The Effect of Anabolic-Androgenic Hormones on Postprandial Triglyceridaemia and Lipoprotein Profiles in Man

Michael Stuart Hislop

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The Effect of Anabolic-Androgenic Hormones on Postprandial Triglyceridaemia and Lipoprotein Profiles in Man

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To my parents

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Declaration

I, Michael Stuart Hislop, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise), and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I empower the University of Cape Town to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

Signature Signed by candidate

Date

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List of Abbreviations

AAS	anabolic-androgenic steroids
ALT	alanine aminotransferase
AP	alkaline phosphatase
apo	apolipoprotein
AST	aspartate aminotransferase
C	triptorelin trial control group
CETP	cholesterol ester transfer protein
CHD	coronary heart disease
GGT	gamma glutamic transaminase
HDL	high density lipoprotein
HDLC	high density lipoprotein cholesterol
HTGL	hepatic triglyceride lipase
HTGLa	hepatic triglyceride lipase activity
IDL	intermediate density lipoprotein
IDLC	intermediate density lipoprotein cholesterol
LCAT	lecithin cholesterol acyl transferase
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LDLC	low density lipoprotein cholesterol
Lp(a)	lipoprotein 'little' a
LPL	lipoprotein lipase
LPLa	lipoprotein lipase activity
OFF	off anabolic-androgenic steroids cycle
ON	on anabolic-androgenic steroids cycle
T	triptorelin trial experimental group
TG	triglyceride
TGRL	triglyceride rich lipoprotein
VLDL	very low density lipoprotein
VLDLC	very low density lipoprotein cholesterol

Terminology

(i) The term *anabolic-androgenic hormones* refers to all endogenous and exogenous (synthetic) anabolic-androgenic hormones.

(ii) The term *anabolic-androgenic steroids*, used in its abbreviated form *AAS*, refers to exogenous (synthetic) anabolic-androgenic hormones only, and includes all AAS administered orally as well as those administered parenterally (parenteral AAS are administered by intra-muscular injection).

(iii) The term *endogenous anabolic-androgenic hormones* refers to all the endogenous anabolic-androgenic hormones, including androstenedione, dihydrotestosterone and testosterone.

(iv) Unless stated directly, all references to *lipoproteins* are to plasma lipoproteins. This also applies to all the other haematology, i.e. a concentration refers to a plasma concentration, unless otherwise indicated.

(v) The term *premature atherosclerosis* implies that atherosclerotic disease becomes evident at an earlier age than expected. As atherosclerosis may be caused by multiple factors acting simultaneously, an additional atherogenic factor will serve to reduce the age at which the disease becomes manifest.

(vi) The term *premature CHD* implies an earlier incidence of CHD (a clinical manifestation of atherosclerosis) due to premature atherosclerosis.

(vii) The terms *men* or *women* imply adult males or females.

(viii) The term 'atherogenic dyslipidaemia' is not in common usage, and is thus enclosed in inverted commas.

Preface

There is controversy in the scientific literature as to whether endogenous testosterone constitutes an atherogenic factor in males, and whether, and in what setting, supplementation with anabolic-androgenic steroids (AAS) may constitute an additional atherosclerotic risk. A better understanding of this question is important because: (i) the use of AAS by males and females, to improve athletic performance or physical appearance is becoming increasingly widespread (Yesalis *et al.*, 1993), and (ii) there is renewed interest in using synthetic testosterone as a male contraceptive (Bagatell and Bremner, 1996), and for hormone replacement therapy in elderly hypogonadal men (Swerdloff and Wang, 1993). Therefore, the aims of this thesis were to: (i) review the current literature, to gain perspective on the basis for the divergent opinions in this field, and (ii) investigate the poorly understood relationship between the anabolic-androgenic hormones and 'atherogenic dyslipidaemia'. This phenotype is characterised by an abnormal triglyceride metabolism, small dense low density lipoproteins and a low high density lipoprotein (HDL) concentration, which confers a high risk of premature atherosclerosis (Grundy, 1996). This thesis comprises; (i) an introductory chapter, in which the relevant literature is reviewed, and the theoretical basis for the present studies is presented; (ii) a methodological chapter, in which the clinical and laboratory procedures are described in detail; (iii) two experimental chapters, one on healthy men undergoing a reversible suppression of testosterone production, and one on bodybuilders using AAS. The experimental chapters include an introduction to the specific study, a brief description of methods and results, and a discussion of the results in the context of current knowledge. The thesis concludes with a general discussion and recommendations for future studies.

Abstract

It has been hypothesised that endogenous testosterone and AAS may predispose humans to premature CHD. However, there is no direct evidence to link these hormones with a greater prevalence of premature CHD. The aim of this thesis was to better describe atherosclerotic risk associated with these hormones by clarifying their effect on additional risk factors for premature atherosclerosis. Little is known about the effect of testosterone and AAS on 'atherogenic dyslipidaemia', a phenotype characterised by elevated postprandial triglyceridaemia, small dense LDL and a low HDLC concentration, which confers a high risk of CHD. Accordingly, the magnitude of postprandial triglyceridaemia, LDL and HDL particle size, and LDLC, HDLC and Lp(a) concentration were compared in male (n=9) and female (n=3) bodybuilders after self administration of AAS for 5-6 weeks (ON cycle) and again after a 4-6 week 'washout' period (OFF cycle), and in normal males (T) (n=10) before and during a reversible suppression of endogenous testosterone, induced using a GnRH agonist (triptorelin), and in a control group (C) (n=8). Lipoprotein size was assessed by gradient gel electrophoresis (GGE), lipoprotein concentrations by immuno and enzymatic assay, and postprandial triglyceridaemia by a standardised oral fat tolerance test (65g/m^2). HDLC decreased in male bodybuilders (0.94±0.30 vs 0.70±0.27 mmol/L, p=0.004; $\bar{x}$ ± SD) and female bodybuilders (1.3±0.5 vs 0.8±0.2 mmol/L) ON cycle. GGE studies suggested that mostly HDL_2 was reduced. There were no significant reductions in LDL particle size ON cycle. Two males had larger LDL species ON cycle. Lp(a) decreased in male bodybuilders (124.7±128.0 to 69.3±73.3 U/L, p=0.008). ON cycle postprandial triglyceride excursion was unchanged in female bodybuilders and reduced (11.6±10.0 vs 7.5±5.4 mmol/L.hr; p=0.027) in male bodybuilders. In the triptorelin study, HDLC was increased in T (1.07±0.18 vs 1.41±0.28 mmol/L, p=0.002) and not in C. GGE studies indicated an increase of HDL_2 in five T subjects and no increase in C. Total cholesterol increased in T (4.77±0.80 vs 5.24±1.04 mmol/L, p=0.039) but not in C. LDL size increased in four T subjects, and not in C. Lp(a) increased in T (277.9±149.1 vs 376.5±222.2 U/L, p=0.004), but not in C. Postprandial triglyceridaemia was unchanged in both T and C. The results of these studies did not show any additional atherogenic effects of endogenous testosterone or AAS in humans. Rather, a suppression of Lp(a) may be an antiatherogenic effect of these hormones. A reduced postprandial triglyceridaemia and increased LDL size in individuals who are predisposed to 'atherogenic dyslipidaemia', may be further antiatherogenic effects of AAS use.

Introduction

Atherosclerosis is a degenerative disease of arteries (Thompson, 1994). The atherosclerotic process is the principal contributor to the pathogenesis of myocardial and cerebral infarction, gangrene and loss of function in the extremities (Ross, 1993). Atherosclerosis is currently the commonest cause of death and disability in most Westernised countries (Thompson, 1994). The pathogenesis of atherosclerosis is exceedingly complex, there being multiple factors which can either predispose to, or protect from, the initiation and/or progression of atherosclerosis. The complex interrelationship between these factors makes it difficult to predict with certainty those who will suffer from this disease.

Endogenous testosterone and AAS are regarded as factors which may predispose to premature atherosclerosis (Bagatell and Bremner, 1995; Glazer, 1991; Kalin and Zumoff, 1990). However, it has proven difficult to establish causality between these hormones and premature atherosclerosis in humans (Melchert and Welder, 1995). To confirm causality, it would be necessary to compare the progression of atherosclerosis between groups of normal individuals having either reduced, unchanged or elevated concentrations of testosterone or AAS. However, ethical and practical considerations constrain the duration of such studies, making it difficult to assess whether the atherosclerotic process has been modified in the vessel wall or in clinical outcome. Indeed, a review of the current literature reveals that evidence to provide a direct link between both endogenous testosterone and AAS, and premature atherosclerosis in humans is limited. Unfortunately, relatively few animal studies have been completed to date, and the results from those that have been published are equivocal (Alexandersen *et al.*, 1996). While animal studies could clarify the role of anabolic-androgenic hormones in atherosclerosis, it remains uncertain whether data obtained in animal atherosclerosis models can be extrapolated to humans.

Another means of establishing a link between the anabolic-androgenic hormones and premature atherosclerosis is by inference. If the anabolic-androgenic hormones adversely affect other risk factors for premature atherosclerosis, it may be inferred that the anabolic-androgenic hormones also constitute an atherogenic risk factor. In this context, a risk factor is defined as any measurable trait or characteristic that predicts an individual's probability of developing clinically manifest disease, but does not necessarily imply a causal relationship (McGill, 1996). Some of

the major risk factors for premature atherosclerosis are hypertension, diabetes, smoking, obesity, a family history of the disease, and an abnormal lipoprotein profile (Thompson, 1994). Currently, the proposal that endogenous testosterone and AAS may be atherogenic stems primarily from the finding that these hormones modify lipoprotein profiles in an apparently atherogenic manner. However, it is unknown whether the lipoprotein changes *per se* induced by endogenous testosterone make a significant contribution to the sex difference in the prevalence of premature coronary heart disease (CHD), or whether the lipoprotein changes evident in AAS users later manifest as premature CHD. In the absence of studies to prove the hypothesis that testosterone and AAS cause premature atherosclerosis in humans, the possibility cannot be excluded that these hormones may induce other anabolic responses which alter the putative deleterious actions of these hormones on circulatory structure and function (Rockhold, 1993). Two observations suggest that the atherogenicity of testosterone and AAS may have been overstated: (i) prospective and cross sectional studies suggest either a neutral or beneficial effect of endogenous testosterone in males (Alexandersen *et al.*, 1996), and (ii) the frequency of reported atherosclerotic complications relative to the total estimated population of AAS users appears to be lower than would be expected, given the apparent atherogenicity of AAS (Wilson, 1988; Windsor and Dumitru, 1988; Street *et al.*, 1996).

Until such time as it becomes possible to perform studies which can provide direct evidence of the association between the anabolic-androgenic hormones and atherosclerosis, it seems prudent to extend the scope of inferential atherosclerotic risk assessment, to permit a better estimation to be made of risk for premature CHD associated with these hormones.

Aims of the Thesis

The aim of this thesis was to provide new insight into the atherosclerotic risk associated with endogenous testosterone and AAS. In order to clarify the present understanding of this association, the relationship between endogenous testosterone, AAS and risk for premature atherosclerosis, is examined in a number of different settings in chapter 1: (i) Does endogenous testosterone increase risk for premature atherosclerosis in males? (ii) Does synthetic testosterone replacement therapy in hypogonadal males increase their risk for premature atherosclerosis? (iii) Does the use of 17β-esterified (parenteral) AAS increase risk for premature atherosclerosis? (iv) Does the therapeutic use of 17α-alkylated (oral) AAS increase risk for premature atherosclerosis? (v) Does the non-therapeutic use of a combination of 17β-esterified (parenteral) and 17α-alkylated (oral) AAS increase risk for premature atherosclerosis? While the distinction drawn between these settings may be somewhat arbitrary, they do serve to illustrate the basis of some of the controversy in this field. The literature review also forms the basis of the assertion that additional research is still required before a consensus can be reached, on whether, and in what setting, endogenous testosterone and AAS constitute a significant atherogenic factor in humans (Rockhold, 1993). Based on this opinion, the studies described in this thesis were performed to better describe the effect of endogenous testosterone and AAS on lipid and lipoprotein risk factors for premature atherosclerosis in humans.

