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Effects of androgen manipulation on postprandial triglyceridaemia, low-density lipoprotein particle size and lipoprotein(a) in men

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Abstract

Although androgenic hormones decrease HDLC concentration, no direct evidence has linked them to atherosclerosis. The present study was undertaken to extend our ability to assess risk associated with androgen induced lipoprotein(Lp) changes by simultaneously gathering information about postprandial triglyceridaemia (PPT), LDL particle size, HDL and Lp(a) in men either taking exogenous androgens or with suppressed endogenous androgen concentrations. The experimental groups comprised nine male bodybuilders who self-administered anabolic-androgenic steroids (AAS) for a mean period of 6.5 weeks, and 10 healthy men whose testosterone concentration had been reversibly suppressed for 5 weeks using the GnRH agonist triptorelin (Decapeptyl®; D-Trp-6-LHRH). A separate group receiving no hormonal treatment provided analytical control ($n = 7$). Lipoprotein size was assessed by gradient gel electrophoresis categorisation (GGE), lipoprotein concentrations by immuno and enzymatic assays and PPT by a standardised oral fat tolerance test (65g /m^2). Testosterone concentration was significantly reduced on triptorelin from 7.32 ± 1.92 to 1.15 ± 0.57 ng/ml ($P = 0.002$). High dose AAS use was confirmed by urinalysis. With AAS use, mean HDLC and Lp(a) concentrations and PPT decreased from 0.9 ± 0.3 to 0.7 ± 0.3 mmol/l ($P = 0.004$), 125 ± 128 to 69 ± 73 U/l ($P = 0.008$) and 11.6 ± 10.0 mmol/l h to 7.5 ± 5.4 mmol/l h ($P = 0.027$) respectively. Mean total cholesterol and LDLC were unchanged. LDL size was unchanged in six AAS users, decreased in one but remaining in the normal size range, and increased in two from small LDL to the normal range. Size changes in the latter two subjects were associated with 42 and 58% reductions in PPT respectively. In the triptorelin group, mean total cholesterol, HDLC and Lp(a) were increased from 4.8 ± 0.8 mmol/l to 5.2 ± 1.0 mmol/l ($P = 0.039$), 1.1 ± 0.2 to 1.4 ± 0.3 mmol/l ($P = 0.002$) and 278 ± 149 to 377 ± 222 U/l ($P = 0.004$) respectively. Mean LDLC concentration and PPT were unchanged. LDL particle size increased in four, decreased in two, and was unchanged in four subjects. LDL size decreased in two and showed no change in the other five control subjects. Other lipid measures were unchanged in the control group. Thus, apart from lowering HDLC concentrations, no other potentially atherogenic effects of endogenous androgens or AAS were observed. A suppression of Lp(a) as well as a reduced PPT and increased LDL size in predisposed individuals may be antiatherogenic effects of AAS. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Anabolic androgenic steroids; Low-density lipoprotein particle size; Lipoprotein(a)

1. Introduction

An explanation of gender differences in the onset of atherosclerosis may lie in sex hormones [1,2]. Evidence for a beneficial effect of reduced androgen concentration on atherosclerosis in men is at best equivocal. Castrated mental institution patients had increased longevity, but this was not attributed to less cardiovascular disease [3]. Historical mortality data in castrated sopranos revealed that prepubertal removal of testes did not influence longevity [4]. Alexandersen et al., in a review of one intervention, eight cohort and several cross-sectional studies concluded that there was either a neutral or a favourable effect of testosterone on CHD in men [5].

The effects of increased androgen concentration on atherosclerosis can be investigated in men who use

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AAS. These substances have been used to enhance athletic performance or physique since 1950 [6]. By 1993 it was estimated that more than one million individuals had used AAS in the USA alone [7]. Currently, data favouring a direct link between AAS and CHD is based on case studies reporting adverse cardiovascular effects; but, many of these events were attributed to acute thrombotic events, few specifically diagnosed premature atherosclerosis, and it is difficult to control for confounding factors, such as genetics, unhealthy lifestyle, other drugs, or underlying disease state [8–10]. Thus, although androgen supplementation may lead to adverse complications in some individuals, these occurrences appear to be rare considering the large number of users, and the prolonged time period since AAS were first used.

