower in regularly menstruating athletes compared with controls. The OAM group had significantly lower levels of E_2 and fT4 than controls and lower levels of prolactin than both regularly menstruating groups. There were no significant differences in hormone values between the three oligomenorrheic and six amenorrheic athletes in the OAM group (data not shown).

None of the subjects reported any eating disorder. Furthermore, no one displayed an EDI-score at risk for eating disorders and the mean EDI-DT score was comparable between groups (OAM 2.1 ± 3.0, RM 2.3 ± 3.7, CTR 0.4 ± 1.1). The 24 h recall showed no significant differences in total caloric intake at baseline between the three groups (OAM 2232 ± 414, RM 2543 ± 573, CTR 2155 ± 450 kcal). However, protein intake was significantly lower in the OAM group compared with the regularly menstruating athletes (84.3 ± 14.5 versus 103.1 ± 17.9 g, $P < 0.05$) and fat intake tended to be lower in the OAM athletes compared with the menstruating groups (53.7 ± 25.0 versus 76.2 ± 22.6 g, $P = 0.057$).

Effects of OCs on body composition

Data pertaining to body composition are presented in Table II. At baseline, there was no significant difference in BMI between groups. However, percentage of fat mass was significantly lower, whereas LBM in legs and the ratio of total LBM/fat mass was significantly higher in the OAM group compared with controls. OC treatment resulted in overall increases in BMI and body weight [both $F(1,22) = 19.0$, $P < 0.001$] but without significant differences between groups. However, there was a significant interaction regarding change in fat mass. Thus, oligo-/amenorrheic athletes displayed an increase in percentage of fat mass with OC, whereas this variable

Table I Baseline characteristics in OAM athletes, RM athletes and CTR

Groups	OAM (n = 9)	RM (n = 8)	CTR (n = 8)	Significance
Age (years)	20.3 ± 5.4	20.4 ± 3.1	21.1 ± 4.8	NS
Menarche (age)	13.8 ± 1.4	12.4 ± 1.4	12.9 ± 1.2	NS
Weight (kg)	57.5 ± 6.8	56.0 ± 4.6	54.6 ± 5.8	NS
Height (cm)	171 ± 4	168 ± 4	168 ± 5	NS
Onset of training (age)	9.9 ± 2.8	10.8 ± 3.3	—	NS
Amount of endurance training (h/w)	7.8 ± 1.3	7.5 ± 1.6	—	NS
VO_2 max (l/min)	3.3 ± 0.3	3.2 ± 0.2	2.4 ± 0.2	b,c***
FSH (IU/l)	6.3 (4.4–7.2)	5.4 (5.3–5.8)	7.3 (6.4–8.1)	c*
LH (IU/l)	5.9 (1.4–8.8)	3.9 (3.0–4.4)	5.9 (5.6–7.6)	NS
Estradiol (pg/ml)	31.6 ± 7.0	32.9 ± 14.7	47.5 ± 15.9	b*
TSH (mIU/l)	2.24 ± 0.60	2.42 ± 0.85	2.34 ± 0.84	NS
Free thyroxin (pg/ml)	8.8 ± 1.2	9.1 ± 1.5	11.4 ± 1.5	b*** c**
Prolactin (μg/l)	5.4 (5.3–6.5)	13.0 (11.5–14.0)	12.0 (10.3–15.0)	a,b**
Testosterone (ng/dl)	34.3 ± 14.0	31.4 ± 9.1	34.3 ± 11.4	NS
SHBG (mg/l)	3.0 ± 0.8	4.1 ± 0.7	3.8 ± 1.0	NS

Values are expressed as mean ± SD or median (P_{25}–P_{75}). VO_2 max, maximal oxygen uptake; TSH, thyroid-stimulating hormone; SHBG, sex hormone-binding globulin. Significant differences between groups are indicated *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$, [a]OAM versus RM, [b]OAM versus CTR, [c]RM versus CTR or NS = not significant (one-way ANOVA followed by Fisher's *post hoc* analysis).

