Acute inhibition of oestrogen biosynthesis does not affect serum leptin levels in young men

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Abstract

Objective: Leptin plays an important role in the regulation of reproduction. To explore the contribution of oestradiol to serum leptin levels in men, we measured the concentrations of serum leptin and insulin after inhibition of oestrogen biosynthesis by selective blockade of the aromatase enzyme.

Design: The study had a double-blind parallel group design.

Methods: The aromatase inhibitor, MPV 2213ad, was given to eight healthy male volunteers as a single dose of 100 mg. Eight men received placebo. Serum leptin and insulin were determined from blood samples collected at 0800 h, 1600 h and 2000 h both on the actual test day (day 0) and on the previous day (day −1), and from single blood samples taken in the morning of days 1, 2, 4 and 7. Changes in serum leptin were correlated with those seen in serum oestradiol, testosterone, LH, FSH, cortisol and aldosterone, which were determined earlier.

Results: After the aromatase inhibitor administration, mean serum oestradiol concentration was reduced by 74% from the baseline compared with a 19% reduction in the placebo group (P for difference < 0.001), and returned to pre-treatment levels within four days. Despite marked changes in serum oestradiol and sustained elevations in serum testosterone, LH and FSH concentrations, serum leptin concentrations were similar in the group receiving the aromatase inhibitor and in the placebo group. We found a weak correlation between serum oestradiol and leptin, which could not be reproduced when the percentage changes in these variables were analysed.

Conclusion: Marked short-term reduction in serum oestradiol concentration has no effect on serum leptin levels in young men.

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Introduction

Leptin is the hormonal product of the ob gene, predominantly produced in adipose tissue but secreted also by human placenta (1) and stomach (2). In addition to its important role in the regulation of energy balance, leptin signals metabolic information to the reproductive system (3).

Leptin administration restores fertility in ob/ob mice lacking endogenous leptin, and accelerates the onset of reproductive functions in normal mice (3). In humans, leptin is needed for the initiation of puberty and development of secondary sexual characteristics (4, 5). Circulating leptin concentration is increased in both boys and girls before pubertal changes in other reproductive hormones (3).

Females have higher concentrations of circulating leptin than men (6–8). The gender difference in circulating leptin and leptin mRNA expression is well established both in rodents (9) and in humans (10), but the reason for this dimorphism is still obscure. It may, at least in part, be explained by a suppressive effect of androgens on leptin. An inverse correlation between testosterone and leptin concentrations has been reported in several studies (11–13), but whether there is an association between serum oestradiol and leptin is not currently known. Earlier studies have, however, suggested that there might be a connection between oestrogens and leptin at the cellular level. According to Shimizu et al., the ob gene has a consensus sequence of the oestrogen-responsive element in its promoter region, and high-affinity oestrogen binding macromolecules are present in the cytosolic fraction of various adipose tissues (14). Leptin is present in follicular fluid and can induce biological response in ovarian cells, suggesting that leptin may have a direct effect on human ovary (15, 16). Both long and short isoforms of leptin receptor are expressed in ovary (15), which raises
the possibility that leptin could influence the hypothalamo–pituitary–gonadal axis at the peripheral level. Oestradiol has been reported to increase leptin mRNA expression twofold in isolated rat adipocytes (17). In rats, ovariectomy decreased serum leptin (14) and leptin mRNA expression in white adipose tissue, and long-term oestradiol supplementation reversed these effects (14, 18). In male rats, oestradiol treatment decreased serum leptin levels (19), which, however, might have been at least partly due to simultaneous reduction in body weight. In human adipose tissue culture, oestradiol stimulated leptin secretion in women but not in men (20). Inhibition of oestrogen biosynthesis by selective blockade of the aromatase enzyme has become an established method of hormonal treatment in oestrogen-dependent malignant conditions (21).

MPV-2213ad (4-(3-(4-fluorophenyl)-2-hydroxy-1-(1,2,4-triazol-1-yl)propyl)benzonitrile a+d) is a new orally active non-steroidal competitive aromatase inhibitor, which has shown high potency and selectivity in phase I studies (22, 23). The aim of this study was to clarify whether oestrogen modulates the circulating leptin concentration in men. Associations of leptin and a number of other hormones potentially affecting serum leptin concentration were also studied.