At present there is a paucity of information about the association between anabolic-androgenic hormones and 'atherogenic dyslipidaemia'. This lipoprotein phenotype is characterised by a low high density lipoprotein cholesterol (HDLC) concentration, increased small dense low density lipoprotein (LDL) particles and an abnormal triglyceride metabolism which can manifest as an exaggerated postprandial triglyceridaemia or a mild fasting hypertriglyceridaemia, leading to an increase in very low density lipoprotein (VLDL) remnants. 'Atherogenic dyslipidaemia' has more recently been identified as one of the two major patterns of atherogenic lipoprotein disorders along with hypercholesterolaemia (elevated low density lipoprotein cholesterol (LDLC) concentration) (Grundy, 1996). In fact, some investigators believe that this phenotype rivals hypercholesterolaemia as a risk factor for CHD (Grundy, 1996). This area was consequently identified as the research focus of the two experimental chapters described in this thesis.

Chapter 1: Literature Review. Endogenous testosterone, AAS and risk for premature atherosclerosis

The following review chapter introduces the reader to endogenous testosterone and AAS, and critically examines whether there is sufficient evidence to conclude the hypothesis that these hormones predispose humans to premature atherosclerosis. This chapter begins with a brief review of lipid and lipoprotein risk factors for premature atherosclerosis, since much of the evidence to show a link between testosterone, AAS and premature atherosclerosis is based on changes in lipoprotein risk factors induced by these hormones.

Lipids, lipoproteins and risk for premature atherosclerosis

Plasma lipoproteins are the prominent constituents of a complex transport system that provides for the movement of exogenous (intestinal) and endogenous (liver) lipid between the organs and tissues. Lipids have important biological functions that include; (i) the use of triglyceride for energy production or as stored fat in adipose tissue and, (ii) the use of cholesterol as a component, in conjunction with phospholipid, of cellular membranes or in the synthesis of steroid hormones or bile (Thompson, 1994). Since lipids are not water soluble, and thus cannot bind receptors, they must combine with apolipoproteins to form micellar lipid-protein complexes or lipoproteins. These water soluble particles are spherical, have finite dimensions, and contain cholesterol (free and esterified), triglyceride, phospholipid, and various apolipoproteins (Thompson, 1994).

The apolipoproteins of the lipoprotein particle have several functions: (i) effecting the aqueous solubility of lipids, (ii) helping to solubilise cholesterol esters and triglyceride by interacting with phospholipid, (iii) regulating the reaction of lipids with enzymes and other proteins, and (iv) binding to cell surface receptors and thus determining the sites of uptake and rates of degradation of other lipoprotein constituents, notably cholesterol (Thompson, 1994). For reference, the apolipoprotein composition of lipoproteins in fasting plasma is included in Appendix I. In brief, apolipoprotein A (apoA) is the chief protein constituent of HDL and is subdivided into apoA-I and apoA-II. Apolipoprotein B (apoB) is heterogeneous; $apoB_{100}$ is found mainly in VLDL and LDL, whereas $apoB_{48}$ is found only in chylomicrons, and represents the amino-terminal half of $apoB_{100}$. $ApoB_{100}$ is a ligand for the LDL receptor, but $apoB_{48}$ is not.

Apolipoprotein C (apoC) comprises at least three distinct apolipoproteins, which occur as major constituents of VLDL and minor constituents of HDL. Apo C-II activates lipoprotein lipase (LPL) and apoC-III inhibits it, whereas the role of apoC-I is not clear. Apolipoprotein E (apoE) is found in VLDL, IDL and HDL, entering plasma mainly in the form of nascent HDL.

Plasma lipoproteins differ according to ultracentrifugal flotation rate (S_f), density (d), size and electrophoretic mobility. Currently, the most popular classification is that based on differences in gravitational density, recognising four basic classes of lipoprotein: (i) chylomicron, derived from intestinal absorption of triglyceride; (ii) VLDL, derived from the liver for the export of triglyceride; (iii) LDL, representing a final stage in the catabolism of VLDL; and (iv) HDL, involved in the reverse transport of cholesterol (Thompson, 1994). Other lipoprotein subfractions do exist, and include intermediate-density lipoprotein (IDL), an intermediary step in VLDL catabolism; HDL that is typically studied as two separate subfractions: HDL_2 and the more dense HDL_3; and lipoprotein(a) (Lp(a)). Lp(a) is a lipoprotein particle that is similar to LDL in terms of lipid and protein composition, but it additionally contains a highly glycosylated protein, designated apolipoprotein(a) (apo(a)), that is linked through a disulphide bridge to $apoB_{100}$ (Gaubatz *et al.*, 1983). Apo(a) is formed by three different structural domains. One of the domains, called kringle 4, is present in multiple copies, the number of which varies and is genetically determined, giving rise to different sizes of apo(a) and consequently Lp(a) (Uterman, 1989; MBewu and Durrington, 1990).

There are a number of lipid and lipoprotein risk factors for premature atherosclerosis. These include: (i) An elevated LDLC concentration; high concentrations of LDLC are known to directly promote atherosclerosis, by allowing excessive cholesterol deposition in the arterial wall, and therapeutic lowering of high LDLC concentrations is known to reduce the risk of CHD, and retard the progression of atherosclerosis (Blankenhorn *et al.*, 1987; Brown *et al.*, 1990; Gordon *et al.*, 1981; Lipid Research Clinics Program, 1984, 1984a; Stamler *et al.*, 1986). (ii) A low HDLC concentration (Assmann and Schulte, 1992; Gordon *et al.*, 1989; Miller and Miller, 1975), although the mechanism whereby HDL influences atherosclerosis remains to be established (Gordon *et al.*, 1989; Vega and Grundy, 1996). Two mechanisms have been proposed to explain this relationship: a direct mechanism, where HDL acts as a 'protective' lipoprotein, providing direct protection against the development of atherosclerosis; and an indirect

mechanism, where a low HDLC concentration is caused by some other metabolic abnormality, which is itself atherogenic. (iii) An elevated plasma triglyceride concentration (Austin and Hokanson, 1996; Criqui, 1996; Drexel *et al.*, 1996; Tenkanen *et al.*, 1996); hypertriglyceridaemia is characterised by the accumulation of chylomicron and VLDL remnants, small dense LDL and a low HDLC concentration, all of which are thought to be atherogenic. (iv) An elevated Lp(a) concentration, although the pathogenicity of Lp(a) appears to be modulated by the concentration of LDLC (Armstrong *et al.*, 1986). The evidence supporting a role for Lp(a) in the atherosclerotic process stems from observations that it accumulates in atherosclerotic plaques, stimulates smooth muscle proliferation, binds apoB-containing lipoproteins, avidly binds to arterial proteoglycans and fibronectin, and promotes cholesterol accumulation in cells (Maher and Brown, 1995). It may also promote thrombosis because it has structural similarities to plasminogen, binds fibrin, competes with plasminogen for binding sites on cells, and exhibits antifibrinolytic actions *in vitro* (Maher and Brown, 1995).

Endogenous anabolic-androgenic hormones

The anabolic-androgenic hormones play an important physiological role in the normal healthy adult male. Endogenous anabolic-androgenic hormones are required for the maintenance of the reproductive tissues, and have a key role in stimulating and maintaining sexual function (Bagatell and Bremner, 1996). Endogenous anabolic-androgenic hormones also play a role in regulating both bone and muscle mass (Swerdloff and Wang, 1993), and in regulating the proportion of depot fat mass in central and peripheral adipose tissue (Mårin *et al.*, 1995). The endogenous anabolic-androgenic hormones are 19-carbon steroid rings derived from cholesterol. The first stage in the synthesis of steroid hormones is the conversion of cholesterol to pregnenolone, from which progesterone is synthesised. The synthesis of endogenous anabolic-androgenic hormones starts with the hydroxylation of progesterone at C-17. The side chain consisting of C-20 and C-21 is then cleaved to yield androstenedione, an endogenous anabolic-androgen. Testosterone, another endogenous anabolic-androgen, is formed by the reduction of the 17-keto group of androstenedione. Oestrogens are synthesised from androstenedione and testosterone by the loss of the C-19 methyl group and the formation of an aromatic A ring. Oestrone, an oestrogen, is derived from androstenedione, whereas oestradiol, another oestrogen, is derived from testosterone (Stryer, 1995).

The hypothalamus secretes gonadotropin-releasing hormone, which stimulates the pituitary to secrete luteinizing hormone and follicle-stimulating hormone. In men, luteinizing hormone stimulates Leydig cells in the testes to produce testosterone. Follicle-stimulating hormone acts on Sertoli cells in the testes, thereby stimulating spermatogenesis. Approximately 7 mg of testosterone is produced daily in men (Bagatell and Bremner, 1996). Testosterone may also be aromatised to oestradiol (E_2) by the aromatase enzyme complex, which is most abundant in adipose tissue, liver, and certain central nervous system nucleii (Bagatell and Bremner, 1996). Men have about one-fifth of the amount of oestrogen found in the nonpregnant woman, much of which is derived from the conversion of testosterone and androstenedione to oestrogens (Longcope *et al.*, 1978). Endogenous oestrogen may play an important role in men by modulating some of the effects of the anabolic-androgenic hormones (Applebaum-Bowden, 1984; Tikkanen and Nikkila, 1987). Moreover, some of the differences in the effects between the various AAS may be due to their relative resistance to aromatisation (Friedl *et al.*, 1990).

The anabolic-androgenic hormones have both a masculinizing (androgenic) effect, coupled with an tissue building (anabolic) effect. The anabolic and androgenic components are not due to different actions of the hormone but result from interaction of the hormone with the same receptor molecule in different tissues (Wilson, 1988). Testosterone is the most abundant circulating endogenous anabolic-androgen in men; smaller amounts of dihydrotestosterone and adrenal androgens are also found in the plasma. Testosterone circulates primarily in a bound form, mostly to sex hormone binding globulin (SHBG); only 1 to 2 percent is unbound. Adrenal androgens are relatively weak in terms of both receptor binding and biological effects; therefore they play only a small role in the normal male physiology (Bagatell and Bremner 1996). At the cellular level, testosterone may be converted to dihydrotestosterone by the 5α-reductase enzymes. Both testosterone and dihydrotestosterone bind to the androgen receptor, but dihydrotestosterone has a higher affinity for the receptor and is therefore a more potent androgen (Bagatell and Bremner, 1995).

The mechanism of action of the anabolic-androgenic hormones is fairly well understood. Once in the bloodstream, the anabolic-androgen passes through the cell wall of its target tissue and attaches to steroid receptors located in the cytoplasm. The binding to these receptors is highly specific, but of relatively low affinity. This hormone-receptor complex is translocated to the

nucleus of the cell and attaches to sites on the nuclear chromatin. Transcription ensues, resulting in the production of specific messenger RNA. Ribosomal translation of the code results in highly specific new proteins that mediate the function of the hormone (Lukas, 1993).

Is endogenous testosterone atherogenic?

The proposal that endogenous testosterone may be atherogenic stems from a number of observations: (I) hypertestosteronaemic women with polycystic ovary syndrome have an increased risk for myocardial infarction (Dahlgren *et al.*, 1992; Kalin and Zumoff, 1990); (ii) hypotestosteronaemic men with cirrhosis of the liver have a decreased prevalence of coronary disease (Kalin and Zumoff, 1990); (iii) experimentally induced male plasma testosterone concentration in female monkeys with diet-induced atherosclerosis, results in coronary atherosclerosis which is approximately twice as extensive as in controls (Adams *et al.*, 1995); (iv) endogenous testosterone is the major determinant of the lower HDLC and HDL_2C concentration in men compared to women (Asscheman *et al.*, 1994). The latter conclusion is derived from the observation that suppression of endogenous testosterone concentration in men results in HDLC concentrations more typically seen in women, and vice versa (Asscheman *et al.*, 1994; Goh *et al.*, 1995). Similarly, male HDLC concentrations fall at puberty, correlating with the rise in plasma testosterone concentration (Kirkland *et al.*, 1987), whereas female HDLC concentrations do not change at puberty, despite the rise in oestrogen concentration (Godsland *et al.*, 1987).

It is, however, difficult to exclude possible confounding factors in (i) and (ii) above: women with polycystic ovary syndrome are characterised by other atherogenic factors (Dahlgren *et al.*, 1992), and cirrhosis in men may reduce the incidence of CHD by decreasing the concentration of coagulation factors. Moreover, in (i) and (iii) above, one wonders if the atherogenic effects of the endogenous sex hormones may differ in women and men. Indeed, in a study on castrated, cholesterol fed male rabbits, Larsen *et al.* (1993) found no consistent significant differences in aortic cholesterol content between the group in which plasma testosterone concentration was restored, compared to the group that was castrated only. In fact, the aortic cholesterol content tended to be lower in the testosterone group than the placebo group, although not significantly. The suppressive effect of endogenous testosterone on HDLC concentration in men compared to women may possibly contribute to the increased risk of CHD

in men (Bagatell and Bremner 1995). It is, however, difficult to determine whether equivalent HDLC concentration confers equivalent risk in men and women. Indeed, a low HDLC concentration appears to be a stronger predictor of atherosclerotic disease in women than in men (Gordon *et al.*, 1989).

