CLINICAL STUDY

Effects of anabolic–androgenic steroid use or gonadal testosterone suppression on serum leptin concentration in men

M S Hislop, B D Ratanjee, S G Soule and A D Marais

Lipid Laboratory, Department of Internal Medicine, University of Cape Town Medical School, Anzio Road, Observatory, 7925, South Africa

(Correspondence should be addressed to A D Marais, Lipid Laboratory, 5th Floor SAMIOT Building, University of Cape Town Medical School, Observatory 7925, South Africa)

Abstract

Objective: Serum leptin concentration shows a sexual dimorphism that is not accounted for by gender differences in adiposity. A strong inverse association exists between serum leptin and testosterone concentrations in men, pointing to a likely influence of gonadal sex steroids on serum leptin concentration. The aim of this study was to investigate whether manipulation of sex steroid hormones in men would alter serum leptin concentration independently of changes in fat mass.

Design and methods: The effects of sex steroid suppression on serum leptin concentration were investigated in nine healthy men in whom testosterone had been reversibly suppressed for 5 weeks after treatment with intramuscular triptorelin. The effects of sex steroid supplementation were investigated in nine male bodybuilders who self-administered anabolic–androgenic steroids (AAS) for a mean period of 6.5 weeks. A control group received no hormonal treatment.

Results: Testosterone concentration was significantly reduced by triptorelin administration (7.32 ± 1.92 ng/ml at baseline compared with 1.15 ± 0.57 ng/ml at 5 weeks, $P = 0.002$). High-dose AAS use was confirmed by urine analysis. Body fat percentage was unaffected by the AAS or triptorelin intervention ($P > 0.19$). Leptin concentration was significantly reduced after one cycle of AAS use (2.40 ± 0.98 ng/ml off cycle compared with 1.63 ± 0.37 ng/ml on cycle, $P = 0.012$), and was significantly increased by triptorelin administration (2.96 ± 1.50 ng/ml at baseline compared with 6.63 ± 4.67 ng/ml at five weeks, $P = 0.004$). No significant change occurred in the control group.

Conclusion: Androgenic sex hormone supplementation decreases serum leptin concentration, whereas suppression increases serum leptin concentration, independently of changes in body fat mass in healthy men. The sexual dimorphism evident in serum leptin concentration is likely to be due to a suppressive effect of testosterone on serum leptin concentration in males.

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Introduction

Leptin is a 167-amino acid product of the *ob* gene, produced mainly in adipose tissue (1, 2). In rodents, leptin appears to have a role as an adipocyte-derived signalling molecule that regulates food intake and energy expenditure. Leptin-deficient (*ob/ob*) or resistant (*db/db*) rodents thus exhibit hyperphagia and reduced metabolic rate, with consequent massive obesity (3, 4). These abnormalities are reversed by administration of leptin in the *ob/ob* mouse (4). The role of leptin in the pathophysiology of human obesity is less clear than it appears to be in rodent models of obesity (5). Serum leptin concentrations are closely correlated with body fat percentage in humans. The high leptin

concentrations that characterise most obese people are suggestive of a deficiency in the feedback loop between leptin, appetite and energy expenditure. Leptin deficiency, resulting from a mutation in the leptin gene, and resistance, resulting from a mutation in the leptin receptor, have been defined in humans, although they appear to be rare events (6, 7).

Besides its metabolic role, leptin may also be closely associated with reproduction and neuroendocrine signalling. Notably, the infertility characteristic of female *ob/ob* mice is corrected by treatment with human recombinant leptin (8). Moreover, leptin appears to trigger the onset of puberty (9) and reproductive function (10) in normal female rodents. In humans, serum leptin increases before the onset of