Table II Body composition before and during treatment with OCs in OAM athletes, RM athletes and CTRs

Groups	OAM (n = 9)		RM (n = 8)		CTR (n = 8)	
	Before	During OC[#]	Before	During OC	Before	During OC
BMI (kg/m²)	19.7 ± 1.9	20.5 ± 2.1	19.7 ± 1.5	20.6 ± 1.4	19.3 ± 1.6	19.7 ± 1.4
Fat mass, total (%)	17.7 ± 4.6 b*	21.2 ± 4.5**	21.7 ± 4.8	23.4 ± 4.3	22.7 ± 4.5	23.0 ± 4.2
LBM, total (kg)	45.9 ± 4.1	46.1 ± 5.3	42.5 ± 2.9	42.5 ± 2.8	39.4 ± 3.7	40.0 ± 3.5
LBM, legs (kg)	16.7 ± 2.3 b**	16.0 ± 1.9	15.3 ± 1.2	15.2 ± 1.1	13.7 ± 1.9	14.2 ± 1.5
Total LBM/fat mass ratio	4.73 ± 1.54 b*	3.70 ± 0.98**	3.62 ± 1.15	3.25 ± 1.13	3.33 ± 0.75	3.27 ± 0.76

Values are expressed as mean ± SD. Significant differences in baseline values between groups are indicated in the first OAM column as: [b]OAM versus CTR (one-way ANOVA followed by Fishers post-hoc analysis). Significant differences within groups are indicated in the right column, respectively (two-way ANOVA followed by paired *t*-test in the case of significant interaction), whereas significant overall treatment effects are described in the text.
Significance levels are *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.
[#]Investigations were performed during the last week of the active OC treatment cycle.

remained unchanged in the regularly menstruating groups. There was no significant change in LBM in any group, whereas the ratio of LBM/fat mass ratio was normalized in the OAM group.

Effects of OCs on fasting levels of IGF-I, IGFBP-2 and CBG

Fasting levels of IGF-I and its binding proteins are shown in Table III. There were no significant differences between groups in fasting levels of total and free IGF-I, IGFBP-2 and binary complex (IGF-I + IGFBP-1) before or during OC treatment. However, there was an overall decrease in levels of free IGF-I and IGFBP-2 by OC [$F(1,22) = 8.0$, $P < 0.01$ and $F(1,22) = 47.1$, $P < 0.001$, respectively], whereas levels of total IGF-I were unchanged during treatment. There was also a tendency to an overall increase in binary complex in the pooled cohort ($F(1,22) = 3.5$, $P = 0.07$].

Fasting levels of CBG before treatment were slightly higher in the OAM group compared with controls (OAM 52.7 ± 7.3, RM 50.2 ± 7.9, CTR 44.6 ± 6.5 μg/l, OAM versus CTR, $P < 0.05$). After treatment, there was a general increase in fasting CBG and mean levels were similar in the three groups [OAM 107.2 ± 24.5, RM 111.6 ± 16.7, CTR 110.1 ± 21.3 μg/l, $F(1,22) = 248$, $P < 0.001$].

Effects of OCs on diurnal secretion of insulin, IGFBP-1, GH and cortisol

Diurnal levels of hormones and IGFBP-1 in OAM, RM and CTR are shown in Table IV. Before OC treatment, OAM displayed significantly lower insulin (AUC and 24 h baseline mean) and higher IGFBP-1 mean

peak amplitude than controls. Furthermore, the AUC insulin/IGFBP-1 ratio was significantly lower in OAM athletes compared with controls [0.51 (0.40–1.48) versus 2.49 (0.05–3.10), $P < 0.01$]. Moreover, pretreatment diurnal levels of GH (24 h baseline mean) were significantly higher in OAM than in regularly menstruating athletes, and cortisol (AUC and 24 h baseline mean) was significantly higher in OAM than in controls.

During OC treatment, there was an overall increase in diurnal secretion of insulin [AUC: $F(1,22) = 15.1$, $P < 0.001$] with no significant difference between groups. However, the change in IGFBP-1 differed between groups with a significant increase in the regularly menstruating subjects (RM and CTR) but not in the OAM athletes with menstrual disturbance. Diurnal profiles of IGFBP-1 before and during OC treatment in individuals representative of the three groups are shown in Fig. 1. OC treatment also decreased AUC insulin/IGFBP-1 ratio in both regularly menstruating groups [RM 1.14 (0.92–1.53) versus 0.75 (0.42–0.94), CTR 2.49 (0.05–3.10) versus 0.96 (0.84–1.22), $P < 0.01$, respectively] but not in the OAM athletes [0.51 (0.40–1.48) versus 0.51 (0.49–0.70)]. Furthermore, there were overall increases in GH [AUC: $F(1,22) = 6.6$, $P < 0.05$, respectively] and cortisol [AUC: $F(1,22) = 146$, $P < 0.001$] by OC treatment with no significant change between groups. After treatment, AUC was similar between groups for insulin, IGFBP-1, cortisol and GH.