If endogenous testosterone had a significant atherogenic effect in men, one would expect to find a lower incidence of premature CHD in men who tended to be hypotestosteronaemic compared to men with normal testosterone concentration. In fact, the opposite trend appears to be the case. In a recent review of studies dealing with the relationship between circulating testosterone concentration and CHD in men, Alexandersen *et al.* (1996) examined five prospective studies, none of which showed any relation, and twenty-nine cross-sectional case-control studies, all but one of which showed either an inverse or a neutral association. They concluded that these studies suggest either a neutral or a favourable effect of endogenous testosterone on CHD in men. The association of endogenous testosterone with more favourable cardiac health in men is consistent with the finding that low concentrations of SHBG and testosterone are associated with the atherogenic smaller, denser LDL in normoglycaemic men (Haffner *et al.*, 1996). This association is also consistent with a review by Bagatell and Bremner (1995) of 18 cross-sectional reports which examined the relationship between plasma testosterone and plasma HDLC concentration. Most of these studies reported a positive relationship. In only two studies were testosterone concentrations associated with lower concentrations of HDLC (although a recent study by Handa *et al.* (1997) must also be added to this total), and in three additional studies, no relationship was observed.

It is likely that the association between HDLC and endogenous testosterone concentration may be influenced by multiple factors, including disease states and lifestyle (e.g., smoking, exercise), and factors such as body mass index, fat distribution and waist to hip ratio (Bagatell and Bremner, 1995). In general, factors that decrease HDLC concentration also decrease testosterone concentration (Plymate and Swerdloff, 1992). In particular, insulin has been shown to suppress SHBG, which in turn results in a reduction in the total plasma testosterone concentration (Bagatell and Bremner, 1995; Plymate and Swerdloff, 1992). Therefore, it is not surprising that on a population basis, men with high plasma insulin concentration might also show lower concentrations of testosterone and HDLC (and higher triglyceride concentration), and

conversely, men with higher testosterone concentration would also have a higher HDLC concentration and a lower triglyceride concentration (Bagatell and Bremner, 1995). It is also possible that the apparent association between low testosterone concentration and poor cardiac health might be accounted for by a secondary reduction in testosterone caused by the disease process. Indeed, rabbits with diet induced atherosclerosis show increased oestradiol and insulin concentrations, and decreased testosterone and thyroid hormone concentrations, which suggests that the hormonal changes may in some cases be a consequence of the disease (Wojcicki *et al.*, 1989).

An increase in Lp(a) concentration in males following orchidectomy indicates that endogenous testosterone has a suppressive effect on Lp(a) concentration (Berglund *et al.*, 1996). Endogenous oestrogen is not likely to influence Lp(a) concentration significantly in males, as synthetic oestrogen (polyoestradiol phosphate 480mg/mth i.m.), causing a twenty fold increase in plasma oestrogen concentration, has no effect on Lp(a) concentration in orchidectomised males (Berglund *et al.*, 1996). In contrast, synthetic testosterone (250mg/wk), causing a doubling of plasma testosterone concentration, significantly reduces Lp(a) concentration in normal males (Berglund *et al.*, 1996). Contradictory results have been reported on the correlation between endogenous testosterone concentration and plasma Lp(a) concentration. One study reported that low plasma testosterone concentrations may be correlated with high plasma Lp(a) concentrations (Dionyssiou and Katimerzi, 1993), whereas two other studies concluded that endogenous testosterone is unlikely to be associated with Lp(a) concentrations in men (Haffner *et al.*, 1994; Marcovina *et al.*, 1996).

In summary, there appears to be only one study on the atherogenicity of endogenous testosterone in male animals. This study compares the progression of atherosclerosis in hypotestosteronaemic compared to eutestosteronaemic rabbits. The results of this study do not favour the hypothesis that endogenous testosterone is atherogenic in males. There also appears to be only one study on the atherogenicity of testosterone in female animals. This study compares the progression of atherosclerosis in female monkeys with male pattern testosteronaemia, compared to controls. The results of this study suggest that an elevated plasma testosterone concentration may be atherogenic in females. Evidence to show an association between endogenous testosterone and premature atherosclerosis in human males is limited. While a lower

HDLC concentration in men compared to women may confer increased risk for atherosclerosis, it is difficult to quantify the magnitude of this risk. A suppressive effect on Lp(a) concentration may be an antiatherogenic effect of endogenous testosterone in males. The neutral relationship between endogenous testosterone and CHD seen in epidemiological studies suggests that testosterone may have beneficial cardiovascular effects in men which may counteract apparently atherogenic effects. On the whole, there appears to be insufficient evidence to conclude that testosterone contributes to the sex difference in the prevalence of premature CHD observed in humans.

Anabolic-androgenic steroids (AAS)

Several synthetic analogues and derivatives of testosterone were developed when it became apparent that testosterone in its natural form cannot be given effectively either orally or by parenteral injection. Oral ingestion is followed by rapid absorption into portal blood and first pass extraction and degradation by the liver so that only small amounts reach the systemic circulation. When testosterone is administered parenterally it is promptly absorbed from the injection vehicle and rapidly degraded so that effective concentrations are not sustained in the plasma. Three general types of modification have been made to the molecule so as to make it useful for therapeutic uses; (i) esterification of the 17β-hydroxyl group, (ii) alkylation at the 17α-position, and (iii) modification of the ring structure of the steroid, particularly substitution at the carbons at position 1, 2, 9, or 11 (Wilson, 1988). Most synthetic steroids contain a combination of structural changes of the ring and either 17β-hydroxy esterification or 17α-alkylation (Wilson, 1988).

Alkylation at the 17α-position with either a methyl or ethyl group is a common feature of most orally active AAS. Such derivatives are effective when given orally because they are more resistant to hepatic metabolism, and hence some of the agent can pass through the liver to reach the systemic circulation in effective amounts. There is no known mechanism for the enzymatic removal of the alkyl groups in the body, and they cannot be aromatised to oestradiol, nor can they be reduced to dihydrotestosterone. Their metabolites are unlike those of endogenous anabolic-androgenic hormones, and some of their physiological effects differ from those of endogenous anabolic-androgenic hormones as well (Wilson, 1988).

Parenteral administration of AAS is made possible by the 17β-esterification of the steroid with various carboxylic acids. This process decreases the polarity of the steroid, making it hydrophobic, and more soluble in the oily vehicles used for injection, thereby slowing the release of the hormone into the circulation (Wilson, 1988). In general, the longer the carbon chain in the ester, the more non-polar and fat-soluble the steroid becomes, and the slower the release of the steroid into the circulation (Wilson, 1988). For example, testosterone propionate (3 carbon chain) must be administered daily whereas testosterone enanthate (7 carbon chain) and testosterone cypionate (8 carbon chain) can be administered every 1 to 3 weeks. After injection, testosterone is hydrolysed from the ester and is metabolically identical to endogenous testosterone (Bagatell and Bremner, 1996).

Alteration of the ring structure of the steroids has been utilised both for oral agents and for parenteral formulations (Wilson, 1988). In some instances the effect is to slow the rate of inactivation; in others it enhances the potency of a given molecule or alters its metabolism. These various agents are not converted in the body to testosterone, and like the 17 alkyl derivatives, circulate in plasma and are excreted as unique metabolites. Alterations of the ring structure also make these steroids resistant to aromatisation (Friedl *et al.*, 1990).

Another important goal of synthetic anabolic-androgenic hormone development was to dissociate the androgenic and anabolic effects so that only the anabolic effects were maintained and the androgenic side effects were minimised. As yet it has not been possible to completely dissociate the two, and since all androgenic hormones also exert anabolic effects to some degree, the term anabolic-androgenic steroid (AAS) is preferred for the synthetic androgenic hormones (Lukas, 1993). Substitutions and alterations of testosterone esters other than at the C17 position have been employed in the development of high anabolic and low androgenic compounds. For instance, 19-nortestosterone esters such as nandrolone decanoate, which have no methyl group at C19, have been found to be long-acting highly anabolic steroids (Hickson, 1989).

Therapeutic and Nontherapeutic use of AAS

AAS may be indicated for a number of therapeutic uses. Men with documented testosterone deficiency due to either primary (hypergonadotropic) or secondary (hypogonadotropic) hypogonadism, as well as boys with constitutional delay of puberty are candidates for

testosterone-replacement therapy (Bagatell and Bremner, 1996). Synthetic testosterone replacement in men with hypogonadism induces greater interest in sexual activity and improvement in other aspects of sexual behaviour (Bagatell and Bremner, 1996). AAS therapy may also be beneficial in patients with haematologic disorders, hereditary angiodema and endometriosis (Bagatell and Bremner, 1996).

Synthetic testosterone replacement therapy may also be beneficial in some elderly men. Testosterone concentrations tend to decrease with aging in men. Testosterone deficiency in elderly men may lead to asthaenia, decrease in muscle mass, osteoporosis, decrease in sexual activity, and in some cases, changes in mood and cognitive function (Swerdloff and Wang, 1993). Synthetic testosterone replacement therapy in elderly hypogonadal men improves muscle mass by stimulating the rate of protein synthesis (Brodsky *et al.*, 1996; Urban *et al.*, 1995), increases muscle strength, decreases bone resorption, increases bone formation markers (Wang *et al.*, 1996) and improves mood (Wang *et al.*, 1996a). Moreover, visceral obesity in males, which is characterised by relative hypogonadism (Mårin *et al.*, 1995), is associated with a greater risk of premature CHD than parietal obesity (Casassus *et al.*, 1992). Synthetic testosterone supplementation inhibits triglyceride uptake and lipoprotein lipase activity (LPLa) and causes a more rapid turnover of triglyceride in the abdominal adipose tissue region only (Mårin *et al.*, 1995).

The possibility has been raised that synthetic testosterone may be used as an effective male contraceptive. Exogenously administered testosterone inhibits gonadotropin secretion, secondarily inhibiting spermatogenesis (Bagatell and Bremner, 1996). A number of clinical trials have been conducted recently to evaluate the safety and efficacy of high dose synthetic testosterone as a male contraceptive (Matsumoto, 1990). Synthetic testosterone therapy may also have a beneficial effect on relieving myocardial ischemia and angina pectoris, by inducing dilation of coronary arteries *in vivo* (Chou *et al.*, 1996; Wu and Weng, 1993; Yue *et al.*, 1995).

The observation that AAS promote nitrogen retention and muscle mass led to their nontherapeutic use to improve physical performance as early as the 1940's, and by the 1950's they were being used by weight lifters (Strauss and Yesalis, 1991). AAS are now widely used by both male and female, professional and recreational athletes alike, to improve athletic performance.

Steroid use is becoming increasingly widespread in individuals, such as body builders, who seek improvement of physical appearance. In a survey in 1993 it was estimated that there were more than one million current or former steroid users in the United States, and that more than three hundred thousand individuals used steroids in the year of the study (Yesalis *et al.*, 1993). Doubt about the true performance enhancing effects of AAS were finally dispelled in a review of the subject by Haupt and Rovere (1984). Their findings were confirmed in a recent study, in which it was concluded that supraphysiological doses of synthetic testosterone, especially when combined with strength training, increase fat-free mass, and muscle size and strength in normal men (Bhasin *et al.*, 1996).

The therapeutic and nontherapeutic use of AAS has been associated with the risk of developing a wide range of temporary and permanent side effects (World Health Organisation, 1993). These can be generalised as follows; effects on secondary sex characteristics, liver toxicity and liver tumours, infertility and virilisation, psychological effects, and cardio- and cerebrovascular effects (World Health Organisation, 1993). The undesired effects of AAS depend on both the type and dose administered. In general, doses used for replacement therapy, and the use of testosterone esters are associated with fewer complications than is the use of 17α-alkylated (oral) AAS, particularly at the high doses of alkylated AAS used by many athletes and bodybuilders (Bagatell and Bremner, 1996). For example, the apparent hepatotoxicity of AAS is unique to the 17α-alkylated (oral) AAS and does not occur with the use of 17β-esterified (parenteral) AAS (Haupt and Rovere, 1984). Similarly, induction of insulin resistance and hyperinsulinaemia by AAS appears to occur with oral but not parenteral agents (Glazer and Suchman, 1994). More recently it has been proposed that the incidence of serious health problems associated with the use of androgens by athletes may have been overstated (Street *et al.*, 1996). Based on the available evidence, it has been suggested that the administration of moderate doses of an injectable androgen, such a testosterone enanthate or nandrolone decanoate, in healthy adult males could induce positive changes in body composition and athletic performance with little or no side effects (Street *et al.*, 1996).