puberty in both sexes (11), suggesting that a critical concentration of leptin may be a permissive factor for the initiation of puberty (12). After Tanner stage 2, serum leptin concentrations diverge in males compared with females, even if body fat percentage is taken into account (11). Accordingly, the possibility exists that leptin concentrations may, in turn, be influenced by gonadal sex steroid hormones. This hypothesis has gained credence through a number of recent studies. First, cross-sectional analyses have defined a negative correlation between testosterone and leptin in both boys (13) and adult men (14–17). Secondly, *in vitro* studies using human adipocytes in culture show that testosterone suppresses leptin mRNA synthesis and leptin secretion (13). Thirdly, increased serum leptin concentration in hypogonadal men is normalised by testosterone substitution (18), and suppression of testosterone in boys is associated with a reduction in serum leptin concentration (19). Finally, manipulation of the hormone milieu in transsexuals reverses the gender disparity in serum leptin concentration (20). In this study, we examined the effect of both an acute increase and an acute decrease in sex steroid hormones on serum leptin concentration in healthy men.

Subjects and methods

Subjects and anthropometry

The effect of androgen supplementation on plasma leptin concentration was investigated in male bodybuilders who self-obtained and self-administered anabolic-androgenic steroids (AAS) on a cyclical basis. The study was advertised locally, and nine male bodybuilders (age 21–27 years, mean 25 ± 2 years) volunteered to participate. Subjects typically trained four to six times a week, with training sessions comprising 1–1.5 h of heavy resistance training and 30 min of 'aerobic' training. Ethical considerations precluded the investigators from advising subjects on cycle durations and AAS dosage. Accordingly, test interventions occurred at the convenience of the participants, at the end of one 'on' cycle (using AAS) and one 'off' cycle (not using AAS). Subjects used a variety of oral and parenteral AAS, in a cyclical fashion, typically spending 6–10 weeks on cycle, followed by 4–8 weeks off cycle. Subjects used, on average, 990 mg 17β -esterified and 375 mg 17α -alkylated AAS per week for an average 6.5 weeks. They agreed to use no AAS during their off cycle. To confirm AAS usage, urine analysis was performed at the end of both on and off cycles, at a control laboratory accredited by the International Olympic Committee (IOC). Subjects acted as their own controls. The effect of androgen suppression on plasma leptin concentration was investigated in healthy men, in whom testosterone production had been reversibly suppressed using the gonadotropin-releasing hormone

(GnRH) agonist, triptorelin (Decapeptyl; D-Trp⁶-luteinising hormone-releasing hormone). These men were participants in an open, randomised, crossover trial, designed to compare the efficacy and tolerance of two triptorelin sustained-release formulations. The trial comprised two parts, the second of which followed 3 months after the commencement of the first, once testosterone concentrations had been within the normal concentration range for several weeks. The study group comprised nine healthy male volunteers (age 23–55 years, mean 31 ± 10 years). Relevant variables were compared during the second part of the crossover, both before triptorelin administration and five weeks after drug administration. The latter time point was established as the nadir of serum testosterone concentration in the first part of the study. A group comprising eight healthy men with normal testosterone concentrations were assessed in parallel with the trial group to provide analytical control. They were matched with the trial group on the basis of age (28 ± 11 years), socioeconomic status and relative physical activity.

The study was approved by the Ethics and Research Committee of the University of Cape Town. Subjects were fully informed about the relevant procedure, and gave written informed consent before commencement of the study. All were healthy as judged by medical history, physical examination, and liver function tests. They were requested not to modify any of their lifestyle habits, exercise regimens or diet, through the duration of the studies. Height was measured to 0.1 cm, and body mass to 0.1 kg. Anthropometry was performed by an individual experienced in anthropometric measurement, using standardised anthropometric landmarks to locate the measuring sites (21). Body fat percentage and muscle mass were calculated as described elsewhere (22, 23).

Biochemical analyses

Blood samples were collected between 0700 and 0730 h, after an overnight fast, and stored at -20°C . Plasma leptin concentrations were measured in duplicate by an RIA (Linco Research, Inc., St Louis, MO, USA) (24). The intra-assay coefficient of variation was 1.4%. Serum testosterone concentrations were measured in duplicate by means of a specific RIA performed on extracts of the plasma samples, using an in-house antiserum and tritiated testosterone label as the tracer. Inter- and intra-assay coefficients of variation were 11.6% and 4.6% respectively. Insulin concentrations were measured using a solid-phase iodine-125 RIA (Coat-A-Count Insulin, Diagnostic Products Corporation, Los Angeles, CA, USA). AAS were detected by gas chromatography with a mass-selective detector after deconjugation and derivatisation of the urine extracts (25). Total and high-density lipoprotein cholesterol (HDL) concentrations were evaluated using

commercially available enzymatic spectrophotometric kits.