That androgens may lead to atherosclerosis is also inferred from surrogate measures of risk. The serum lipid effects of AAS first became evident as early as the 1960s [11]. At first, AAS showed therapeutic benefit for hypertriglyceridaemia through their triglyceride lowering effects [12,13]. Subsequently the potentially adverse effect of an increased LDLC:HDLC ratio was found. In normal men it was also shown that lower HDLC concentrations relative to women could be attributed to a higher endogenous androgen concentration [14,15]. These lipid effects suggested that androgens might contribute to the increased prevalence of premature atherosclerosis in men [16].

A new atherogenic lipoprotein phenotype, characterised by small dense LDL, a low HDLC concentration, and an impaired triglyceride metabolism which can manifest as a postprandial hypertriglyceridaemia, has recently been described [17]. Given the difficulties inherent in providing a direct link between the androgens and premature atherosclerosis, the present study was undertaken to gain more insight into androgen induced lipoprotein changes by simultaneously gathering information about PPT, LDL particle size, HDLC and Lp(a) in men either taking exogenous androgens or by reducing endogenous androgen production.

2. Methods

2.1. Subjects and hormonal interventions

This study was approved by the Ethics and Research Committee of the University of Cape Town. Subjects gave written informed consent prior to commencement. Anthropometry was measured as described [18]. Subjects were asked to complete 3-day dietary records, including 1 weekend day, as close as possible to the 2 test days. Dietary analysis was performed using nutritional analysis software (Foodfundi®, South African Medical Research Council). Subjects were asked to not modify their lifestyle habits, exercise regimens or diet during the studies.

The effects of androgen supplementation were investigated in nine male bodybuilders, mean age 25 ± 2 years, who self-obtained and self-administered anabolic-androgenic steroids. Mean bodybuilding history was 5 years. Subjects typically performed 1.5 hours of heavy resistance and 0.5 hour of 'aerobic' training four to six times a week. With AAS believed to have side effects, and non-prescribed use being unlawful in South Africa, the initiation of the study was conditional on (i) all subjects being informed of potential side effects, and (ii) being discouraged from using AAS by an independent medical practitioner. Ethical considerations precluded the investigators from advising subjects on cycle lengths and AAS dosage. But for one, all bodybuilders had used AAS for longer than 6 months. Investigations were done at the end of one on cycle (using AAS) and one off cycle (not using AAS). Subjects used a combination of oral and parenteral AAS in a cyclical fashion, typically spending 6–10 weeks on cycle, followed by 4–8 week off cycle. Mean AAS dosage was 990 mg 17β-esterified and 375 mg 17α-alkylated AAS per week for an average 6.5 weeks. To confirm AAS usage, urinalysis was performed after both on and off cycles at an IOC-accredited laboratory.

The effects of androgen suppression were investigated in 10 healthy male volunteers, mean age 31 ± 10 years, who had normal testosterone concentrations. Testosterone production was reversibly suppressed using the GnRH agonist triptorelin (Decapeptyl®; D-Trp-6-LHRH). While physiological GnRH concentrations stimulate gonadotropin release, supra-physiological GnRH concentrations decrease gonadotropin secretion and reduce plasma testosterone concentration. Variables were compared before triptorelin administration and 5 weeks later when serum testosterone concentration reached a nadir.

A group comprising seven healthy men with normal testosterone concentrations were assessed in parallel with the triptorelin group to provide analytical control. They were matched on the basis of age (27 ± 11 years), socio-economic status and relative physical activity. We were unable to find a group of bodybuilders who abstained from AAS.

2.2. Biochemical analyses

Blood samples were collected between 7.00 and 7.30 h after an overnight fast, and stored at -20°C. The concentration of total, LDL and HDL cholesterol was evaluated by a routine pathology lab practising conventional quality control and using commercially available enzyme kits on automated machines. The LDLC concentration calculation was by the Friedewald equation. Lipoprotein(a) was measured in the plasma as Units/l

using the Mercodia Apo(a) ELISA, enzyme immunoassay (Mercodia AB, Seminariegatan 29, S-752 28 Uppsala, Sweden). Serum testosterone concentrations were measured using a specific RIA on plasma sample extracts, employing an in-house antiserum and tritiated testosterone label as the tracer. Inter- and intra-assay coefficient of variation was 11.6 and 4.6% respectively. Insulin concentrations were measured using a solid-phase 125I RIA (Coat-A-Count Insulin, Diagnostic Products Corporation, Los Angeles, CA 90045-5597). AAS were detected by gas chromatography with a mass selective detector after deconjugation and derivatisation of the urine extracts [19].