Are AAS atherogenic?

Case reports of adverse cardiovascular events associated with the use of AAS

To date there have been nineteen case reports documenting adverse cardiovascular events in twenty-one subjects self-administering AAS (Akter *et al.*, 1994; Appleby *et al.*, 1994; Bowman *et al.*, 1989; Bowman, 1990; Dickerman *et al.*, 1995; Ferenchick *et al.*, 1991; Ferenchick *et al.*, 1992; Fisher *et al.*, 1996; Frankle *et al.*, 1988; Huie, 1994; Jaillard *et al.*, 1994; Kennedy, 1993; Kennedy *et al.*, 1993a; Laroche, 1990; Luke *et al.*, 1990; Lyngberg, 1991; McNutt *et al.*, 1988; Mewis *et al.*, 1996; Mochizuki and Richter 1988; Siekierzynska-Czarnecka *et al.*, 1990). All of these subjects were male, sixteen were bodybuilders, four 'weight lifters' and one a 'college athlete'. Ten subjects were in their third decade, six in their fourth, two in their fifth and the rest unknown. Myocardial infarction was diagnosed in twelve cases, cerebrovascular accident in five and cardiopulmonary arrest, sudden cardiac death, pulmonary embolism and congestive heart failure in the remainder. There have been eight case reports, documenting adverse cerebrovascular events in fourteen patients receiving therapeutic administration of AAS (De Stefano *et al.*, 1988; Krauss *et al.*, 1991; Nagelberg *et al.*, 1986; Reeves *et al.*, 1976; Shah *et al.*, 1987; Shiozawa *et al.*, 1982; Shiozawa *et al.*, 1986; Tully *et al.*, 1990). Many of these appear to have been acute thrombotic events.

Information obtained from these case studies is, however, difficult to interpret. It is seldom possible to assess whether AAS induced premature atherosclerosis may have contributed to the aetiology of the event. An attempt was made to assess the extent of atherosclerosis in only seven of these cases; no evidence of atherosclerosis was found in four cases (Ferenchick and Adelman, 1992; Fisher *et al.*, 1996; Kennedy *et al.*, 1993a; Luke *et al.*, 1990), 'mild' atherosclerosis was reported in one (Dickerman *et al.*, 1995) and marked atherosclerosis was reported in two (Bowman *et al.*, 1989; Mewis *et al.*, 1996). Moreover, in cases where individuals may have premature atherosclerosis, it is seldom possible to account in full for other potential confounding factors, such as a genetic predisposition to cardiovascular disease, unhealthy lifestyle habits, or an underlying disease state which may predisposes to premature atherosclerosis.

The data obtained from case reports may be used to indicate that AAS might predispose particular individuals to premature atherosclerosis. The observation that adverse cardiovascular events appear to be precipitated by an acute thrombosis in many of the cases, indicates that particular attention should be paid to the proposal that AAS may be thrombogenic in some individuals (Ferenchick, 1990; Ferenchick, 1991; Ferenchick *et al.*, 1992). It is possible that the use of AAS together with other drugs and supplements may create a particularly thrombogenic setting in some weightlifters and bodybuilders (Welder and Melchert, 1993). It should be noted, however, that the data obtained from these case reports provides no indication of whether AAS users are more likely to suffer from premature atherosclerotic disease than non-users.

Does synthetic testosterone replacement in hypogonadal males increase risk of premature atherosclerosis?

It is unknown whether synthetic testosterone replacement will increase the incidence of CHD in hypogonadal males. An indication of potential risk for premature atherosclerosis is currently sought in the traditional lipoprotein risk factors. In a recent study by Zgliczynski *et al.* (1996), testosterone enanthate (200mg/2wk i.m.) was administered to eleven men (18-77yr.) with hypopituitarism and eleven otherwise healthy elderly men with low testosterone concentrations for one year. It was found that synthetic testosterone replacement therapy may have a beneficial effect on lipid metabolism in hypogonadal and elderly men, by decreasing LDLC concentration without significant alterations in HDLC concentration. These data are consistent with earlier studies on elderly hypogonadal men, in which a similar dose of synthetic testosterone was administered for a period of three months, resulting in a decrease in total cholesterol without a change in HDLC concentration (Morley *et al.*, 1993; Tenover, 1992).

Results from studies on young hypogonadal men have been equivocal. Two studies have examined young hypogonadal men, having either idiopathic hypogonadism or Klinefelters syndrome. In one study, LDLC concentration increased by 14% and HDLC concentration was unchanged, four weeks after a testosterone ester implant (Jones *et al.*, 1989). In the other study, LDLC concentration was increased by 6% and HDLC concentration by 13%, three weeks after a course of synthetic testosterone (Sustanon 250mg/wk) (Ozata *et al.*, 1996). One other study has examined young hypopituitary males. A four week treatment with testosterone enanthate

(100mg/2wk) resulted in non-significant decreases in both HDLC and LDLC concentration (Sorva *et al.*, 1988).

In summary, it remains unclear whether synthetic testosterone replacement therapy will increase risk for premature atherosclerosis in young hypogonadal men. Preliminary assessment of lipoprotein risk factors does not indicate an increased risk for premature atherosclerosis in elderly hypogonadal men receiving synthetic testosterone replacement therapy. Since cardiovascular disease is a major cause of morbidity and mortality in older men, further research will be required before an evaluation can be made on the practicality of synthetic testosterone replacement therapy on a widespread basis (Bagatell and Bremner, 1995).

Does the administration of 17β-esterified (parenteral) AAS increase risk for premature atherosclerosis?

There appears to be no direct evidence linking the use of parenteral AAS with premature atherosclerosis in humans. Accordingly, an indication of atherosclerotic risk associated with parenteral AAS use in humans, is currently sought in the traditional lipoprotein risk factors. The effects of testosterone enanthate (200mg/wk) on HDLC and LDLC concentration have been monitored in five studies for up to twelve months, (Anderson *et al.*, 1995; Bagatell *et al.*, 1994; Meriggiola *et al.*, 1995; Thompson *et al.*, 1989; Zmuda *et al.*, 1993). These studies reported HDLC concentration reductions of between 9-21%, with no change in LDLC concentration in four studies, and a significant decrease in LDLC concentration in the other (Thompson *et al.*, 1989). One other study has examined the effects of testosterone enanthate (280mg/wk) on HDLC and LDLC concentration, finding no significant changes in either after a twelve week course of treatment (Friedl *et al.*, 1990).

Two studies have examined the effects of testosterone cypionate on HDLC and/or LDLC concentration (Crist *et al.*, 1986; Kouri *et al.*, 1996). In one study testosterone cypionate (100mg/wk) was found to reduce HDLC concentration by 13% (Crist *et al.*, 1986), and in the other, testosterone cypionate (300mg/wk) was found to reduce HDLC concentration by 21%, with no further reductions after doubling the dose to 600mg/wk for an additional three weeks (Kouri *et al.*, 1996). Testosterone cypionate had no effect on LDLC concentration (Kouri *et al.*, 1996). An additional two studies have examined the effect of nandrolone decanoate

(100mg/wk) on HDLC and LDLC concentration (Glazer and Suchman, 1994; Crist *et al.*, 1986). In one study no effect was detected (Glazer and Suchman, 1994) and in the other a 16% reduction in HDLC concentration was reported (Crist *et al.*, 1986).

The effects of testosterone enanthate (200mg/wk) on Lp(a) concentration has been examined in three studies, all of which reported a significant reduction in the mean Lp(a) concentration, of between 22-37% (Anderson *et al.*, 1995; Marcovina *et al.*, 1996; Zmuda *et al.*, 1996). The Lp(a) response to synthetic testosterone treatment was found to vary widely among subjects and appears to be dependent on the pretreatment concentration, with no significant change being seen in those with a concentration less than 25 nmol/L, and a significant reduction being seen in those with a concentration greater than 25 nmol/L (Marcovina *et al.*, 1996).

A literature search revealed only one study which has examined the atherosclerotic effect of testosterone supplementation in male animals. In this study, excess synthetic testosterone was administered to one week-old male chicks (Toda *et al.*, 1984). A seven week treatment of 30mg synthetic testosterone per day had little effect on plasma lipid metabolism. However, this treatment resulted in the activation of fibroblasts in the comb, activation of fibroblast-like and smooth muscle cells in the aorta, and degeneration of smooth muscle cells in the aorta. In the same study, treatment of one week-old male chicks with 150mg of synthetic testosterone per day for seven weeks induced hyperlipidaemia and lipid-rich aortic lesions (Toda *et al.*, 1984). The high doses of testosterone administered, and the early developmental stage of these animals makes the relevance of this data to adult humans uncertain.

A literature search revealed only one study which has examined the atherosclerotic effect of parenteral AAS in female animals. In this study, nandrolone decanoate (25 mg/3 wk) was administered to female cynomolgus monkeys fed a moderately atherogenic diet for two years (Obasanjo *et al.*, 1996). It was found that the extent of coronary artery atherosclerosis was significantly greater in the group administered nandrolone decanoate compared to controls. Interestingly, the nandrolone decanoate group had significantly larger arteries and lumen area than the control monkeys. Longer treatment with nandrolone decanoate did however result in increased atherosclerotic plaque size and the possible benefit of increased lumen size was compromised (Obasanjo *et al.*, 1996).

In summary, the reduction of HDLC concentration by the parenteral AAS may indicate an increase in risk for premature atherosclerosis in humans. In contrast, the reduction in Lp(a) concentration may indicate a decrease in risk for premature atherosclerosis. It is unknown if these factors will counteract each other. Evidence to provide a direct link between the 17β-esterified (parenteral) AAS and premature atherosclerosis in humans is limited. One study in male animals indicates an atherogenic effect of supraphysiological doses of synthetic testosterone in one week-old chicks, and one study in female animals indicates an atherogenic effect of nandrolone decanoate in monkeys. Although it is uncertain whether this data can be extrapolated to humans, close attention needs to be paid to the possibility that the effects of the 17β-esterified (parenteral) AAS may differ in adolescents and women compared to men.

Does the therapeutic use of 17α-alkylated (oral) AAS increase risk for premature atherosclerosis?

Ten studies have examined the effect of AAS therapy on lipid and lipoprotein risk factors in humans, using 17α-alkylated (oral) AAS (Albers *et al.*, 1984; Applebaum-Bowden *et al.*, 1987; Cheung *et al.*, 1980; Crook *et al.*, 1992; Farish *et al.*, 1995; Friedl *et al.*, 1990; Haffner *et al.*, 1983; Olsson *et al.*, 1974; Taggart *et al.*, 1982; Thompson *et al.*, 1989). All of these studies reported significant reductions in HDLC concentration of between 35-50%, and significant elevations in LDLC concentration of between 10-30% (excluding Albers *et al.* who reported a non-significant increase in apoB concentration). The Lp(a) concentration was measured in three studies, all of which reported significant reductions of between 38-79% (Albers *et al.*, 1984; Crook *et al.*, 1992; Farish *et al.*, 1995).

There appears to be no direct evidence linking the use of 17α-alkylated (oral) AAS with premature atherosclerosis in humans. One animal study has examined the effect of stanozolol on HDLC concentration, LDLC concentration and atherosclerotic development in cholesterol fed rabbits (Fogelberg *et al.*, 1990). No significant influence of stanozolol on either atherosclerotic extent or on HDLC or LDLC concentration was found. The authors did, however, comment that the influence of stanozolol on the development of atherosclerosis could not be excluded, because of the finding that two out of ten stanozolol treated rabbits on normal diet developed macroscopic atherosclerosis compared to none out of the seventy-two rabbits of the same age from previous studies given the same normal diet.

In summary, the combination of a significant increase in LDLC concentration and significant decrease in HDLC concentration is strongly suggestive of an increase in risk for premature atherosclerosis in humans. It is uncertain how the reduction in Lp(a) concentration will modify this relationship. Evidence to provide a direct link between the therapeutic use of 17α-alkylated (oral) AAS and premature atherosclerosis in humans and animals is limited.

Does the non-therapeutic use of combined 17β-esterified (parenteral) and 17α-alkylated (oral) AAS increase risk for premature atherosclerosis?