Statistics

A statistical software package (Instat, Graphpad Software, Inc. San Diego, CA, USA) was used for data analysis. Data are expressed as a mean $\pm$ s.d. Data were analysed using non-parametric methods, with the Wilcoxon matched pairs test. Correlation (non-parametric, Spearman) and linear regression were assessed using another software package (Graphpad Prism). A value of $P < 0.05$ was accepted to define statistical significance.

Results

Anthropometry

Body mass of the AAS users had increased significantly by the end of the on cycle, from 96.2 ± 11.1 kg to 99.7 ± 11.1 kg ($P = 0.004$). Muscle mass increased from 60.8 ± 7.6 to 62.5 ± 7.5 kg ($P = 0.03$). There was no change in fat percentage on cycle ($13.5 \pm 3.5\%$ compared with $13.0 \pm 2.8\%$, $P = 0.36$). Body mass (80.8 ± 12.2 kg and 81.4 ± 12.7 kg, $P = 0.65$), muscle mass (43.3 ± 6.1 kg and 43.5 ± 6.2 kg, $P = 0.91$) and fat percentage ($20.1 \pm 6.8\%$ and $19.9 \pm 7.1\%$, $P = 0.20$) did not change between tests 1 and 2 in the triptorelin-treated group. Likewise, body mass (76.1 ± 10.7 kg and 76.3 ± 11.6 kg, $P = 0.69$), muscle mass (42.0 ± 6.0 kg and 41.8 ± 5.7 kg, $P = 0.47$) and fat percentage ($16.7 \pm 7.7\%$ and $16.3 \pm 8.4\%$, $P = 0.69$) did not change between tests 1 and 2 in the control group.

Anabolic-androgenic steroids

AAS use was confirmed in all nine of the bodybuilders, by urine analysis. During the on cycle, testosterone esters were detected in all of them, and nandrolone was detected in eight. Low concentrations of nandrolone could be detected in seven of the subjects, at the end of their off cycle 'washout period'; these were, most probably, remnants of the previous cycle. In one of the group, testosterone and stanozolol from a previous cycle could be detected during his 'washout period'. Subsequent to the on cycle period, one bodybuilder admitted supplementing his AAS use with growth hormone (GH) i.m. 25 IU/week for 7 weeks. The other men denied use of GH.

Lipoproteins

In the AAS users, mean HDLC concentration decreased significantly when subjects were on cycle, from 0.9 ± 0.3 mmol/l to 0.7 ± 0.3 mmol/l ($P = 0.004$). Mean total cholesterol (5.40 ± 1.99 mmol/l compared with

4.83 ± 1.17 mmol/l, $P > 0.3$) and low-density lipoprotein cholesterol (3.97 ± 1.97 mmol/l compared with 3.66 ± 1.27 mmol/l, $P > 0.5$) were unchanged on cycle. Fasting triglyceride concentrations were unchanged between the off and on cycles (1.1 ± 0.5 mmol/l compared with 1.0 ± 0.3 mmol/l, $P = 0.73$).

Mean total cholesterol increased after 5 weeks in the triptorelin group (4.8 ± 0.8 mmol/l compared with 5.2 ± 1.0 mmol/l, $P = 0.04$), and did not change in the control group (4.9 ± 1.1 mmol/l compared with 4.9 ± 0.9 mmol/l, $P > 0.94$). Mean HDLC concentration increased significantly after 5 weeks in the triptorelin group (from 1.1 ± 0.2 mmol/l to 1.4 ± 0.3 mmol/l, $P = 0.002$); it did not change in the control group (1.2 ± 0.2 mmol/l compared with 1.2 ± 0.1 mmol/l). Fasting triglyceride concentrations were unchanged between the two test dates in both the triptorelin (1.2 ± 0.2 mmol/l compared with 1.3 ± 0.7 mmol/l, $P = 0.63$) and control groups (1.0 ± 0.5 mmol/l compared with 1.0 ± 0.4 mmol/l, $P = 0.47$).