Subjects and methods

Study design and subjects

Previously, we have studied and reported the effect of a new orally active non-steroidal aromatase inhibitor (MPV-2213ad) (23) on oestradiol biosynthesis. In the work reported here, we determined serum leptin and insulin concentrations in the samples originating from that study. The study had a double-blind, parallel group design. Eight healthy male volunteers were randomised to receive a single dose of placebo, whereas another eight volunteers received a single 100 mg dose of MPV-2213ad. The study drugs were given orally with 200 ml water after an overnight fast.

The mean age of the volunteers was 23 years (range 21–34 years), and the mean body weights in the placebo and active treatment groups were 79.6 ± 7.0 kg and 73.4 ± 6.3 kg, respectively. The mean body mass index (BMI) was 24.0 ± 0.8 kg/m² in the placebo group and 22.6 ± 1.6 kg/m² in the MPV-2213ad group. The general health of the subjects was ascertained by laboratory determinations and by ongoing physical examination. The volunteers were required to keep to their normal dietary habits and to abstain from smoking, any use of alcohol or strenuous physical activity during the test days. Any use of medication was prohibited for two weeks prior to the study. Orion Corporation (Turku, Finland) manufactured MPV-2213ad, the clinical development of which is performed by Hormos Medical Ltd (Turku, Finland). The study was conducted at the Department of Clinical Pharmacology, University of Turku. The procedures followed were approved by the Joint Ethics Committee of the Turku University and the Turku University Central Hospital, and were in accordance with the ethical standards of the Helsinki Declaration and its revisions. The National Agency for Medicines was notified prior to the start of the study, according to the local regulations. Written informed consent was obtained from every subject.

Experimental procedures

Because of circadian variation in serum concentrations of many hormones, blood samples for hormone measurements were collected at 0800 h, 1600 h and 2000 h both on the day preceding the drug test day (day −1) and on the actual test day (day 0). Blood samples were drawn through an indwelling cannula inserted into a forearm vein. On days 1, 2, 4 and 7 after the drug test day a blood sample was collected at 0800 h by venipuncture. Blood samples were drawn into glass tubes without additives, and the sera were kept frozen at −20°C until analysed. The subjects were followed in the laboratory for 8 h after drug administration. During the observation period, all volunteers were allowed to move freely but were not allowed to leave the laboratory. They were required to be in a sitting position for 15 min before each blood sampling. Standard lunch was served 4 h after the drug administration.

Assays

Serum leptin was analysed with a human leptin RIA kit (Linco Research, St Charles, MO, USA), using a polyclonal antibody raised in rabbits against highly purified human leptin. The assay had a sensitivity of 0.5 µg/l. The interassay coefficient of variation (CV) was 3.6% at a mean concentration of 2.7 µg/l, and 9.8% at a mean concentration of 21.0 µg/l. Serum insulin was determined with a Phadeseph insulin RIA kit (Pharmacia, Uppsala, Sweden) with a detection limit of 2.5 mU/l. The intra-assay CV was 0.5% at a mean concentration of 21.0 mU/l. Serum oestradiol, testosterone, luteinising hormone (LH), follicle-stimulating hormone (FSH), aldosterone and cortisol were measured as published earlier by Ahokoski et al. (23). All analyses were carried out according to the manufacturers’ guidelines.

Statistical analyses

Results are expressed as means ± S.D. BMI was calculated by dividing body weight (kg) by square height (m). Percentage changes in the hormones were used in the calculations, as given later. Logarithmic transformations were made when necessary to obtain normality. The differences between the two treatment groups after
drug administration were tested with 2-way (treatment × time) ANOVAs for repeated measurements, using treatment (MPV-2213ad or placebo) as the between variable and time (time of blood sampling) as the within variable. The value recorded at 0800 h on day 0 was defined as 100%. For the same purpose, we also calculated the area under the curve (AUC) of percentage change in serum leptin and oestradiol concentration for each subject from day 0 to day 7, and then used t-test to compare the two treatment groups. The calculation was performed with TopFit 2.0 pharmacokinetic and pharmacodynamic software (Gustav Fischer, Stuttgart, Germany). Days −1 and 0 were compared with 3-way (treatment × day × time) ANOVAs, where the 0800 h value on day −1 was defined as 100%. t-test was used to compare the reductions in serum oestradiol on day −1 and day 0 from the morning value to the nadir at 1600 h between the treatment groups. Correlations were calculated by Pearson’s method using actual hormone concentrations. In the stepwise multiple regression analysis, all the variables were first analysed together, and only significant variables were included in the next step of analysis. P values less than 0.05 were considered statistically significant. Statistical analysis was performed using Statistica (StatSoft, Tulsa, OK, USA).