Athletes and bodybuilders who use AAS, typically self-administer high doses of a combination of several different oral and parenteral agents (Lukas, 1993). The possible atherosclerotic effect of this practise has been the subject of intense interest. To date, twenty-two studies have examined the relationship between the (self-administered) non-therapeutic use of AAS and lipid and lipoprotein profiles (Alen and Rahkila, 1984; Alen *et al.*, 1985; Baldo-Enzi *et al.*, 1990; Baumstark *et al.*, 1988; Cohen *et al.*, 1986; Cohen *et al.*, 1988; Cohen *et al.*, 1996; Costill *et al.*, 1984; Faber *et al.*, 1986; Hurley *et al.*, 1984; Kantor *et al.*, 1985; Kiraly, 1988; Kleiner *et al.*, 1989; Kuipers *et al.*, 1991; Lajarin *et al.*, 1996; Lenders *et al.*, 1988; McKillop and Ballantyne, 1987; Moffat *et al.*, 1990; Peterson and Fahey, 1984; Webb *et al.*, 1984; Yeater *et al.*, 1996; Zuliani *et al.*, 1988). All of these studies report that this pattern of AAS use is associated with a marked reduction in HDLC concentration (except for Cohen *et al.* (1988) who did not report HDLC concentration). Studies which have compared HDLC concentration before and after mixed non-therapeutic steroid use, report HDLC concentration reductions of between 50-70% (Alen and Rahkila, 1984; Alen *et al.*, 1985; Baldo-Enzi *et al.*, 1990; Faber *et al.*, 1986; Hurley *et al.*, 1984; Kleiner *et al.*, 1989; Kuipers *et al.*, 1991; Lajarin *et al.*, 1996; Lenders *et al.*, 1988; Peterson and Fahey, 1984; Webb *et al.*, 1984; Zuliani *et al.*, 1988). Studies which have compared LDLC concentration before and after mixed non-therapeutic steroid use, report LDLC concentration increases of between 25-35% (Baldo-Enzi *et al.*, 1990; Faber *et al.*, 1986; Hurley *et al.*, 1984; Kleiner *et al.*, 1989; Lajarin *et al.*, 1996; Lenders *et al.*, 1988; Webb *et al.*, 1984).

Two studies have examined the effects of mixed non-therapeutic AAS use on Lp(a), both of which reported a suppression of Lp(a) concentration (Cohen *et al.*, 1996; Lajarin *et al.*, 1996). One study found that Lp(a) was undetectable in their subjects four weeks after commencing

steroid use (Lajarin *et al.*, 1996). There appear to be no controlled studies to provide a direct link between the non-therapeutic use of AAS with an increased incidence of premature atherosclerosis in athletes or bodybuilders. Moreover, there appear to be no animal studies in which the pattern of steroid use typically seen in athletes and bodybuilders is simulated.

In summary, (self-administered) non-therapeutic use of AAS results in marked reductions in HDLC and Lp(a) concentration, and elevations in LDLC concentration. Reduction of HDLC concentration tends to be slightly greater (approximately 10%) than the reductions seen in controlled studies. The combination of a significant increase in LDLC concentration and significant decrease in HDLC concentration is a strong indicator of an increase in risk for premature atherosclerosis. However, the lack of prospective or retrospective data to establish a link between AAS and premature atherosclerosis makes the significance of an AAS induced lipoprotein profile change in otherwise healthy individuals uncertain.

It has been estimated that if the contributions to CHD risk of HDLC concentration depression and LDLC concentration elevation are considered together, CHD risk is increased about 3-6 times in athletes and bodybuilders who use AAS (Glazer, 1991). Given the apparent increase in atherosclerotic risk, one would expect to have seen a significant increase in atherosclerotic complications in AAS users (Wilson, 1988). It is perhaps surprising, that in spite of there having been more than one million AAS users in the United States alone (Yesalis *et al.*, 1993), and that AAS were first used by athletes as early as the 1950's (Strauss and Yesalis, 1991), little direct evidence has been forthcoming to support the hypothesis that AAS use will cause premature atherosclerosis. In mitigation, the relative paucity of reports linking AAS with atherosclerotic complications may be accounted for by a number of factors: (i) individuals may be reluctant to admit AAS use, since non-therapeutic use of these agents is illegal in many countries (including South Africa), (ii) the identification of AAS as a causative factor in cardiovascular events is difficult, (iii) it is possible that a lag phase may be required for the true prevalence of vascular complications to become evident (Wilson, 1988), and (iv) ethical and funding constraints have impeded efforts to conduct definitive studies on the role played by AAS in atherosclerosis (Cowart, 1989). Nevertheless, it has also been argued that the intensity of medical interest in reporting assumed cases is likely to compensate for the reluctance of AAS users to admit use

(Rockhold, 1993). Moreover, the possibility cannot at present be excluded that the adverse cardiovascular effects of AAS may have been overstated (Street *et al.*, 1996).

Mechanism of anabolic-androgenic hormone induced lipoprotein concentration changes

The higher endogenous testosterone concentration in men compared to women induces a relatively increased hepatic triglyceride lipase activity (HTGLa) (Applebaum-Bowden, 1984). HTGL is responsible for the catabolism of HDL and for conversion of lipoprotein particles from the HDL_2 density fraction to the HDL_3 fraction (Eisenberg, 1984). Increased lipolytic degradation due to an increased HTGLa is believed to be the principal cause of the reduced HDLC and particularly HDL_2C concentration in men compared to women (Applebaum *et al.*, 1987; Baldo-Enzi *et al.*, 1990; Crook *et al.*, 1992; Haffner *et al.*, 1983; Kantor *et al.*, 1985). Endogenous testosterone appears to have relatively little effect on plasma LDLC, VLDLC or on fasting triglyceride concentrations (Asscheman *et al.*, 1994; Bagatell and Bremner, 1995). The sex difference in LDLC concentration is more likely to be due to the higher oestrogen concentration in women, than to a high testosterone concentration in men. Oestrogen appears to lower LDLC concentration by accelerating the rate of LDL catabolism, possibly by increasing LDL receptor messenger RNA transcription and thereby increasing the number of hepatic LDL receptors (Sacks *et al.*, 1995).

The cause of the disparity in the magnitude of HDLC concentration depression between the parenteral and the oral AAS remains unclear. It is likely that differences in their routes of administration and their metabolic pathways contribute to the observed difference. Orally administered agents are absorbed into the portal circulation but are somewhat resistant to hepatic metabolism, resulting in high hepatic AAS concentrations shortly after ingestion (Bagatell and Bremner, 1995). HTGL, is found in highest concentration in the liver, and it is likely that high local concentrations of AAS stimulate its activity (Bagatell and Bremner, 1995). Indeed, significant elevations in HTGLa are consistently reported in studies in which oral AAS are administered, whereas elevations in HTGLa are relatively small with parenteral AAS use (Bagatell and Bremner, 1995; Glazer and Suchman, 1994). The significant increase in HTGLa caused by the 17α-alkylated AAS is believed to increase LDLC concentration as the final product of an accelerated VLDL catabolism (Baldo-Enzi *et al.*, 1990). Modification of apoB synthesis,

and a decreased lecithin cholesterol acyl transferase (LCAT) activity, could also be partially responsible for the increase in LDLC concentration in users of AAS (Albers *et al.*, 1984; Crook *et al.*, 1992).

The aromatisation of AAS to oestrogens also accounts in part for the lesser suppression of HDLC concentration by the 17β-esterified AAS compared to the 17α-alkylated AAS (Bagatell and Bremner, 1995). Testosterone esters readily aromatise to 17β-oestradiol while the 17α-alkylated AAS do not form potent oestrogens (Friedl *et al.*, 1990). Since oestrogens decrease HTGLa and AAS increase HTGLa, each producing inverse changes in HDLC concentration, aromatisation of synthetic testosterone to oestradiol may mitigate an anabolic-androgenic effect. Blocking synthetic testosterone aromatisation augments the elevation in HTGLa and the reductions in HDL_2C and apoA-I concentrations (Thompson *et al.*, 1989). The net effect of AAS administration on HDLC concentration may thus be related to the metabolic fate of that particular AAS, particularly in the degree of aromatisation to oestrogens (Friedl *et al.*, 1990).

Anabolic-androgenic hormones may also reduce HDLC concentration by reducing HDL synthesis, and by increasing removal by receptors (Baldo-Enzi *et al.*, 1990). AAS use reduces HDLC concentration by affecting apolipoprotein metabolism, causing a significant decrease in apoA-I concentration in the HDL_2 subfraction and a significant decrease in both apoA-I and apoA-II concentration in the HDL_3 subfraction (Baldo-Enzi *et al.*, 1990). Treatment with the oral AAS, stanozolol, is associated with a decrease in residence times for apoA-I and apoA-II, as well as a marked decrease in the rate of apoA-I synthesis but essentially no change in the rate of apoA-II synthesis (Haffner *et al.*, 1983). The low apoA-I concentration could explain the reduced percentage of cholesterol esterification observed in steroid users, because this apolipoprotein acts as a cofactor of LCAT (Baldo-Enzi *et al.*, 1990). LCAT concentration also decreases markedly during treatment with stanozolol (Albers *et al.*, 1984). A significant decrease in both LCAT substrate particles and LCAT could potentially retard reverse cholesterol transport.

HDLC concentrations fall to their nadir within one week after initiating AAS use and tend to return to pretreatment concentrations 3-5 weeks after cessation of AAS use with no significant residual HDLC concentration depression (Glazer, 1991). In particularly heavy users, the HDLC concentration may not reach the baseline value until 12 to 14 weeks after cessation

(Alen *et al.*, 1985; Lajarin *et al.*, 1996). There is a lack of dose relationship in AAS induced HDLC concentration depression, which may be explained by a similar increase in HTGLa, regardless of whether AAS dose is therapeutic or supra-therapeutic. Similar hepatic triglyceride lipase (HTGL) induction by the various AAS doses may reflect a saturation of receptors mediating AAS induced HTGL increase that occurs at or below therapeutic AAS dosages (Glazer, 1991).

Exogenous anabolic-androgenic hormones have a profound suppressive effect on Lp(a) concentration (Crook *et al.*, 1992; Farish *et al.*, 1995). The mechanisms for Lp(a) synthesis and clearance have, however, not been clarified (Berglund *et al.*, 1996). AAS may affect Lp(a) concentration by reducing apo(a) synthesis or secretion by impairing Lp(a) assembly, or by affecting Lp(a) catabolism (Crook *et al.*, 1992). Anabolic steroids modify the action of various enzyme systems, including hepatic hydrolytic enzymes, which could lead to an effect on synthesis or processing of Lp(a) (Crook *et al.*, 1992). Because apoB usually does not decrease with anabolic-androgenic hormone treatment, it is likely that Lp(a) is under separate metabolic control from VLDL and LDL, and furthermore Lp(a) is probably not a catabolic product of VLDL (Albers *et al.*, 1984).

Conclusion of literature review

It has been hypothesised that testosterone may contribute to the sex difference in the prevalence of premature CHD in humans. Evidence reviewed in this chapter suggests that male pattern testosteronaemia may be atherogenic in females. However, there appears to be insufficient evidence to conclude that endogenous testosterone is atherogenic in males. Additional animal studies, particularly in hypogonadal compared to eugonadal male animals, will help to clarify whether endogenous testosterone is atherogenic. Although equivalent human studies are limited by ethical and practical constraints, the recent development of drugs which allow a reversible suppression of endogenous testosterone may provide new opportunities to investigate the effect of endogenous testosterone on atherosclerotic risk factors in human males.

Anecdotal accounts indicate a widespread belief that AAS users will suffer from premature atherosclerotic disease. The recent publication of a number of case reports showing adverse

cardiovascular events associated with AAS use, may be viewed as supportive of this belief. However, it is seldom possible to conclude from these reports whether AAS induced premature atherosclerotic disease caused the adverse cardiovascular events. Studies seeking inferential evidence for an atherosclerotic effect of AAS, indicate that 17β-esterified (parenteral) AAS may have fewer potentially atherogenic effects than 17α-alkylated (oral) AAS. There appear to be few animal studies which have investigated the atherosclerotic effects of AAS. Data obtained in female animals suggests that close attention needs to be paid to the possibility that the adverse effects of AAS may differ in males compared to females. On the whole, adverse cardiovascular events associated with AAS use in humans appear to be rare. Moreover, there appears to be insufficient evidence to prove the hypothesis that AAS users have a greater prevalence of premature atherosclerotic disease compared to matched non-users. Until such time as data becomes available to allow a comparison of atherosclerotic progression in AAS users compared to non-users, additional studies into the effect of AAS on risk factors for premature atherosclerosis will allow a better prediction to be made about the potential adverse cardiovascular events associated with the use of these agents.