Table III Fasting levels of total and free IGF-I and IGFBP before and during treatment with OC in OAM athletes, RM athletes and CTRs

Groups	OAM (n = 9)		RM (n = 8)		CTR (n = 8)	
	Before	During OC	Before	During OC	Before	During OC
Total IGF-I (μg/l)	356 ± 104	316 ± 38	315 ± 81	276 ± 32	313 ± 68	320 ± 112
Free IGF-I (μg/l)	1.44 ± 0.64	1.39 ± 0.42	1.58 ± 0.47	1.19 ± 0.36	1.75 ± 0.51	1.42 ± 0.59
IGFBP-2 (μg/l)	193 ± 43	129 ± 38	178 ± 51	134 ± 41	141 ± 51	105 ± 38
Binary complex (μg/l)	7.0 (3.2–15.0)	18.7 (8.6–28.2)	11.4 (9.6–14.8)	14.0 (6.0–31.0)	8.4 (5.1–16.0)	9.2 (5.0–17.6)

Values are expressed as mean ± SD or median (P_{25}–P_{75}). There were no significant differences within and between groups (one-way and two-way ANOVA). Significant overall treatment effects are described in the text. Binary complex is IGF-I + IGFBP-1.

Correlations

Pretreatment diurnal baseline mean levels of GH were negatively correlated with percentage of total and trunk fat mass ($r_s = -0.47$, $P < 0.05$ and $r_s = -0.45$, $P < 0.05$, respectively). Furthermore, pretreatment GH levels were positively correlated with change in percentage of total fat mass ($r_s = 0.67$, $P < 0.01$) and trunk fat mass ($r_s = 0.49$, $P < 0.01$) during OC treatment. Diurnal cortisol levels before treatment were also positively correlated with change in the corresponding fat mass variables ($r_s = 0.64$, $P < 0.01$ and $r_s = 0.69$, $P < 0.01$, respectively). There were no significant correlations between insulin or IGFBP-1 and body composition variables.

Discussion

We have demonstrated that OC treatment in OAM endurance athletes increases body fat mass and results in diurnal levels of insulin, IGFBP-1, cortisol and GH that are comparable to those in regularly menstruating athletes and controls. The change in fat mass correlated with pretreatment diurnal levels of GH and cortisol, which together with insulin and IGFBP-1 differed significantly between oligo-/amenorrheic athletes and regularly menstruating subjects at baseline.

Before treatment, OAM athletes had lower diurnal levels of insulin and higher levels of IGFBP-1 (mean peak amplitude) and cortisol (AUC and 24 h baseline mean) than regularly menstruating subjects, as well as lower amount of body fat than controls. The production of IGFBP-1 is negatively regulated by insulin (Fernqvist-Forbes *et al*., 1999) and the low insulin levels in the athletes with menstrual disturbance most likely explain the increased IGFBP-1 secretion. However, free and total IGF-I was not significantly different between OAM athletes and subjects with regular menstruations. Both short-term fasting (Chen *et al*., 2005) and anorexia nervosa (Gianotti *et al*., 2002) have

Table IV Diurnal levels of hormones and IGFBP-1 before and during treatment with OC in OAM athletes, RM athletes and CTRs