Results

The percentage changes in serum leptin, oestradiol, testosterone, LH and FSH concentrations during the study are shown in Fig. 1. The changes in serum leptin concentration after the drug administration were similar in the placebo and aromatase inhibitor groups (treatment F = 0.002, P = 0.96, 2-way-ANOVA for repeated measurements), and the shapes of the leptin concentration curves did not differ between the groups (treatment × time interaction F = 0.78, P = 0.60). The AUC of the percentage changes in leptin concentrations calculated over day 1 and up to 7 days did not differ between the two treatment groups (P = 0.59 and P = 0.82 respectively, t-test). There were clear between group differences in the shapes of the concentration vs time curves in serum oestradiol (treatment × time interaction F = 19.47, P < 0.001), LH (treatment × time interaction F = 2.93, P = 0.008) and FSH (treatment × time interaction F = 15.89, P < 0.001). Serum oestradiol concentrations decreased markedly in response to drug administration, reaching pre-treatment levels within four days. On day −1, the decreases in the oestradiol concentrations from the baseline value to the nadir were similar in the MPV-2213ad and in the control group (18% and 16% respectively, P = 0.75, t-test). On the day of aromatase inhibitor administration, however, the reductions in the oestradiol concentrations in the MPV-2213ad and the control group were 74% and 19% respectively (P < 0.001, t-test). The AUC of the percentage changes in oestradiol concentration was significantly smaller in the MPV-2213ad group compared with the control group when calculated over day 1 or up to day 7 (P < 0.0001 and P < 0.01 respectively, t-test). Serum testosterone was slightly increased in the MPV-2213ad group on days 2 to 7, but the changes did not differ from those in the placebo group. Sustained elevations were seen in serum LH and FSH concentrations after the active drug administration. Their concentrations returned to the pre-treatment level by day 7. The shapes of the concentration curves of serum testosterone, aldosterone, cortisol and insulin did not differ between the two study groups.

To see whether there were changes in the diurnal patterns of hormone concentrations in serum after aromatase inhibitor administration, study days −1 and 0 were compared with each other. Despite the marked reduction in serum oestradiol on day 0, the normal diurnal pattern of serum leptin was unchanged (treatment × day × time interaction F = 0.59, P = 0.50). Comparison of days −1 and 0 also showed that the aromatase inhibitor treatment had no effect on the diurnal patterns of serum testosterone, aldosterone or cortisol. Serum LH tended to be higher on the day of MPV-2213ad administration (treatment × day × time interaction F = 2.66, P = 0.08) in the active treatment group. Elevation of serum FSH was slightly greater in the MPV-2213ad group than in the placebo group on day 0 when compared with the previous day (treatment × day × time interaction F = 4.03, P = 0.02).

When measurements from all time points were pooled together, serum leptin concentrations correlated with BMI (r = 0.27; P < 0.001), serum oestradiol (r = 0.28, P < 0.001), aldosterone (r = 0.16, P = 0.03), and insulin (r = 0.33; P < 0.001), but not with serum testosterone, LH, FSH or cortisol. Including all measured variables in a multiple linear regression model, serum oestradiol (r = 0.28, P = 0.001), insulin (r = 0.43, P < 0.001), testosterone (r = −0.27, P = 0.003) and aldosterone (r = 0.20, P = 0.03) were all significant determinants of serum leptin concentration, and explained 35% of the total variability in leptin. After stepwise multiple linear regression analysis only insulin remained a significant predictor of serum leptin (r = 0.45, P < 0.001), explaining 22% of the variation in serum leptin. When percentage changes in hormone concentrations were analysed, changes in leptin correlated with those of insulin (r = 0.16, P = 0.03), aldosterone (r = 0.26, P = 0.001) and cortisol (r = 0.2, P = 0.009).