There is presently very little information to show whether endogenous testosterone and AAS may influence the lipid and lipoprotein components of the 'atherogenic dyslipidaemia' phenotype. The effect of AAS on plasma triglyceride clearance has been investigated in only two studies (Olsson *et al.*, 1974; Thompson *et al.*, 1989), neither of which discussed their data in relation to 'atherogenic dyslipidaemia'. It is also unknown how AAS influence LDL particle size. There is a paucity of information about the effect of endogenous testosterone suppression in healthy men, on postprandial triglyceridaemia and LDL size. Moreover, it is unknown whether the increased HDLC concentration associated with a reduced testosterone concentration is related, at least in part, to the magnitude of postprandial triglyceridaemia. There appears to be only one study which has investigated the effect of endogenous testosterone suppression on Lp(a) concentration (von Eckardstein *et al.*, 1997). Accordingly, the studies described in the subsequent experimental chapters were undertaken to clarify these issues.

Chapter 2: The theoretical basis for the experimental chapters

The underlying cause of 'atherogenic dyslipidaemia' appears to be an overproduction and/or an impaired clearance of triglyceride rich lipoproteins (TGRL). The following chapter describes the metabolism of TGRL, the potential atherogenic effects of these lipoproteins, and their potential to influence the atherogenicity of LDL and HDL. Because the exact relationship between HDL and atherosclerosis remains unclear, this chapter also provides a brief review of the present understanding of the role played by HDL in atherosclerosis.

Digestion and absorption of dietary triglyceride

Enzymatic hydrolysis of triglycerides begins in the stomach, where as much as 30% of the dietary triglyceride may be digested (Carey *et al.*, 1983). The stomach chyme is propelled into the intestine, where bile and pancreatic secretions (lipase and colipase) mix. Triglyceride is hydrolysed into a 2-monoglyceride and two free fatty acids, which cross the microvillus membrane into the enterocyte by passive diffusion (Guyton, 1986). Normally, over 90% of triglyceride is absorbed, which means that, in an average adult, roughly 80-170 mmol (70-150g) of exogenous triglyceride enters the circulation per day. Short chain fatty acids can at this point pass through the cell and be accepted onto albumin which will carry them to the liver by the portal circulation (Guyton, 1986). The long chain fatty acids and to some extent also short chain fatty acids that have been elongated, are re-esterified to triglyceride in the smooth endoplasmic reticulum of the enterocyte (Guyton, 1986). The re-esterified triglyceride forms oil droplets within the smooth endoplasmic reticulum which are associated with phospholipid, cholesterol and cholesterol ester. Some apolipoproteins, notably apoB_{48}, apoA-I, and apoA-IV, are added, and the vesicles are secreted through the basolateral aspect of the enterocytes into lacteals (Thompson, 1994). These particles, which are now assembled lipoproteins, are termed chylomicrons, and act as the transporters of most of the dietary triglycerides. The lacteals converge and ultimately drain into the cysterna chyli just below the diaphragm. From here the thoracic duct takes the chyle upwards until it joins the left brachiocephalic vein, where it mixes with the systemic venous blood (Guyton, 1986).

Chylomicron and chylomicron remnant metabolism

Once in the general circulation, these very large (>100 nm in diameter), triglyceride enriched lipoproteins undergo several modifications associated with triglyceride hydrolysis and are ultimately converted to chylomicron remnants, which are then cleared from the blood primarily by the liver (Mahley and Hussain, 1991). There are three primary intra-vascular modifications: (i) The chylomicrons acquire C apolipoproteins from other circulating lipoproteins. ApoC-II facilitates binding of chylomicrons to LPL, which is found mainly in adipose tissue and skeletal and cardiac muscle, where it is attached to the luminal surface of the capillary endothelium. LPL catalyses the hydrolysis of triglyceride, while the particles are bound to the capillary endothelium, resulting in the liberation of free fatty acids, which are taken up, modified and stored as triglycerides in adipose tissue, or are used as an energy source in muscle and other tissues (Olivecrona and Bengtsson-Olivecrona 1990; Olivecrona and Bengtsson-Olivecrona 1990a). The rate of removal of chylomicrons from plasma is rapid, with a half-life of less than one hour (Thompson 1994). (ii) The particles lose lipids and proteins from their surface as a result of a reduced volume caused by the loss of the triglyceride core. It is believed that sheets of phospholipid and apolipoproteins (especially apoA-I) are shed from the partially hydrolysed chylomicrons. These sheets give rise to HDL particles and represent one source of intra vascular HDL formation (Tall, 1996). Residual triglyceride in the chylomicron remnants is hydrolysed by HTGL located on the luminal surface of hepatic endothelial cells (Mahley and Hussain, 1991). Although less active than LPL, HTGL is unaffected by the inhibitors and activators of the of the extrahepatic enzyme. (iii) The hydrolysed chylomicrons (chylomicron remnants) acquire apoE from other plasma lipoproteins. This modification targets the remnants primarily to the liver, where they are catabolized (Mahley and Hussain, 1991). The $apoB_{48}$ associated with chylomicron lacks the receptor-binding domain present in $apoB_{100}$ thus, the apoE on chylomicron remnants appears to be the critical protein determining their plasma clearance and hepatic uptake, binding to LDL receptor and the related receptor, LDL receptor related protein.

VLDL metabolism

Although similar to chylomicrons in structure and composition, VLDL are smaller (40-100 nm), contain less triglyceride and more cholesterol, phospholipid and protein, primarily apoC, apoE and $apoB_{100}$ (Thompson, 1994). VLDL particles show a considerable variation in size, smaller particles having a lower ratio of apoC:apoB than larger ones (Thompson, 1994).

VLDL are mainly synthesized in the liver and their chief function is the transport of endogenously synthesised triglyceride (Thompson, 1994). VLDL synthesis is promoted by (i) an increase in the flux of free fatty acid to the liver, originating from the hydrolysis of exogenous lipid, (ii) an increase in the rate of hepatic synthesis of endogenous fatty acids, as occurs during a period of high carbohydrate intake (Thompson, 1994), and (iii) when supplied by incoming fat in the form of chylomicron remnants (Bradley and Gianturco, 1994).

Triglyceride hydrolysis of VLDL is mediated by apoC-II interaction with LPL, which reduces the size of these particles, and they are then referred to as VLDL remnants or intermediate density lipoprotein (IDL) (Thompson, 1994). The ability of LPL to effect removal of triglyceride is potentially saturable and excessive accumulation of VLDL in plasma can impair chylomicron metabolism (Karpe *et al.*, 1993). The function of HTGL in VLDL clearance is less well established, but it is probably involved in the hydrolysis of most of the residual triglyceride in the VLDL remnant particles, resulting in the formation of LDL. There is evidence that a greater proportion of small VLDL particles (S_f20-60) are converted into LDL via IDL as compared with large VLDL (S_f60-400), which are converted to a form of IDL which is removed from plasma before undergoing conversion to LDL (Packard *et al.*, 1984). The rate of VLDL clearance in humans is less rapid than that of chylomicrons, with a half life of 2 - 4 hours. LDL particles have a much longer half life. The catabolism of VLDL into IDL and then into LDL thus leads to the transport of cholesterol by the LDL receptor pathway (Thompson, 1994). Any cell with a cholesterol requirement can upregulate the LDL receptor to increase cholesterol uptake.

Postprandial triglyceridaemia

Postprandial triglyceridaemia comprises the (non-steady) state of plasma lipoproteins between fat ingestion and the postabsorptive state, until triglyceride metabolism has again reached a state of equilibrium. The time taken between swallowing and digestion is a minimum of about 25 minutes. The rise of plasma triglyceride concentration occurs about 1 hour after ingestion of a fatty meal, peaks between 3 and 6 hours later, and usually reaches baseline by 8-12 hours. This means that the systemic circulation is exposed to absorbed fat, in a pulsed fashion after each meal. Both chylomicron and VLDL contribute to postprandial triglyceridaemia (Cohn *et al.*, 1989), and are collectively known as the triglyceride rich lipoproteins (TGRL). Increase in plasma triglyceride concentration in healthy human subjects usually peaks once or

twice in the twelve-hour period following the ingestion of a fat-rich meal, and intestinal lipoproteins make a significant contribution to both earlier and later peaks (Cohn *et al.*, 1989; Karpe *et al.*, 1993).

The magnitude of postprandial triglyceridaemia is defined as the extent and duration of the rise of plasma triglycerides in response to a standardised fatty meal. While a number of factors may influence the magnitude of postprandial triglyceridaemia (in the absence of gastrointestinal disease, variations in fat digestion and absorption can be ignored), the efficiency of triglyceride clearance by LPL and HTGL is clearly the most important (Ebenbichler *et al.*, 1995). A deficiency in triglyceride clearance leads to the accumulation of TGRL in plasma and an elevated postprandial triglyceridaemia. It important to emphasise that an exaggerated postprandial triglyceridaemia is not necessarily associated with an elevated fasting triglyceride concentration or hypertriglyceridaemia (Patsch *et al.*, 1983). An exaggerated postprandial triglyceridaemia could however be viewed as a transient hypertriglyceridaemia (Karpe *et al.*, 1994).

The role of TGRL in the development of atherosclerosis

Until recently, the close metabolic relationship between triglyceride, HDL and LDL subfractions has been poorly understood, because lipid and lipoprotein analysis has traditionally been confined to the post-absorptive (overnight fasting) state. Studies into postprandial lipid metabolism were largely neglected, which is surprising, since we spend most of our lives in the postprandial state. The fact that epidemiological studies did not include determinations of postprandial lipoproteins, may have contributed to the persisting confusion concerning the role of triglyceride as a risk factor for CHD, and has led historically to a downgrading of plasma triglyceride as a coronary risk factor in multivariate analyses (Karpe *et al.*, 1994). In the past decade, however, studies on lipoproteins in the postprandial state have provided compelling evidence that TGRL play a central role in the pathogenesis of atherosclerosis (Groot *et al.*, 1991; Uiterwaal *et al.*, 1994), exerting their effect both directly on the arterial wall, and indirectly by modifying other lipoprotein classes in an atherogenic fashion (Ryu *et al.*, 1992; Zilversmit, 1979).

Under normal metabolic conditions the liver favours the production of smaller VLDL (S_f<60), which appear to be the precursors of LDL (approximately 70% of VLDL are

S_f 20-60 in normal subjects) (Karpe *et al.*, 1993a). The impaired peripheral clearance of triglyceride leads to the return of remnants to the liver, which promotes the formation of larger VLDL's (S_f 60-400), enriched in cholesterol and cholesterol ester. These are resistant to lipase activity and can further impede the undisturbed passage of other TGRL through the lipolytic cascade. Potentially atherogenic cholesterol-enriched chylomicron remnants and IDL, may accumulate. Larger VLDL may also be removed directly from plasma, and not lipolysed to form LDL (Packard *et al.*, 1984). It has been hypothesised that large VLDL and some dietary TGRL may have an abnormal ability to interact with arterial cells through rapid receptor mediated processes. Thus only a portion of the VLDL apoB enters the LDL pool, unlike in normal subjects, where most VLDL apoB is converted into LDL apoB. Although it was traditionally believed that TGRL could not participate in endothelial cholesterol deposition, because their large size precluded entry into the arterial wall, it has recently been confirmed that TGRL are indeed present in atherosclerotic lesions, and can thus contribute to the initiation and progression of atherosclerosis (Rapp *et al.*, 1994). A large VLDL carries five times or more total cholesterol per lipoprotein particle than LDL, and by this criterion, has a greater potential for the deleterious delivery of cholesterol (Bradley and Gianturco, 1994).