Groups	OAM (*n* = 9)		RM (*n* = 8)		CTR (*n* = 8)	
	Before	During OC	Before	During OC	Before	During OC
Insulin (mIU/l)						
AUC (mIU/l × 24 h)	312 (209–390) b*	401 (372–462)	329 (289–374)	435 (365–490)	449 (381–494)	527 (425–571)
24 h baseline mean	7.0 ± 2.3 b**	8.7 ± 3.2	8.5 ± 2.0	9.0 ± 2.6	11.4 ± 3.0	12.6 ± 2.4
Peaks/24 h	10 (9–12)	9 (8–11)	10.5 (10–13)	9 (8.5–10)	10 (8–11.5)	10.5 (9–11)
Mean peak amplitude	15.4 (12.1–25.3)	22.7 (19.7–24.5)	11.6 (11.1–14.1)	20.6 (17.1–27.6)	17.0 (14.6–26.5)	22.2 (15.6–25.9)
IGFBP-1 (μg/l)						
AUC (μg/l × 24 h)	520 (230–808)	722 (500–844)	259 (214–387)	654 (454–1250)***	201 (147–358)	568 (377–702)***
24 h baseline mean	10.4 (4.2–14.0)	12.9 (7.1–22.4)	4.7 (4.0–5.3)	9.4 (7.2–29.4)	3.6 (2.5–6.2)	12.8 (6.8–18.5)
Peaks/24 h	5.8 ± 1.9	5.3 ± 2.1	6.4 ± 1.1	5.6 ± 2.5	8.2 ± 1.8	4.0 ± 1.5 ***
Mean peak amplitude	23.6 ± 13.6 b*	28.4 ± 11.5	16.3 ± 9.2	37.2 ± 22.5**	8.6 ± 3.9	29.3 ± 16.7***
GH (μg/l)						
AUC (μg/l × 24 h)	50.9 (40.7–60.2)	66.0 (35.2–76.7)	56.9 (39.0–71.3)	56.1 (41.9–71.9)	33.2 (27.0–58.2)	54.2 (34.7–62.3)
24 h baseline mean	0.35 (0.29–0.60) a*	0.72 (0.36–1.07)	0.16 (0.13–0.24)	0.23 (0.18–0.85)	0.15 (0.11–0.22)	0.22 (0.17–0.35)
Peaks/24 h	10.0 (9.0–11.00)	10.0 (9.0–11.0)	9.0 (8.5–9.5)	9.0 (8.5–10.0)	8.0 (7.5–8.5)	9.0 (8.0–12.0)
Mean peak amplitude	3.6 (3.1–4.3)	3.2 (2.8–4.8)	5.4 (3.5–7.1)	4.8 (4.4–5.7)	3.5 (2.9–5.0)	3.9 (3.0–5.4)
Cortisol (μg/dl)						
AUC (μg/dl × 24 h)	211 ± 41 b*	353 ± 74	173 ± 33	305 ± 56	161 ± 34	293 ± 72
24 h baseline mean	5.2 ± 1.1 b*	9.7 ± 2.4	3.8 ± 1.0	7.8 ± 2.0	3.8 ± 1.1	7.8 ± 2.9
Peaks/24 h	4.8 ± 1.0	3.1 ± 0.6	4.2 ± 0.5	3.5 ± 1.2	5.0 ± 1.5	3.9 ± 2.3
Mean peak amplitude	7.8 (7.2–9.5)	14.6 (12.9–16.6)	8.0 (7.1–9.0)	13.8 (11.7–16.7)	7.1 (5.9–9.0)	14.9 (9.1–17.7)

Values are expressed as mean ± SD or median (P_{25}–P_{75}). Significant differences between groups before OC treatment are indicated in the first OAM column as: [a]OAM versus RM and [b]OAM versus CTR (one-way ANOVA followed by Fisher's *post hoc* analysis). Significant differences within groups are indicated in the right column, respectively (two-way ANOVA followed by paired *t*-test in the case of significant interaction), whereas significant overall treatment effects are described in the text, AUC, area under curve.