2.3. Oral fat tolerance test

The oral fat tolerance test was performed as described [20]. Subjects abstained from exercise and alcohol the day before the test, and from food and beverages (excluding water) for 12 h before the test. At 06.45 h the body surface area was derived by nomogram (Geigy Scientific Tables, 1984). Subjects were seated and fasted blood samples were drawn. The test meal was given at 07.00 h and consumed within 3 min. The content of the test meal, per m^2 of body surface area was; cream 175 ml (39.5% fat), powdered chocolate flavouring 15 ml, granulated sugar 2.5 ml, fat free milk powder 7.5 ml. This delivers about 3050 J of which 83% (65 g) is from fat. No food was permitted during the day apart from water or energy-free drinks. Subjects refrained from exercise, and underwent venesection at two-hourly intervals for the next 10 h. Triglyceride concentration was measured with a commercially available GPO PAP kit (Human, Germany, Kit No. 10164.), and plotted against time. PPT (mmol/l h) was calculated as the area under the curve. Postprandial triglyceride excursion was calculated by subtracting the minimal triglyceride concentration, multiplied by 10 h, from the total area under the curve.

2.4. Non-denaturing gradient gel electrophoresis (GGE) for lipoproteins

Changes in low-density and high-density lipoprotein particle size were investigated by means of non-denaturing gradient gel electrophoresis (GGE), using the Biorad minigel apparatus along a method similar to Li et al. [21], but using 2–8 and 4–18% separate glass plate sandwiches for LDL and HDL respectively. Visual interpretation of the gels was confirmed by densitometric scan (Hoeffer Scientific Instruments GS300).

2.4.1. LDL gel

Plasma samples (100 μl) are mixed with 50 μl of Sudan black in ethylene glycol, incubated for 1 h at 4°C and spun for 20 min at $10\,000 \times G$. An equal volume of this preparation is mixed with a saturated sucrose/Bromophenol blue solution and 12 μl is loaded per well, before running overnight at 4°C at 130 V (current < 60 mA). Samples from each subject were placed in adjacent lanes. The gels were inspected without any information. This technique reproducibly identifies about five species of LDL, ranging from A (largest), to I (intermediate), to B (smallest), with intermediate to large species regarded as the normal range. To allow comparisons between gels, marker lanes of A and B patterns are carried over from gel to gel.

2.4.2. HDL gel

Plasma samples are prepared as above, but are not mixed with the bromophenol blue solution; 16 μl is loaded per well and run at 130 V for 4 h at 4°C. In our system we find mostly two peaks; the smaller corresponds to HDL3 and the larger to HDL2. Markers of HDL2 and HDL3 are carried over from gel to gel. The description is semi quantitative, judging a species to be dominant in either one of the two bands or equivalent, a process which correlates with the area under the curve and the peak intensities on densitometric scanning.

2.5. Statistical analysis

A statistical software package (Instat, Graphpad Software, Inc. San Diego, CA 92121) was used for data analysis. Data are expressed as a mean $\pm$ standard deviation (S.D.). Data were analysed using non-parametric methods, with the Wilcoxon matched pairs test. A value of $P < 0.05$ was accepted to define statistical significance.

3. Results

3.1. Subject characteristics and diet

All of the subjects were in good health as judged by medical history, physical examination and liver function tests. No clinically significant adverse effects of AAS or triptorelin use were observed. Resting blood pressure was within the normal range for all subjects. One cycle of AAS use had no measurable effect on blood pressure. Liver function tests revealed minor derangements in the study groups across treatment periods. The triptorelin group had a mean LDH concentration (351 ± 99 U/l) which was elevated compared to the control group (257 ± 54) and the upper reference range limit (290 U/l).