Virtually all major lipoprotein classes, including VLDL, IDL, LDL and HDL, are influenced by postprandial triglyceridaemia, and its magnitude and duration determines how much these lipoproteins are affected. These effects are mediated by cholesterol ester transfer protein (CETP), which catalyses the transfer of cholesterol esters from HDL and LDL to chylomicrons, VLDL and IDL and the reciprocal transfer of triglycerides. During postprandial triglyceridaemia, the action of CETP increases remnant-cholesterol, VLDLC and IDLC concentrations and decreases the HDLC concentration. Triglycerides transferred to LDL and HDL are susceptible to lipase-mediated hydrolysis, so that the size of these lipoproteins is reduced in impaired triglyceride tolerance. Frequently occurring elevations of TGRL species could provide favourable conditions for the sequential actions of lipid transfer proteins and HTGL which lead to accumulation of dense, lipid-depleted LDL particles. A predominance of small, dense low LDL in plasma is thought to be particularly atherogenic, and has been associated with up to a three-fold increase in risk of acute myocardial infarction (Austin *et al.*, 1988), and the presence of coronary artery disease (Campos *et al.*, 1992). Another reason for the increased atherogenicity of postprandial

LDL appears to be a greater susceptibility to chemical modification such as oxidation (Chait *et al.*, 1993; Lechleitner *et al.*, 1994).

HDL metabolism

The plasma HDL are small lipoproteins consisting of about 50% protein and 50% lipid. The major lipids are phospholipids, cholesterol, and cholesterol esters. The major apolipoproteins are apoA-I and apoA-II. The HDL also contains proteins of central importance in lipoprotein metabolism, namely, the enzyme LCAT and the plasma lipid transfer proteins, CETP and phospholipid transfer protein (PLTP) (Tall, 1996). ApoA-I is synthesised in approximately equal proportions in the liver and small intestine, whereas apoA-II is made exclusively in the liver (Eisenberg, 1984; Tall, 1996). ApoA-I forms three types of stable structure with lipids: small lipid-poor complexes (prebeta-1 HDL); flattened discoidal particles (prebeta-2 HDL) containing only polar lipids (phospholipid and cholesterol); and spheroidal particles (HDL) containing both polar and nonpolar lipids which form the bulk of the HDL (Fielding and Fielding, 1995). ApoA-I is present in at least one copy (and usually two to four copies) in each HDL particle of normal plasma (Fielding and Fielding, 1995). HDL can be formed as a direct result of cellular secretion, or is derived from surface remnants shed during the catabolism of TGRL. A major proportion of the apolipoprotein and phospholipid destined to become HDL is initially secreted on VLDL and chylomicrons.

During lipolysis of chylomicrons and VLDL, surface lipids (phospholipid and cholesterol) and proteins (apoA-I, apoA-II, and apoC's) are transferred into the HDL fraction. These components may form nascent discoidal particles, which are rapidly acted on by LCAT to generate mature spherical HDL_3, or they may be incorporated into preexisting HDL particles. LCAT modifies HDL particles by transforming the cholesterol into cholesterol ester. This neutral lipid accumulates in the HDL core, making the HDL particle spherical. The depletion of surface cholesterol by LCAT causes the development of a chemical gradient which favours the continual transfer of excess cholesterol from cellular membranes to HDL. This constant flow of free cholesterol, succeeded by esterification and movement of the ester into the core, together with surface remnants from TGRL, expands the HDL_3 particle and shifts the density range to the less dense HDL_2 particle. As this HDL_2 particle increases in size, two other enzymatic reactions

occur. Firstly, CETP facilitates the transfer of the cholesterol ester to other lipoproteins (chylomicron and VLDL remnants) in exchange for triglyceride. This allows the movement of cholesterol ester originating from HDL to the liver by chylomicron and VLDL remnants, where they are catabolised and the cholesterol is excreted into the small intestine as bile. Secondly, the HDL_2, now enriched in triglyceride, provides a good substrate for HTGL, which converts the particle back to the HDL_3 size range. Thus the small dense HDL_3 can originate by LPL action; be modified by LCAT, resulting in HDL_2 formation; undergo further processing mediated by CETP and HTGL; and be transformed into HDL_3 in the liver and released back into the circulatory system to begin the process once again (Eisenberg, 1984; Tall, 1996).

Recently it has been shown that HDL subclasses may be further subdivided according to a functional classification, into HDL containing apoA-I only (LpA-I) and HDL containing both apoA-I and apoA-II (LpA-I/LpA-II) (Leroy *et al.*, 1993). A number of studies indicate that LpA-I and LpA-I/LpA-II are metabolically distinct and may perform different functions. ApoA-I injected as part of LpA-I particles is catabolised at a higher rate than apoA-I injected as part of LpA-I/LpA-II particles (Mowri *et al.*, 1994). Particles containing only apoA-I may be the physiologic acceptor of cellular cholesterol, and HDL_2 containing both apoA-I and apoA-II constitute a better substrate for HTGL than HDL_2 containing apoA-I only.

HDLC concentration is known to be closely related to a number of lifestyle factors (Heiss *et al.*, 1980). Cigarette smoking is associated with substantially lower HDLC concentrations (Criqui *et al.*, 1980). There is a strong positive gradient of HDLC concentration with reported alcohol intake in both men and women (Ernst *et al.*, 1980). Body mass is significantly and inversely associated with plasma HDLC concentration and positively associated with plasma triglyceride concentration (Glueck *et al.*, 1980). Correlation of HDLC concentration with obesity and physical activity appear to be affected primarily through their associations with triglycerides, and alcohol use and smoking through associations with apoA-I (Patsch *et al.*, 1992).

Many epidemiological studies have demonstrated that the risk for CHD is inversely correlated with plasma HDLC concentration (Gordon *et al.*, 1989; Miller and Miller, 1975). This has created the widespread impression that the association between HDL and CHD is the opposite to that existing between LDL and CHD, in which a high concentration of LDL directly promotes

CHD, and therapeutic lowering reduces the risk for CHD. The mechanisms underlying the relationship between HDL and CHD do however, appear to be far more complex, and is still unclear whether therapeutic intervention to raise HDLC concentration will decrease CHD risk (Vega and Grundy, 1996). There are several different theories that have attempted to explain the relationship between HDL and atherogenesis. These may be divided into theories that postulate a direct antiatherogenic action of HDL versus those that say that HDL is merely a marker for some other event, such as a defect in the metabolism of triglyceride rich lipoproteins (Tall, 1996).

Low HDL as a direct cause of atherosclerosis

There is strong *in vitro* evidence that HDL provides direct protection against the development of atherosclerosis, through a process termed 'reverse cholesterol transport' (Fielding and Fielding, 1995). In brief, HDL removes excess cholesterol from peripheral cells by esterification, and either transfers the esterified cholesterol to TGRL triglyceride-rich lipoproteins, which are subsequently removed for catabolism in the liver by hepatic receptors, or itself delivers cholesterol directly to the liver. Accordingly, a low HDL concentration would be directly atherogenic by not counteracting the deposition of cholesterol at sites where an excessive cholesterol load could produce atherosclerosis. Despite the appeal of this theory, it has been difficult to gain supporting evidence *in vivo* (Tall, 1996). A direct link between HDL and CHD has been sought in genetic disorders of humans and animals which cause either very high or very low HDL concentrations. Three main genetic disorders have been investigated, those associated with abnormal apoA-I and apoA-II concentrations, abnormal CETP concentration, and abnormal HTGLa and LPLa.

Humans having genetic mutations resulting in complete deficiency of apoA-I have been described (Lackner *et al.*, 1993; Matsunaga *et al.*, 1991; Ng *et al.*, 1994). While some individuals suffer from premature atherosclerosis, other family members with the same genotype appear to be healthy, suggesting that the rate of disease development may be modified by other factors such as elevated LDLC concentrations (Tall, 1996). There are other gene mutations which result in partial deficiency of apoA-I. Carriers of the apoA-I_{Milano} mutation, are characterised by an average 40% decrease in apoA-I and apoA-II concentrations, and a 67% decrease in HDLC concentration (Franceschini *et al.*, 1980). Despite the low HDLC concentrations, subjects with the apoA-I_{Milano} mutation do not have increased coronary artery disease; in fact, the opposite is suspected, with

longevity noted in some family members. Complete or partial LCAT deficiency is another genetic mutation showing no apparent increased risk of premature atherosclerotic disease, despite conferring markedly low HDLC concentrations, and a partial deficiency of apoA-I, and apoA-II (Norum *et al.*). An overexpression of the human apoA-I gene in transgenic mice does appear to reduce the progression of atherosclerosis when these animals are challenged with a hypercholesterolaemic diet (Rubin *et al.*, 1993), whereas induction of high concentrations of apoA-II appears to mitigate or reverse the apparent protective effect of apoA-I (Warden *et al.*, 1993). Surprisingly, mice which lack the apoA-I gene fail to show increased aortic lesions even when fed an atherogenic diet (Li *et al.*, 1993).

A genetic deficiency of CETP results in a marked increase in HDLC concentration, resulting from defective transfer of cholesterol ester from HDL to TGRL. It might be anticipated that, if high HDLC concentration directly protect against CHD, CETP-deficient patients would not manifest CHD. To date, however no consistent evidence from human and animal studies has been produced to support this hypothesis. Rather, limited data in humans suggest that changes in HDLC concentrations based on differences in plasma CETP may not be closely linked to atherosclerosis (Vega and Grundy, 1996). Interestingly, individuals with abnormally elevated HDL concentration due to a combined reduction of CETP and HTGL may be susceptible to CHD (Hirano *et al.*, 1995).

The association between abnormal HTGLa and LPLa and reduced HDLC and HDL_2C concentration are well known. High LPLa apparently raises HDLC concentration by lowering triglyceride concentration and by promoting transfer of cholesterol from TGRL to HDL. HTGL has the opposite effect on HDL; a high activity lowers HDLC concentration by hydrolysing triglycerides and phospholipid in HDL particles. Of the two lipases, HTGL appears to be more tightly linked to HDLC concentration than LPL, and higher HTGL activities in men appear to be largely responsible for the sex related differences in HDLC concentration (Blades *et al.*, 1993). There is some evidence that elevated HTGL might give rise to an atherogenic form of low HDLC concentration, as increases in HTGL activities frequently occur in patients having both low HDLC concentrations and premature CHD (Vega and Grundy, 1996). Two possibilities may explain the apparent association between high HTGLa, low HDLC concentration and premature atherosclerosis: (i) the high HTGL may be part of a more generalised metabolic disorder which

promotes atherosclerosis in other ways, and (ii) a change in the HTGL gene may cause a high HTGLa, indeed it has been shown that genetic factors may account for as much as 50% of the variation of HDLC concentration in the general population, and allelic variation in the HTGL gene alone could explain at least half of the genetic contribution (Cohen *et al.*, 1994). In contrast, the absence of HTGL confers a phenotype, with high IDL and high and abnormal HDL concentrations, which may be atherogenic (Connelly *et al.*, 1990).

Other genetic abnormalities provide some additional insight. A marked reduction of HDLC concentration occurs in individuals having apoA-I gene cluster mutations, which results in a complete deficiency of apoA-I and apoC-III; this rare disorder is accompanied by premature atherosclerosis (Norum *et al.*, 1982). Although the near absence of HDL in this condition might directly promote development of atherosclerosis, the concurrent deficiency of apoC-III could also induce an atherogenic change in VLDL metabolism, which makes it difficult to conclude that a low HDLC concentration *per se* is the cause of enhanced atherogenesis (Vega and Grundy, 1996). Tangier disease is an anomaly characterised by extremely low plasma HDLC concentrations, caused by an excessive catabolism of apoA-I (Schmitz *et al.*, 1985). Despite a high cholesterol/HDLC ratio, these patients do not have a high incidence of atherosclerosis. The hepatic secretion of nascent HDL may be normal in these patients, which could explain why the majority of Tangier patients do not appear to accumulate cholesterol esters in their vessels (Vega and Grundy, 1996).

Although evidence from studies on humans and animals with abnormal HDLC concentrations due to genetic mutations is confusing, studies in animal models and in humans, show that regression of atherosclerosis occurs in settings where LDLC concentration is lowered and HDLC concentration is raised, which suggests that it is likely that regression involves removal of cholesterol from lesions by HDL (Tall, 1996). Together with *in vitro* evidence, it can be concluded that the balance of evidence indicates that it is likely that HDL is directly involved in a process of reverse cholesterol transport from atheromata into plasma and back into the liver (Tall, 1996). What is less clear is the extent to which the 'protective' effect of HDL noted in human epidemiological studies is mediated by changes in the efficiency of the reverse cholesterol transport system. For example, it has never been shown that variations in HDLC concentration similar to those in plasma, modulate cholesterol removal in the arterial wall (Tall, 1996). While

there may be a correlation between HDLC concentration and the rate of reverse cholesterol transport, it is not possible to derive a flux from a concentration without knowing the rate of entry of cholesterol from atheromata into the plasma cholesterol ester pool, and the rate of clearance via the liver.