Significance levels are *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

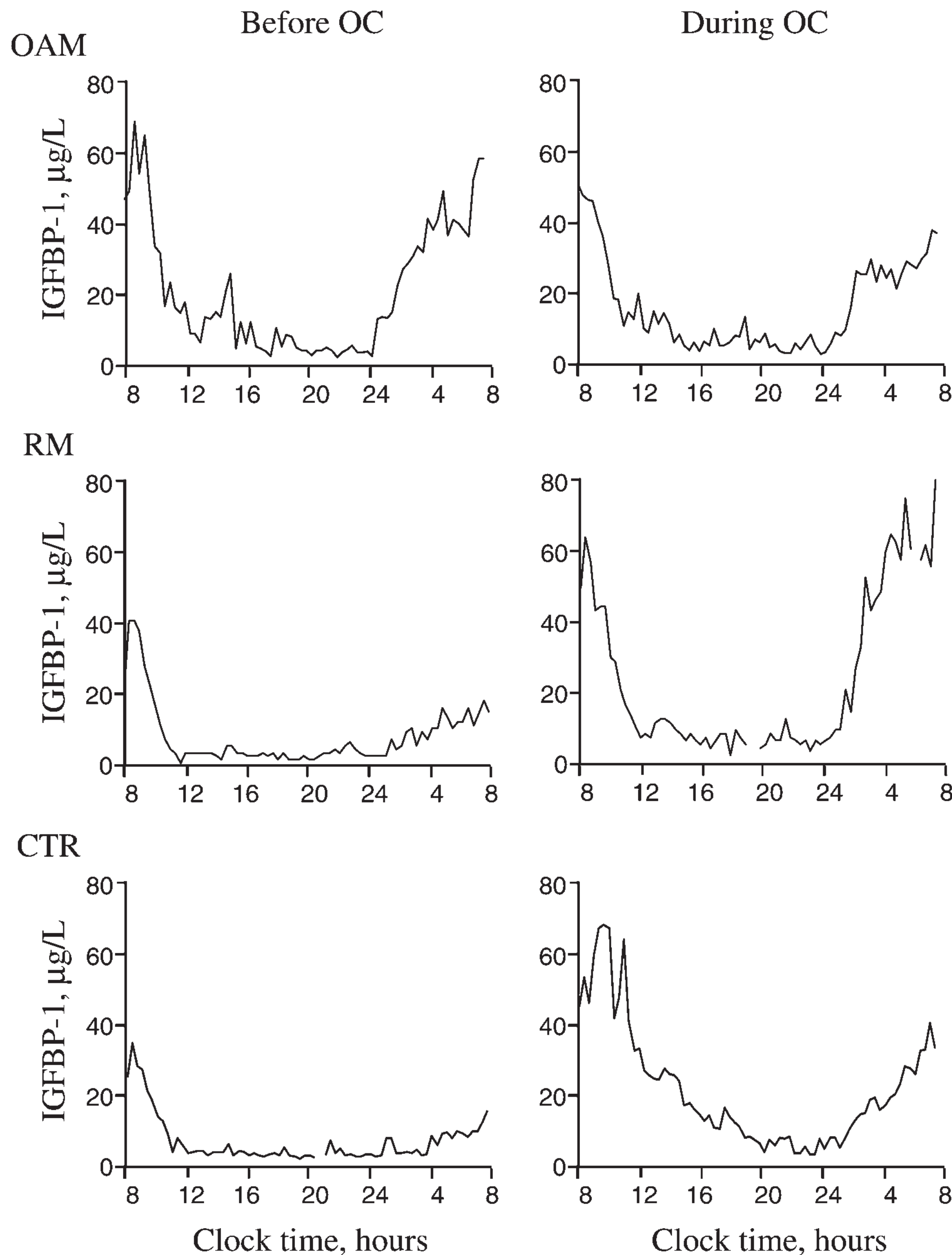

Figure 1 Diurnal profiles of IGFBP-1 before and during treatment with OC (30 µg ethinyl etradiol + 150 µg levonorgestrel) in three representative study individuals: one athlete with OAM, one athlete with RM, and one regularly menstruating CTR.

been shown to be associated with significantly decreased levels of free and total IGF-I. In contrast, more modest reduction in caloric intake appears to have minor or no impact on IGF-I levels (Fontana *et al.*, 2008). This is in agreement with the findings in our OAM athletes who had numerically lower caloric intake and significantly lower protein intake (on average 18%) than the regularly menstruating athletes. Although free IGF-I was lowest in the OAM group, the difference did not reach significance with the limited sample size. The higher GH secretion in OAM athletes may reflect a relative GH resistance as a result of nutritional deficits. This could be explained by the lower insulin secretion, as insulin is an important regulator of the hepatic GH receptor density (Leung *et al.*, 2000).

The women in the present study were treated with an OC containing 30 µg ethinyl estradiol and 150 µg of the androgenic progestagen, levonorgestrel, for a mean period of 7 months. It has been shown from studies of normal subjects of different ages that oral administration of estrogens and androgens have partly opposite effects on the GH–IGF– IGFBP axis (Heald *et al.*, 2005; Rooman *et al.*, 2005; Veldhuis *et al.*, 2005). Oral estrogens impair the metabolic actions of GH in the liver causing a fall in circulating-free IGF-I, which via

negative feedback regulation may increase GH secretion (Rooman *et al*., 2005; Veldhuis *et al*., 2005). This fall of IGF-I appears to be caused both by depression of post-receptor GH signaling in the hepatocytes and by increase in circulating GH-binding protein, reducing the availability of GH to the receptor (Leung *et al*., 2004). Furthermore, IGFBP-1 is stimulated and IGFBP-2 is suppressed by oral estrogens. Androgens on the other hand have been reported to stimulate free IGF-I and have no effect on IGFBP-1 and -2 (Rooman *et al*., 2005). It has been shown that the decrease in IGF-I during administration of combined OCs containing the androgenic progesterone levonorgestrel is lower than with the anti-androgenic dienogest (Balogh *et al*., 2000). In the present study, we found no change in fasting levels of total IGF-I but a general decrease in free IGF-I with a non-significant increase in the IGF-I–IGFBP-1 binary complex as well as a decrease in IGFBP-2. Thus, the effects of ethinyl estradiol may have been partly obviated by levonorgestrel.