Body mass of the AAS group increased significantly on cycle, from 96.2 ± 11.1 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 ($13.5 \pm 3.5\%$ vs. $13.0 \pm 2.8\%$, $P =$

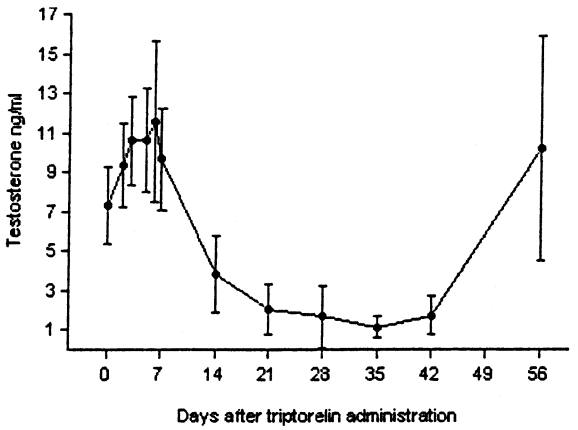

Fig. 1. Effect of triptorelin on testosterone concentration in normal men ($n = 10$, mean ± S.D.).

0.36). Neither body mass (80.8 ± 12.2 vs. 81.4 ± 12.7 kg, $P = 0.65$), muscle mass (43.3 ± 6.1 vs. 43.5 ± 6.2 kg, $P = 0.91$), nor fat percentage (20.1 ± 6.8 vs. 19.9 ± 7.1%, $P = 0.20$) changed between tests in the triptorelin subjects. None of these parameters changed in the control group.

Nine AAS users completed one dietary record, and four completed the second. The remainder were unwilling to complete the second dietary record, maintaining that their diet had not changed since the first test. Mean energy intake was 15 201 ± 4817 kJ. The percentage of energy made up by protein, carbohydrate and fat was 27.3 ± 3.9, 53.0 ± 5.2 and 19.9 ± 3.5% respectively. Seven of the triptorelin subjects completed both dietary records. There were no significant differences in energy consumption (8218 ± 3362 kJ vs. 8874 ± 3061 kJ), percentage protein (16.3 ± 3.5 vs. 16.7 ± 5.1%), percentage carbohydrate (54.2 ± 12.3 vs. 55.6 ± 8.0%), percentage fat (31.7 ± 9.1 vs. 29.2 ± 4.4%) between tests.

3.2. Testosterone, AAS and insulin

Baseline testosterone concentration was similar in the triptorelin (7.3 ± 1.9 ng/ml) and control group (7.7 ± 2.0 ng/ml), and was significantly reduced with triptorelin therapy to 1.2 ± 0.6 ng/ml after 5 weeks, $P = 0.002$. Testosterone concentration did not change in the control group (7.0 ± 1.8 ng/ml, $P = 0.69$). A plot of testosterone concentration versus time in the triptorelin group (Fig. 1), shows that test 2, performed 5.3–5.8 weeks after triptorelin administration, coincided with the nadir of testosterone concentration. Plasma testosterone concentration was not measured in the AAS group.

AAS use was confirmed in all of the bodybuilders by urinalysis. During the on cycle, testosterone esters were detected in all, and nandrolone was detected in eight. Low concentrations of nandrolone could be detected in seven subjects, at the end of their off cycle 'washout period', most likely remnants of the previous on cycle. In one subject, testosterone and stanozolol from a previous cycle could be detected during his off cycle.

Insulin concentration was unchanged in the AAS users (5.2 ± 1.7 μU/ml off cycle, vs. 4.9 ± 1.7 μU/ml on cycle, $P > 0.24$), triptorelin (7.3 ± 7.1 μU/ml vs. 7.1 ± 4.3 μU/ml, $P > 0.70$), and control groups (3.6 ± 1.5 μU/ml vs. 3.9 ± 1.6 μU/ml, $P = 1.00$).

3.3. Lipoprotein values

With AAS use, mean HDLC and Lp(a) concentrations decreased from 0.9 ± 0.3 to 0.7 ± 0.3 mmol/l ($P = 0.004$) and 125 ± 128 to 69 ± 73 U/l ($P = 0.008$) (median; 80 U/l off and 46 U/l on cycle) respectively (Table 1). Across the group, Lp(a) concentration did not decrease as a fixed percentage of the initial concentration. Mean total cholesterol (5.4 ± 2.0 vs. 4.8 ± 1.2 mmol/l, $P = 0.301$) and LDL cholesterol (4.0 ± 2.0 vs. 3.7 ± 1.3 mmol/l, $P = 0.496$) were unchanged.