Testosterone and insulin

Plasma testosterone concentration was not measured in the AAS group. Baseline testosterone concentration in the triptorelin group (7.32 ± 1.92 ng/ml) was similar to that in the control group (7.30 ± 2.08 ng/ml; normal range 3–10 ng/ml) (26). Testosterone concentration was significantly reduced with triptorelin therapy, to 1.15 ± 0.57 ng/ml after 5 weeks ($P = 0.002$), but did not change in the control group (6.50 ± 2.18 ng/ml at 5 weeks, $P > 0.38$). A plot of testosterone concentration against time in the triptorelin group (Fig. 1), shows that test 2, performed 5.3–5.8 weeks after triptorelin was administered, coincided with the nadir of testosterone concentration.

Figure 1 Effect of triptorelin on testosterone concentration in nine healthy men (mean $\pm$ s.d.). Leptin concentrations were measured at day 0 and day 35 (5 weeks) after triptorelin administration, when testosterone concentrations were at their nadir.

Insulin concentration was unchanged after one cycle of AAS use ($5.22 \pm 1.69 \mu\text{U/ml}$ off cycle compared with $4.86 \pm 1.67 \mu\text{U/ml}$ on cycle, $P > 0.24$), and was unchanged 5 weeks after administration of triptorelin in the experimental ($7.34 \pm 7.05 \mu\text{U/ml}$ at baseline and $7.10 \pm 4.28 \mu\text{U/ml}$ at 5 weeks, $P > 0.70$) and control groups ($3.71 \pm 1.43 \mu\text{U/ml}$ at baseline and $5.15 \pm 3.79 \mu\text{U/ml}$ at 5 weeks, $P > 0.58$).

Leptin

Leptin concentration was significantly increased by triptorelin administration ($2.96 \pm 1.50 \text{ ng/ml}$ at baseline and $6.63 \pm 4.67 \text{ ng/ml}$ at 5 weeks, $P = 0.004$; Fig. 2), did not change in the control group ($2.31 \pm 1.12 \text{ ng/ml}$ at baseline and $2.94 \pm 1.89 \text{ ng/ml}$ at 5 weeks, $P > 0.46$), and was significantly reduced after one cycle of AAS use ($2.40 \pm 0.98 \text{ ng/ml}$ off cycle compared with $1.63 \pm 0.37 \text{ ng/ml}$ on cycle, $P = 0.012$; Fig. 3).

There was a significant correlation between fat percentage and leptin for all participants before intervention ($P < 0.001$, $n = 24$, $y = 0.14x + 0.35$, $r^2 = 0.53$, 95% confidence interval (CI) of slope 0.09 to 0.20). The association between fat percentage and leptin was significantly strengthened after the suppression of testosterone in the triptorelin group (before intervention: $n = 8$, $r^2 = 0.87$, $P = 0.002$; $y = 0.21x - 0.92$, 95% CI 0.11 to 0.31; after intervention: $n = 8$, $r^2 = 0.90$, $P = 0.001$; $y = 0.66x - 5.71$, 95% CI 0.39 to 0.93). The association between fat percentage and leptin was unchanged after one cycle of AAS use, and in the control group. When the percentage change in leptin concentration in response to triptorelin was analysed as a function of the baseline concentration, linear regression indicated that the baseline concentration explained 59% of the variance, with $P < 0.02$.

Figure 2 Plasma leptin concentration before and 5 weeks after triptorelin treatment in nine healthy men.

Figure 3 Plasma leptin concentration before and after one cycle (mean 6.5 weeks) of AAS self-administration in nine male bodybuilders.