Diurnal levels of insulin (AUC) increased during OC treatment in all groups. This is most likely a result of decreased insulin sensitivity known to occur during OC treatment (Crook and Godsland, 1998). Regularly menstruating athletes and sedentary controls had a substantial increase in IGFBP-1 (AUC and mean peak amplitude) and a decrease in the ratio of insulin/IGFBP-1. In contrast, this ratio was unchanged in athletes with menstrual disturbance and levels of IGFBP-1 displayed unchanged diurnal variations. Furthermore, there were general increases of GH (AUC), probably attributed to the decrease in free IGF-I. Diurnal levels of cortisol (AUC) were also increased in the three groups, which was most likely the result of a concomitant increase in CBG since free cortisol does not increase during OC treatment (Simunkova *et al*., 2008). The significant differences between groups before OC treatment in insulin (AUC), IGFBP-1 (mean peak amplitude) and cortisol (AUC and 24 h baseline mean) were abolished after treatment. OCs significantly increased fat mass in athletes with oligo-/amenorrhea but not in regularly menstruating athletes and sedentary controls. The increase in body fat was positively related to diurnal levels of cortisol and GH before treatment. Thus, high pretreatment levels of GH and cortisol indicate reduced energy stores, which can be reversed by OC treatment.

The precise mechanisms for the OC-associated increase in weight and fat mass in the athletes with menstrual disturbance but not in regularly menstruating subject are unknown. Sex steroids may interfere with appetite and metabolic functions. E_2 is known to inhibit feeding in animals and to increase the activity of the cholecystokinin (CCK) satiation signaling pathway (Geary, 2001), whereas high-dose progestagens are appetite stimulating (Maltoni *et al*., 2001). We have previously demonstrated a suppressed secretion of the satiety peptide CCK during OC treatment related to an increase in body fat in young women (Hirschberg *et al*., 1996). Thus, some women may experience changes in appetite and weight by OC treatment, although studies so far in general have failed to show any effect on body weight and body composition (Franchini *et al*., 1995; Reubinoff *et al*., 1995; Lloyd *et al*., 2002; De Melo *et al*., 2004). Despite extensive clinical experience of OC treatment, effects on appetite and metabolism still remain to be explored.

In anorexia nervosa and other conditions with severe undernutrition, OC treatment has not been shown to have significant effect on weight recovery (Miller *et al*., 2006). However, in the present study, none of the subjects suffered from any eating disorder that would restrain food intake. Moreover, similar physical activity was reported during the study period. It is therefore possible that the OC treatment might have brought about a change in appetite and subsequently increased food intake. However, an alternative explanation is that OC treatment induces metabolic changes, such as decreased fat oxidation, resulting in increased fat mass as a source for energy utilization. Subjects with reduced energy stores may be more prone to gain weight than those with adequate nutrition.

A limitation with the present study was the relatively small sample size. However, the study comprised a well-defined cohort of female endurance athletes. The strength of the study was the prospective design by which the subjects were rigorously studied during 8 months of OC treatment.

In summary, this study demonstrates that OC treatment in endurance athletes with menstrual disturbance increases body fat mass and results in diurnal levels of insulin, IGFBP-1, GH and cortisol that are comparable to those in regularly menstruating subjects. These results suggest that OCs improve metabolic balance in OAM athletes. The treatment could therefore be beneficial for athletes with oligo-/amenorrhea resulting from hypothalamic inhibition. However, adequate nutrition should be encouraged as the first line of strategy.

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References

Åstrand PO, Rodahl K. Evaluation of physical performance on the basis of tests. *Textbook of Work Physiology*. New York: McGraw-Hill Book Co., 1986, 354–387.

Balogh A, Kauf E, Vollanth R, Graser G, Klinger G, Oettel M. Effects of two oral contraceptives on plasma levels of insulin-like growth factor I (IGF-I) and growth hormone (hGH). *Contraception* 2000;**62**:259–269.

Chen JW, Hojlund K, Beck-Nielsen H, Sandahl Christiansen J, Orskov H, Frystyk J. Free rather than total circulating insulin-like growth factor-I determines the feedback on growth hormone release in normal subjects. *J Clin Endocrinol Metab* 2005;**90**:366–371.

Crook D, Godsland I. Safety evaluation of modern oral contraceptives. Effects on lipoprotein and carbohydrate metabolism. *Contraception* 1998;**57**:189–201.

De Melo NR, Aldrighi JM, Faggion D Jr, Reyes VR, Souza JB, Fernandes CE, Larson E. A prospective open-label study to evaluate the effects of the oral contraceptive Harmonet (gestodene75/EE20) on body fat. *Contraception* 2004;**70**:65–71.