In the triptorelin group, mean total cholesterol, HDLC and Lp(a) were increased from 4.8 ± 0.8 to 5.2 ± 1.0 mmol/l ($P = 0.039$), 1.1 ± 0.2 to 1.4 ± 0.3 mmol/l ($P = 0.002$) and 278 ± 149 to 377 ± 222 U/l ($P = 0.004$) respectively (Table 1). Mean LDLC concentration and PPT were unchanged. None of these measures changed in the control group.

Table 1
Lipoprotein values of the experimental groups[a]

	Total Chol. (mmol/l)		LDLC (mmol/l)		HDLC (mmol/l)		Lp(a) (Units/l)	
	Off	On	Off	On	Off	On	Off	On
AAS	5.4 ± 2.0	4.8 ± 1.2	4.0 ± 2.0	3.7 ± 1.3	0.9 ± 0.3	0.7 ± 0.3	125 ± 128	69 ± 73
$n = 9$	$P = 0.301$		$P = 0.496$		$P = 0.004$*		$P = 0.008$*	
Triptorelin	4.8 ± 0.8	5.2 ± 1.0	3.1 ± 0.7	3.3 ± 0.8	1.1 ± 0.2	1.4 ± 0.3	278 ± 149	377 ± 222
$n = 10$	$P = 0.039$*		$P = 0.203$		$P = 0.002$*		$P = 0.004$*	
Control	4.6 ± 0.6	4.6 ± 0.5	3.0 ± 0.5	3.0 ± 0.3	1.2 ± 0.2	1.2 ± 0.1	237 ± 241	248 ± 321
$n = 7$	$P = 0.813$		$P = 0.813$		$P = 0.875$		$P = 0.938$	

[a] Data indicates mean ± S.D. * $P < 0.05$ considered to be statistically significant difference.

Table 2
Fasting and postprandial triglyceride concentration in the experimental groups[a]

	Fasting TG (mmol/l)		Area under curve (mmol/l h)		TG excursion (mmol/l h)	
	Off	On	Off	On	Off	On
AAS	1.1 ± 0.5	1.0 ± 0.3	20.1 ± 13.3	15.7 ± 7.5	11.6 ± 10.0	7.5 ± 5.4
$n = 9$	$P = 0.734$		$P = 0.039$*		$P = 0.027$*	
Triptorelin	1.2 ± 0.2	1.3 ± 0.7	16.6 ± 3.8	16.7 ± 4.6	8.5 ± 3.6	8.7 ± 2.6
$n = 10$	$P = 0.625$		$P = 0.846$		$P = 1.000$	
Control	1.0 ± 0.4	1.0 ± 0.4	12.4 ± 4.5	16.8 ± 4.1	6.3 ± 2.3	8.7 ± 2.0
$n = 7$	$P = 0.469$		$P = 0.078$		$P = 0.078$	

[a] Data indicates mean $\pm$ S.D. *$P < 0.05$ considered to be statistically significant difference.

3.4. Triglycerides and PPT

Fasting triglyceride concentration was unchanged with AAS use (1.1 ± 0.5 vs. 1.0 ± 0.3 mmol/l, $P = 0.734$). Mean PPT was significantly decreased on cycle ($P < 0.04$) (Table 2). The two subjects with small LDL off cycle respectively showed 42 and 58% reductions in PPT when on cycle.

Fasting triglyceride concentrations were unchanged in both the triptorelin (1.2 ± 0.2 vs. 1.3 ± 0.7 mmol/l, $P = 0.625$) and control group (1.0 ± 0.4 vs. 1.0 ± 0.4 mmol/l, $P = 0.469$) (Table 2). PPT was unchanged in the triptorelin and control groups.

3.5. LDL and HDL particle size by GGE

LDL size was unchanged in six AAS users, decreased in one but remaining in the normal size range, and increased in two from small, B pattern LDL to the normal range. In the triptorelin group, LDL particle size increased in four, decreased in two, and was unchanged in four subjects. LDL size decreased in two and showed no change in the other five control subjects.