Discussion

This study shows that manipulation of the sex steroid hormones in normal healthy men causes significant changes in serum leptin concentration, independently of changes in body fat mass or insulin concentration. Specifically, we show that sex hormone supplementation, with high doses of AAS, causes a significant reduction in serum leptin concentration, whereas sex hormone suppression causes a significant increase in serum leptin concentration in adult men. It is notable that the suppression of sex steroid hormones significantly enhances the strength of the correlation between body fat percentage and serum leptin concentration in men.

Investigation of the effects of AAS on serum leptin concentration was complicated by ethical considerations that precluded investigator involvement in drug administration. To be sure that subjects had self-administered AAS, urine samples collected at the end of the on and off cycle periods were sent for urine analysis at an IOC-accredited laboratory. These tests showed that all subjects had used AAS during the on cycle period. Residual concentrations were also detectable at the end of the washout period, in seven of the nine bodybuilders, indicating that traces of AAS remained from the previous cycle (data not shown). It is likely that the change in leptin concentration between the on and off cycle periods would have been greater than that observed had the individuals been entirely free of AAS during the off cycle period. Additional proof for AAS usage was provided by the change in HDLC concentration, which is very sensitive to the presence of AAS (27). In the bodybuilders we studied, HDLC concentration showed the anticipated marked decline during the on cycle period, and increase during the off cycle period. We are thus confident that the

self-administration of AAS was consistent with that reported by our subjects.

To our knowledge, a reduction in serum leptin concentration as a result of AAS use in healthy men has not previously been described. This result is consistent with the finding that testosterone substitution reduces serum leptin concentration in hypogonadal males (18). AAS use appears to have a similar suppressive effect on serum leptin concentration in females (unpublished data) – an observation in keeping with the finding that testosterone enanthate substitution suppresses serum leptin concentration in female-to-male transsexuals (20). By comparison, and in view of the first finding, the increase in serum leptin concentration after endogenous testosterone suppression would be anticipated. This result is consistent with reports that serum leptin concentration is suppressed in male-to-female transsexuals receiving cyproterone acetate (antiandrogen) and ethinyl oestradiol therapy (20), and in boys treated with a GnRH agonist for central precocious puberty (19).

Because body fat mass is a strong determinant of serum leptin concentration in humans (28, 29), and because gonadal steroids influence body fat mass and distribution, an important consideration in studies of this kind is whether changes in serum leptin concentration are the result of changes in sex steroid concentration *per se*, or of changes in body fat mass in the period between leptin measurements. Gonadal sex hormone supplementation appears to have minimal effect on body fat mass in men with testosterone concentration in the normal range (30). This treatment does, however, tend to increase lean body mass (30, 31) which can result in a reduction in body fat expressed as a percentage. In the present study, no change in body fat mass could be detected, and there was only a very slight and non-significant decrease in body fat percentage during AAS use. It has been demonstrated that non-visceral adipose tissue influences leptin concentration more than does visceral adipose tissue (32, 33). The lean, active young men included in this study had relatively little total fat and thus also little visceral fat. Furthermore, in the short course of the study, neither visceral nor non-visceral fat is likely to change appreciably. Given the magnitude of the decrease in serum leptin concentration with AAS use, the small changes in body fat percentage are unlikely to account for the significant reduction in serum leptin concentration seen in these men.

The possibility that sex steroid supplementation might influence serum leptin concentration, through its effect on body fat mass, is of greater concern in studies on hypogonadal males. Sex steroid supplementation has a more pronounced effect on body fat mass in these individuals, possibly because they tend to have a body fat percentage that is greater than that in eugonadal males (34). These changes do, however, only appear to become evident after a number of

months of steroid treatment. Investigators report no changes in body fat mass after 2–3 (35) and 6 months of testosterone supplementation (36), and significant reductions only after 6 (37) to 18 months (34) of testosterone supplementation. We are aware of only one study that has investigated the effect of sex steroid supplementation on serum leptin concentration in hypogonadal males (18). Although percentage body fat was not reported in that study, body mass index showed no decrease after 100 days of testosterone replacement therapy. This finding is consistent with the reports showing no reduction in body fat percentage within 6 months of starting testosterone replacement therapy in hypogonadal males (35, 36), supporting the contention that the effects of testosterone supplementation on serum leptin are independent of changes in body fat mass.