De Souza MJ, van Heest J, Demers LM, Lasley BL. Luteal phase deficiency in recreational runners: evidence for a hypometabolic state. *J Clin Endocrinol Metab* 2003;**88**:337–346.

Fernqvist-Forbes E, Ekberg K, Lindgren BF, Brismar K. Splanchnic exchange of insulin-like growth factor binding protein-1 (IGFBP-1), IGF-I and acid-labile subunit (ALS) during normo- and hyper-insulinaemia in healthy subjects. *Clin Endocrinol (Oxf)* 1999;**51**:327–332.

Fontana L, Weiss EP, Villareal DT, Klein S, Holloszy JO. Long-term effects of calorie or protein restriction on serum IGF-I and IGFBP-3 in humans. *Aging Cell* 2008;**7**:681–687.

Franchini M, Caruso C, Nigrelli S, Poggiali C. Evaluation of body composition during low-dose estrogen oral contraceptives treatment. *Acta Eur Fertil* 1995;**26**:69–73.

Frystyk J, Skjaerbaek C, Dinesen B, Orskov H. Free insulin-like growth factors (IGF-I and IGF-II) in human serum. *FEBS Lett* 1994;**348**:185–191.

Frystyk J, Dinesen B, Orskov H. Non-competitive time-resolved immunofluorometric assays for determination of human insulin-like growth factor I and II. *Growth Regul* 1995;**5**:169–176.

Frystyk J, Hojlund K, Rasmussen KN, Jorgensen SP, Wildner-Christensen M, Orskov H. Development and clinical evaluation of a novel immunoassay for the binary complex of IGF-I and IGF-binding protein-1 in human serum. *J Clin Endocrinol Metab* 2002;**87**:260–266.

Garner DM. *Eating Disorder Inventory-2, professional manual*. Odessa, FL: Psychological Assessment Resources, Inc., 1991.

Geary N. Estradiol, CCK and satiation. *Peptides* 2001;**22**:1251–1263.

Gianotti L, Lanfranco F, Ramunni J, Destefanis S, Ghigo E, Arvat E. GH/IGF-1 axis in anorexia nervosa. *Eat Weight Disord* 2002;**7**:94–105.

Heald A, Kaushal K, Anderson S, Redpath M, Durrington PN, Selby PL, Gibson MJ. Effects of hormone replacement therapy on insulin-like growth factor (IGF)-I, IGF-II and IGF binding protein (IGFBP)-1 to IGFBP-4: implications for cardiovascular risk. *Gynecol Endocrinol* 2005; **20**:176–182.

Hirschberg AL, Bystrom B, Carlstrom K, von Schoultz B. Reduced serum cholecystokinin and increase in body fat during oral contraception. *Contraception* 1996;**53**:109–113.

Karlsson R, Eden S, von Schoultz B. Altered growth hormone secretion during oral contraception. *Gynecol Obstet Invest* 1990;**30**:234–238.

Krassas GE, Pontikidis N, Kaltsas T, Dumas A, Frystyk J, Chen JW, Flyvbjerg A. Free and total insulin-like growth factor (IGF)-I, -II, and IGF-binding protein-1, -2 and -3 serum levels in patients with active thyroid disease. *J Clin Endocrinol Metab* 2003;**88**:132–135.

Laughlin GA, Yen SS. Nutritional and endocrine-metabolic aberrations in amenorrheic athletes. *J Clin Endocrinol Metab* 1996;**81**:4301–4309.

Leung K-C, Doyle N, Ballesteros M, Waters MJ, Ho KKY. Insulin regulation of human hepatic growth hormone receptors: divergent effects on biosynthesis and surface translocation. *J Clin Endocrinol Metab* 2000; **85**:4712–4720.

Leung K-C, Johannsson G, Leong GM, Ho KKY. Estrogen regulation of growth hormone action. *Endocr Rev* 2004;**25**:693–721.

Lloyd T, Lin HM, Matthews AE, Bentley CM, Legro RS. Oral contraceptive use by teenage women does not affect body composition. *Obstet Gynecol* 2002;**100**:235–239.

Loucks AB, Thuma JR. Luteinizing hormone pulsatility is disrupted at a threshold of energy availability in regularly menstruating women. *J Clin Endocrinol Metab* 2003;**88**:297–311.

Maltoni M, Nanni O, Scarpi E, Rossi D, Serra P, Amadori D. High-dose progestins for the treatment of cancer anorexia-cachexia syndrome: a systematic review of randomised clinical trials. *Ann Oncol* 2001; **12**:289–300.