Five of the AAS users had a reduction in the HDL2 size range. The other four subjects showed no change in HDL profile on cycle. HDL was undetectable by GGE in one subject when on cycle and in another both off and on cycle. In the triptorelin group, HDL profiles showed a relative increase of high density lipoprotein in the HDL2 size range in five triptorelin subjects and no change in the other five. HDL profile did not change in seven of the control subjects, and one showed a decrease in the HDL2 size range.

4. Discussion

Although measures of risk point to a possible association between the androgenic hormones and premature atherosclerosis, a direct link has not yet been shown. This study was undertaken to extend our ability to assess risk associated with androgen induced lipoprotein changes by simultaneously gathering information about PPT, LDL particle size, HDLC and Lp(a) in men either taking exogenous androgens or by reducing endogenous androgen production. The results show that androgen supplementation with AAS reduces PPT, particularly in those with an elevated PPT. Suppression of endogenous androgens had no effect on PPT in the short term. Although androgen manipulation had no effect on LDLC concentration, it did have a pronounced effect on HDLC and Lp(a) concentration, respectively increasing them with androgen suppression and decreasing them with androgen supplementation. This study confirms previous findings that androgens can ameliorate hypertriglyceridaemia and provides the observation that such improved postprandial triglyceride excursions may permit favourable size changes in LDL.

The present study has the disadvantage of small group sizes but, knowing that the opportunity for such an examination will not readily be repeated, the findings are worth recording. All subjects were in good health during the study. In particular, the AAS users showed neither elevated blood pressure nor abnormal hepatic function. The study depended on a change in the androgen status of the participants. Analysis of testosterone in the triptorelin group showed a significant and sustained reduction in concentration prior to the second assessment. Androgen status was more difficult to control in the AAS group as ethical considerations precluded investigator involvement in drug administration. On cycle AAS use could, however, be confirmed by urinalysis. An increased on cycle muscle mass corroborated an anabolic effect. If anything, the impact of AAS may be underestimated in this study because the off cycle urinalysis revealed residual AAS metabolites.

A reduced HDLC concentration with androgen supplementation is well documented [22]. The relatively low mean HDLC concentration at the end of the off cycle is attributed to residual plasma AAS. In the triptorelin group, testosterone suppression increased

HDLC concentration, confirming observations in similar studies [15,23]. HDLC concentration can be restored during testosterone suppression through synthetic testosterone replacement, showing that the testosterone suppressant itself has minimal intrinsic effect on plasma lipids [15,23]. While this does not exclude an intrinsic effect unique to triptorelin, this possibility is unlikely. The inverse association between androgens and HDLC concentration is likely to be mediated through hepatic triglyceride lipase (HTGL). The higher endogenous androgen concentration in males is related to a relatively increased HTGL activity compared to females [24]. Further increases in HTGL activity are associated with use of 17-alkylated (oral) AAS, and to a lesser extent with 17-esterified (parenteral) AAS [16,25]. Increased HTGL activity would tend to decrease HDLC concentration through increased lipolytic degradation [26–28]. It is possible also that androgens may reduce HDL concentration to an extent by reducing HDL synthesis, and by increasing removal by receptors [27]. Our GGE studies confirm that AAS induce primarily HDL2 sub fraction reductions [22,27]. Conversely, we observed an increase in the HDL2 size range in half of the triptorelin subjects, consistent with reports showing an increased cholesterol concentration in the HDL2 rather than HDL3 sub-fraction following manipulation of endogenous testosterone concentration [14,15].

AAS use was associated with a reduction in Lp(a) concentration. This effect is in accordance with previous reports, having been observed with AAS use [29,30], as well as with testosterone supplementation in hypogonadal [31] and normal males [32,33]. We found that testosterone suppression had the inverse effect on Lp(a) concentration, in keeping with previous studies in men [34,35]. There was a wide inter-individual variability in percentage Lp(a) reduction. Given the strong influence of androgens on Lp(a), it is surprising that there appears to be no gender difference in Lp(a) concentration [36]. Although a high Lp(a) concentration is associated with an increased risk of myocardial infarction [37,38], it is presently unknown whether reduction of Lp(a) concentration will reduce the incidence of atherosclerosis and CHD.