Discussion

Our results clearly show that short-term reduction of oestradiol biosynthesis and serum oestradiol do not affect circulating leptin in young men. We found a statistically significant correlation between serum oestradiol and leptin when measurements from different time points were pooled together, which is in agreement with one earlier report (24) but not with others (25–28). This
A weak positive correlation between serum leptin and oestradiol concentrations could not be reproduced when the percentage changes in these variables were used in the analysis.

One clear advantage of our study is that we specifically modulated serum oestradiol, with minimal effects on many other important hormones. Despite a marked reduction in serum oestradiol concentration after MPV-2213ad, no change in the normal diurnal pattern of serum leptin was noted. However, there are important functional differences between genders in the physiological function of the gonadal–hypophysial axis. Therefore, the connection between leptin and oestradiol may be different in females.

Earlier studies on the effect of oestrogens on leptin biosynthesis and availability have given conflicting results. While in female rats ovariectomy reduces serum leptin and long-term oestradiol supplementation reverses this effect (14), in male rats oestradiol treatment decreases serum leptin levels and body weight (19). In normal-weight women, hysterectomy (27), postmenopausal hormone replacement therapy (27, 29, 30) or oral contraceptives (29, 31) do not affect serum leptin. Changing androgenic/oestrogenic status in hyperandrogenic women by oral contraceptives has no effect on plasma leptin concentration (32). In men, oestradiol treatment in combination with an anti-androgen increased serum leptin levels independently.

Figure 1 Changes in hormone concentrations in MPV-2213ad- and placebo-treated groups. A 100 mg single dose of the drug, or placebo, was given at 0800 h on day 0 (arrow). Samples were taken at 0800, 1600 and 2000 h on the day preceding the drug test day (day −1) and on day 0, and at 0800 h on days 1, 2, 4 and 7 after treatment. The basal value on day −1 was set at 100%. The error bars have been omitted for clarity. ● leptin, ○ oestradiol, × testosterone, □ luteinizing hormone, « follicle-stimulating hormone.
of changes in body fatness (33); whether the effect was
due to oestrogenic or antiandrogenic actions could not
be resolved.

MPV-2213ad is a potent and highly selective
inhibitor of the aromatase enzyme (22, 23). Aromatase
enzyme converts testosterone to oestradiol, which
explains the slight increase seen in serum testosterone
in the group treated with the aromatase inhibitor. Large
elevation of serum testosterone is associated with
lowered serum leptin (13). However, the increase in
serum testosterone in the present study was most likely
too subtle to have any effect on serum leptin concentra-
tion, and it occurred first on day 2 and thereafter.
Likewise, there were sustained elevations in serum LH
and FSH after aromatase inhibitor administration,
probably as a feedback response to the reduction in
serum oestradiol (23), but these changes were not
reflected in serum leptin concentrations during the
one-week follow-up.

Leptin concentration in human plasma shows a
circadian fluctuation (34). Licinio et al. have studied the
24-h leptin release profiles in women, and they found
synchronicity of leptin and oestradiol, and of leptin and
LH (35), which may explain the weak correlation between
serum leptin and oestradiol concentrations found here.
They proposed that leptin was not only a trophic factor for
the reproductive system, but also that the pulse patterns
or concentrations of leptin may organise the function-
ing of the hypothalamo–pituitary–ovarian axis, by
regulating the minute-to-minute oscillations of LH
and oestradiol concentrations.

We studied the effect of acute inhibition of oestradiol
biosynthesis by an aromatase inhibitor in males.
Circulating serum oestradiol concentration was power-
fully reduced, whereas only slight changes occurred in
FSH and LH concentrations. Serum leptin concentra-
tion was not affected by the short-term reduction in
serum oestradiol, suggesting that oestradiol, at least in
the acute setting, is not a major determinant of leptin
secretion in men.

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