Merriam GR, Wachter KW. Algorithms for the study of episodic hormone secretion. *Am J Physiol* 1982;**243**:E310–E318.

Miller KK, Lee EE, Lawson EA, Misra M, Minihan J, Grinspoon SK, Gleysteen S, Mickley D, Herzog D, Klibanski A. Determinants of skeletal loss and recovery in anorexia nervosa. *J Clin Endocrinol Metab* 2006;**91**:2931–2937.

Povoa G, Roovete A, Hall K. Cross-reaction of serum somatomedin-binding protein in a radioimmunoassay developed for somatomedin-binding protein isolated from human amniotic fluid. *Acta Endocrinol (Copenh)* 1984;**107**:563–570.

Reubinoff BE, Grubstein A, Meirow D, Berry E, Schenker JG, Brzezinski A. Effects of low-dose estrogen oral contraceptives on weight, body composition, and fat distribution in young women. *Fertil Steril* 1995; **63**:516–521.

Rickenlund A, Carlstrom K, Ekblom B, Brismar TB, von Schoultz B, Hirschberg AL. Hyperandrogenicity is an alternative mechanism underlying oligomenorrhea or amenorrhea in female athletes and may improve physical performance. *Fertil Steril* 2003;**79**:947–955.

Rickenlund A, Thoren M, Carlstrom K, von Schoultz B, Hirschberg AL. Diurnal profiles of testosterone and pituitary hormones suggest different mechanisms for menstrual disturbances in endurance athletes. *J Clin Endocrinol Metab* 2004a;**89**:702–707.

Rickenlund A, Carlstrom K, Ekblom B, Brismar TB, Von Schoultz B, Hirschberg AL. Effects of oral contraceptives on body composition and physical performance in female athletes. *J Clin Endocrinol Metab* 2004b;**89**:4364–4370.

Rooman RP, De Beeck LO, Martin M, van Doorn J, Mohan S, Du Caju MV. Ethinylestradiol and testosterone have divergent effects on circulating IGF system components in adolescents with constitutional tall stature. *Eur J Endocrinol* 2005;**152**:597–604.

Simunkova A, Starka L, Hill M, Kriz L, Hampl R, Vondra K. Comparison of total and salivary cortisol in a low-dose ACTH (Synacthen) test: influence of three-month oral contraceptives administration to healthy women. *Physiol Res* 2008;**57**(Suppl 1):193–199.

Song S, Chen JK, He ML, Fotherby K. Effect of some oral contraceptives on serum concentrations of sex hormone binding globulin and ceruloplasmin. *Contraception* 1989;**39**:385–399.

Stoving RK, Chen JW, Glintborg D, Brixen K, Flyvbjerg A, Horder K, Frystyk J. Bioactive insulin-like growth factor (IGF) I and IGF-binding protein-1 in anorexia nervosa. *J Clin Endocrinol Metab* 2007;**92**:2323–2329.

Veldhuis JD, Frystyk J, Iranmanesh A, Orskov H. Testosterone and estradiol regulate free insulin-like growth factor I (IGF-I), IGF binding protein 1 (IGFBP-1), and dimeric IGF-I/IGFBP-1 concentrations. *J Clin Endocrinol Metab* 2005;**90**:2941–2947.

Waters DL, Qualls CR, Dorin R, Veldhuis JD, Baumgartner RN. Increased pulsatility, process irregularity, and nocturnal trough concentrations of growth hormone in amenorrheic compared to eumenorrheic athletes. *J Clin Endocrinol Metab* 2001;**86**:1013–1019.

Waters DL, Dorin RI, Qualls CR, Ruby BC, Baumgartner RN, Robergs RA. Estradiol effects on the growth hormone/insulin-like growth factor-1 axis in amenorrheic athletes. *Can J Appl Physiol* 2003;**28**:64–78.

Westwood M, Gibson JM, Pennells LA, White A. Modification of plasma insulin-like growth factors and binding proteins during oral contraceptive use and the normal menstrual cycle. *Am J Obstet Gynecol* 1999;**180**:530–536.

Wiegratz I, Kutschera E, Lee JH, Moore C, Mellinger U, Winkler UH, Kuhl H. Effect of four different oral contraceptives on various sex hormones and serum-binding globulins. *Contraception* 2003a;**67**:25–32.

Wiegratz I, Kutschera E, Lee JH, Moore C, Mellinger U, Winkler UH, Kuhl H. Effect of four oral contraceptives on thyroid hormones, adrenal and blood pressure parameters. *Contraception* 2003b;**67**:361–366.

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