Oral fat tolerance improved after one cycle of AAS use, but was unaffected by testosterone suppression. We are unaware of previous studies which have investigated the effects of either AAS or reversible testosterone suppression on oral fat tolerance. Our findings are however consistent with AAS lowering fasting triglyceride concentration in hypertriglyceridaemic patients [13], and reversible hypoandrogenism having no effect on fasting triglyceride concentrations [15,23]. Olsson et al. [13] reported increased intravenous fat clearance with oxandrolone in hypertriglyceridaemic patients, and Thompson et al. [39] reported a non-significant increase in intravenous fat clearance in normotriglyceridaemic males. In our study, improvements in oral fat tolerance in the AAS group were most evident in individuals who had an elevated PPT, even though they displayed a normal fasting triglyceride concentration [40]. The improved oral fat tolerance with AAS is most likely due to an increase in hepatic lipase activity. Lipoprotein lipase (LPL) is unlikely to have played a role; changes in LPL activity reported with AAS use are minimal [27,39,41,42]. Exercise, being similar off and on AAS is unlikely to have influenced LPL activity significantly. A reduction of testosterone with triptorelin would most likely have had insufficient effect on lipase enzymes to significantly influence triglyceride clearance.

Small LDL is associated with coronary artery disease [43], and a three-fold increase in the risk of acute myocardial infarction [44]. We are unaware of LDL particle size evaluations with AAS use or testosterone suppression. The small groups in the present study make statistical inference difficult. What is clear is that responses in LDL size varied; possible trends in LDL size were the persistence of the same size in controls, a larger size in the triptorelin group and increase in size of small LDL in the AAS subjects whose larger postprandial hypertriglyceridaemia at baseline improved on treatment. Increased androgen concentrations certainly did not influence LDL size in an apparently atherogenic manner. Male hormonal status could influence LDL particle size by various mechanisms. For some, reduced PPT would be an important mechanism, there being a strong and inverse association between plasma triglyceride concentration and LDL particle size [45,46]. This effect appears to be largely mediated by cholesterol ester transfer protein, which catalyses the transfer of cholesterol ester from LDL and HDL to triglyceride rich lipoproteins, and the reciprocal transfer of triglyceride. Triglycerides transferred to LDL and HDL are susceptible to lipase mediated hydrolysis, so that the size of these lipoproteins is reduced [47]. Thus, sustained increases in the residence time of triglyceride rich lipoproteins may lead to reductions in LDL size by reciprocal lipid transfer [48]. It was interesting to note that the two AAS subjects with small dense LDL had an exaggerated PPT. AAS use in these individuals was associated with a significant reduction in PPT and an increase of LDL size into the normal size range. Different factors may influence LDL size in other subjects: a different kind of VLDL may increase LDL size or loss of hepatic lipase modulation of LDL. The decrease in LDL size with age might be accounted for by decreasing testosterone and increasing triglyceride.

AAS had no effect on total cholesterol or LDLC concentration, in contrast to previous findings [22,27]. The significant increase in total cholesterol with triptorelin is accounted for largely by the elevated HDLC

concentration. We confirm that suppression of endogenous testosterone had no effect on LDLC concentration [15].

In summary, we investigated the effects of androgen supplementation or suppression on PPT and LDL particle size, as well as Lp(a), HDL and LDL concentration in men. Androgen supplementation was investigated in AAS users, and androgen suppression in other men who had received triptorelin, a powerful GnRH agonist. This study shows that androgen supplementation could reduce PPT, particularly in individuals characterised by an elevated PPT, whereas androgen suppression had no effect on PPT. Androgen manipulation had no effect on either LDL particle size or LDLC concentration, but had a marked effect on HDLC and Lp(a) concentration, respectively increasing these parameters with androgen suppression and decreasing them with androgen supplementation. Endogenous testosterone may well be the major determinant of the lower HDLC and particularly the lower HDL2C concentration in men compared to women. Decreased Lp(a) concentration may be an antiatherogenic effect of the androgenic hormones. An increase in LDL size and a reduced PPT may be additional antiatherogenic effects of AAS use in individuals who are predisposed to 'atherogenic dyslipidaemia'. More studies, particularly on subjects who are predisposed to 'atherogenic dyslipidaemia', are required to confirm these observations.

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