Gonadal steroid suppression can also lead to marked changes in body fat percentage in healthy males. In the study reported by Elbers *et al.* (20), body fat mass increased significantly in male-to-female transsexuals in the period between serum leptin measurements. It was, however, possible to show, by analysis of covariance, that changes in serum leptin concentration were independent of changes of body fatness in this group, indicating that changes in serum leptin concentration were likely to be determined by sex steroid hormones rather than changes in body fat composition. Similarly, Palmert *et al.* (19) did not report body fat mass, and commented that the possibility could not be excluded that changes in body composition had occurred over the 6-month period of their study. Nonetheless, they found only small differences among the body mass indexes of boys across the time-points of the paired analysis, indicating that changes in serum leptin concentration were unlikely to be accounted for by changes in body fat composition. In our study, no changes in body fat percentage could be detected during the relatively short duration of the study. This finding confirms the previous conclusions that changes in serum leptin concentration are mainly due to changes in testosterone concentration, rather than to changes in body fat percentage.

A number of other endocrine factors may also have influenced serum leptin concentrations in our study. Prolonged exposure of adipocytes to insulin appears to increase leptin secretion (38). In the present study, fasting insulin concentration did not change with either triptorelin therapy or anabolic steroid use, which argues against this hypothesis. Furthermore, GH replacement results in a decrease in serum leptin (39). Accordingly, one cannot exclude the possibility that covert GH administration in some of the AAS users may have played a part in the fluctuations in serum leptin; however, interviews with these men indicated that this was unlikely in all but one of them.

It is notable that the percentage change in serum leptin concentration with sex steroid manipulation

differed between individuals in the present study. There was no correlation between the initial or percentage change in testosterone concentration and the percentage change in serum leptin concentration in the triptorelin group. There was, however, a correlation between the initial leptin concentration and the percentage change in leptin concentration following androgen manipulation in both the triptorelin and AAS groups. Although it was our impression that persons with more body fat had higher initial concentrations of leptin, the group was too small to analyse further the determinants of leptin concentration and its change.

All participants were asked to keep dietary records for analysis during the study. Dietary records taken in the AAS group indicated a strict commitment in each individual to a selected putative anabolic diet that contained little fat (20%). The dietary records from the triptorelin group indicated that the group had a more traditional western style diet (about 50% as fat). None of the participants reported a change in appetite and no change in eating habits was reflected in the records. This raises the question of whether serum leptin concentration has any relevance in the control of appetite in man in the short term, although it may have in the longer term (40).

The powerful suppressive effect of gonadal sex steroids on serum leptin concentration observed in these studies lends support to the hypothesis that male sex hormones are the main cause of the sexual dimorphism in serum leptin concentration (41–43). This finding is also consistent with the observation that there is a strong negative correlation between serum leptin concentration and testosterone concentration in men (14–16). The fact that leptin concentration changed without a concomitant measureable change in adipose mass supports an independent effect of testosterone on serum leptin concentration *in vivo*; the mechanism for such an effect *in vivo* is unknown at present. The change is likely to be due to a suppressive effect of gonadal sex steroids on leptin mRNA, thereby reducing leptin secretion from adipose tissue, as has been found *in vitro* (13), or possibly to alterations in the metabolic clearance of leptin, mediated perhaps by changes in binding proteins.

In conclusion, our studies have confirmed and extended the observation of a direct modulatory effect of androgenic steroids on serum leptin concentration *in vivo*. By studying the effects of exogenous androgen supplementation and the impact of GnRH agonist-induced hypogonadism on leptin concentrations in normal men, we confirm the effect of androgen supplementation on reducing serum leptin and demonstrate that suppression of physiological concentrations of serum testosterone results in a marked increase in serum leptin concentration in normal men, independently of changes in body fat mass.

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