Low HDL as a marker of other atherogenic lipoproteins

Zilversmit (1979) originally proposed that some chylomicrons bearing dietary cholesterol would be hydrolysed on large blood vessels and subsequently enter the arterial wall and promote atheroma formation. The atherogenic remnant hypothesis was subsequently modified to say that low HDLC concentrations might be a marker for the accumulation of chylomicron or VLDL remnants in plasma (Patsch *et al.*, 1983). TGRL may affect HDL_2 concentration in two ways: (i) Rapid lipolysis of TGRL by the enzyme LPL generates excess surface material, predominantly phospholipid, which is assimilated by HDL. This uptake of lipid promotes the formation of the large HDL_2 and thereby raises the cholesterol carrying capacity of HDL. The assimilation of lipolytic surface remnants by HDL not only effects the formation of HDL_2, but may also protect arterial wall cells, as surface remnants, when not incorporated into HDL, are cytotoxic to macrophages in culture (Chung *et al.*, 1989). (ii) Delayed lipolysis of TGRL increases the opportunity for the reciprocal transfer of triglycerides from TGRL into HDL and of cholesterol esters from HDL into TGRL. This neutral lipid exchange reaction is catalysed by CETP. Triglycerides transferred to HDL are hydrolysed by HTGL. In this way, HDL cholesterol is lost to TGRL, large HDL are converted into small dense HDL, and the cholesterol-carrying capacity of HDL decreases (Patsch, 1994). Hence, rapid lipolysis of TGRL keeps HDL_2C concentration (and thus HDLC concentration) high by promoting the formation of the larger HDL_2 as well as by preventing their catabolism.

The response of HDLC concentration to the rapid fluctuations of the concentrations of TGRL is relatively slow and constitutes the biochemical basis for the 'memory function' of HDL with respect to triglyceride transport. Therefore, HDLC concentration can be viewed as an integrative marker of triglyceride transport in all states of absorption, which explains why HDLC concentration tends to eliminate the rapidly fluctuating triglycerides as risk factor in multivariate analyses (Miesenböck and Patsch, 1992).

While little doubt exists that hypertriglyceridaemia and exaggerated postprandial triglyceridaemia usually result in low HDLC concentrations, there is some uncertainty about the prevalence of impaired triglyceride metabolism as a cause of low HDLC concentrations in the general population (Vega and Grundy, 1996). Indeed, recent reports from Cohen *et al.* (1991, 1992) failed to identify exaggerated postprandial lipaemic responses in either long distance runners with relatively low HDLC concentrations, or in patients with categorically low HDLC concentrations. It is thus likely that many individuals with low HDLC concentrations can have a normal postprandial triglyceridaemia. More extensive epidemiological studies will be required to resolve the true prevalence of impaired triglyceride metabolism as a cause of low HDLC concentrations in the general population.

Additional protective effects of HDL

A large body of evidence suggests that oxidation of LDL is involved in atherogenesis (Chisolm *et al.*, 1991; Hessler *et al.*, 1979; Witzum and Steinberg, 1991; Witzum, 1994). Both extensively oxidised and minimally oxidised LDL may play roles in stimulating atherogenesis. *In vitro* experiments indicate that HDL can inhibit many of the effects of LDL oxidation. Parthasarathy *et al.* (1990) have showed that HDL may play a protective role in atherogenesis by preventing the generation of oxidatively modified LDL. Navab *et al.* (1991) have shown that HDL inhibits the stimulation of macrophage chemotaxis induced by minimally modified LDL, and Hessler *et al.* (1979) have shown that HDL inhibits the LDL-mediated cytotoxicity of vascular smooth muscle and endothelial cells. There are several potential mechanisms by which HDL may modify the formation or properties of oxidised LDL. These include (i) an exchange of lipid peroxidation products between HDL and LDL, sequestering oxidised lipids away from apoB and limiting apoB modification (Parthasarathy *et al.*, 1990), (ii) binding of oxidation-promoting transition metals by apoA-I (Kunitake *et al.*, 1992), and (iii) two enzyme systems, platelet-activating factor acetylhydrolase and paraoxonase, associated with HDL may be able to inhibit the generation of oxidised lipids (Mackness *et al.*, 1993; Mackness and Durrington, 1995; Watson *et al.*, 1995; Watson *et al.*, 1995a).

ApoJ, also known as clusterin, is a glycoprotein that is present in a subpopulation of HDL together with paraoxonase and apoA-I, and has been reported to have diverse functions, including roles in complement regulation and prevention of cytolysis, lipid transport, apoptosis, membrane

protection at fluid-tissue interfaces where it is expressed, and may also reduce the oxidative potential of LDL and of the arterial wall cells (Navab *et al.*, 1997). Plasma apoJ concentrations increase significantly during inflammatory conditions, including in atherogenesis, which may allow it to serve a protective effect through its diverse biological interactions. Since antioxidant enzymes and apoJ are associated with only a small fraction of HDL particles, this may explain in part why some patients with low HDLC concentrations may not have clinically significant atherosclerosis, and others with relatively normal HDLC concentrations may have premature atherosclerosis (Navab *et al.*, 1997).

Summary

'Atherogenic dyslipidaemia' is a lipoprotein phenotype which has more recently been identified as one of the two major patterns of atherogenic lipoprotein disorders along with hypercholesterolaemia. The underlying cause of this phenotype is usually an excessive dietary intake (of fat in particular), excessive VLDL production, and/or an impaired triglyceride clearance. This results in a prolonged plasma residence of high concentrations of chylomicron and VLDL, which manifests as a mild fasting hypertriglyceridaemia or an exaggerated postprandial triglyceridaemia. These TGRL can play a central role in atherosclerosis, both directly, and by modifying other lipoprotein classes, particularly LDL and HDL, in an atherogenic fashion. Delayed lipolysis of TGRL increases the opportunity for a neutral lipid exchange reaction catalysed by CETP, which allows the reciprocal transfer of triglyceride from TGRL into LDL and HDL, and of cholesterol esters from LDL and HDL into TGRL. Triglycerides transferred to LDL and HDL are hydrolysed by HTGL, and these lipoproteins become smaller and denser. In this way, the HDLC concentration decreases, and the predominance of small dense (atherogenic) LDL increases. Small dense LDL are susceptible to oxidation, which further increases their atherogenicity. A reduced HDL concentration may limit the inhibition of LDL oxidation by HDL associated antioxidant enzymes. An elevated TGRL concentration is also characterised by an increased concentration of atherogenic chylomicron and VLDL remnants. A reduced HDL concentration may exacerbate this increase, as many of these remnants are normally incorporated into HDL.

A low HDLC concentration may not necessarily be caused by an exaggerated postprandial triglyceridaemia, but may also be caused by other factors such as an elevated HTGLa. The

suppressive effect of testosterone and AAS on HDLC concentration appears to be due primarily to an elevated HTGLa. Although a low HDLC concentration may be closely related to a reduction in reverse cholesterol transport, it is unclear whether changes in HDLC concentration reflect the efficiency of reverse cholesterol transport in all cases. At present, the possibility cannot be excluded that a low HDLC concentration may reflect an increased flux of cholesterol through the HDL cholesterol pool. Indeed, individuals having gene mutations which confer a partial suppression of HDLC concentration, are not necessarily predisposed to premature atherosclerotic disease. Until it is possible to show that therapeutic lowering of HDLC concentration in otherwise healthy individuals causes premature atherosclerosis, a measure of caution should be brought into the interpretation of the atherogenicity of testosterone and AAS induced HDLC concentration reduction.

Chapter 3: The general methodology for the experimental chapters

Similar clinical and laboratory methods were used in both experimental chapters. Therefore, the general methodological chapter provides detailed descriptions of these methods to avoid repetition. The methods described here are for triglyceride analysis, cholesterol analysis, Lp(a) analysis, the oral fat tolerance test (used to assess postprandial triglyceridaemia), and non-denaturing gradient gel electrophoresis (GGE) for assessment of LDL and HDL particle size.

Sample Collection and Processing

Blood samples for analysis of total cholesterol, Lp(a) concentration and for GGE were taken from the fasted subjects between 06h45 and 07h00, together with the first blood sample for the oral fat tolerance test. Subjects were seated, and fasted blood samples were drawn from an arm vein into Vacutainers containing EDTA (ethylenediaminetetraacetic acid), by a trained phlebotomist. Samples were immediately placed upright in crushed ice. On completion of the oral fat tolerance test at 17h00, all samples were centrifuged at 3000 rpm in a Beckman GS-6R for 15 minutes. Plasma was aspirated and divided between four cryovials. Samples were stored at -20°C until it was possible to perform a batch analysis. Analysis took place within three weeks after the second test day. Analysis of duplicate samples for test days 1 and 2 of the same individual were performed within the same assay.

Triglyceride analysis

General

Triglyceride concentration was assessed using a commercially available GPO-PAP kit (Human, Germany, Kit no:10164). Lipases hydrolyse triglyceride to yield glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate by glycerokinase and adenosine triphospate. The glycerol-3-phosphate is reacted with oxygen by glycerophosphate oxidase yielding dihydroxyacetone-phosphate and H_2O_2. Two molecules of the latter is used by peroxidase as well as 4-aminoantipyrine and 4-chlorophenol to generate quinoneimine, HCl and H_2O, yielding a pink-red colour, read spectrophotometrically at 500 nm in a 1 cm path (reference, package insert).

Materials

Buffer solution contains: PIPES buffer (piperazine-N,N'-bis[2-ethanesulfonic acid]; 1,4-piperazinediethanesulfonic acid) (pH=7.5) 40 mmol/L; magnesium ions 5 mmol/L; 4-chlorophenol 5 mmol/L and sodium azide 0.095 g%. Enzyme reagent contains 4-aminoantipyrine 0.4 mmol/L; adenosine triphospate 1 mmol/L; lipases ≥150 U/ml; glycerol kinase ≥0.4 U/ml; glycerol-3-phosphate oxidase ≥ 1.5 U/ml; peroxidase ≥0.5 U/ml.

Method

The enzyme lyophilisate is reconstituted with 15 ml buffer. This is stable at 4 °C for 21 days or 20 °C for 3 days. The design is for a final volume of about 750 μl, which allows for reading in a microcuvette in a spectrophotometer. Blank has no plasma while 10 μl of plasma sample is reacted. In turbid plasma it may be necessary to dilute the sample by a known amount. Add 250 μl of saline, vortex. Add 500 μl of reagent, vortex. Incubate at room temperature for 15 minutes and read absorbance within an hour. Duplicate test samples are read spectrophotometrically (500 nm) against the standards provided with the kit. Analysis is by linear regression.

Intra-assay coefficient of variation was 3.8%.

Cholesterol analysis

General

Cholesterol and cholesterol ester are released from lipoproteins by detergents. Cholesterol ester is hydrolysed by cholesterol esterase to yield cholesterol. Cholesterol oxidase is used to oxidise cholesterol, yielding delta-4 cholestenone, and releasing hydrogen peroxide in the process. The Boehringer Mannheim kit (290319) uses 2 molecules of hydrogen peroxide to react 4-aminophenazone and phenol to yield 4-(*p*-benzoquinone-monoimino)-phenazone and 4 H_2O by the enzyme peroxidase (reference, package insert). Note that bilirubin above 70 μmol/L and haemoglobin above 200 mg/dl may interfere with the test.

Materials

The concentrations of the reagent solution are: Tris buffer 100 mmol/L (pH=7.7); magnesium ions 50 mmol/L; 4-aminophenazone 1 mmol/L; sodium cholate 10 mmol/L; phenol 6 mmol/L;

3,4-dichlorophenol 4 mmol/L; hydroxypolyethoxy-alkanes 0.3%; cholesterol esterase ≥0.4 U/ml, cholesterol oxidase ≥0.25 U/ml and peroxidase ≥0.2 U/ml. The reagent powder is dissolved in 32 ml redistilled water, and is ready to use after 10 minutes. The reagent solution is stable at 4 °C for 4 weeks, and for 7 days at 15-20 °C.

Calibration and standards

Calibration is done by using a commercial plasma preparation (Precinorm) and/or gravimetrically prepared standards which have to be dissolved into the solution by means of ethanol and Triton X-100 (1% in distilled water). Standards are prepared by dissolving 10.0 mg cholesterol (BDH Chemicals Ltd. Poole England) in 10.0 ml of benzene (analytical grade, SAARCHEM, Krugersdorp, South Africa); mix thoroughly for 1 μg/μl (solution A). Exactly 1.0 ml of this solution is brought to 10 ml in benzene for 0.1 μg/μl (solution B). Appropriate volumes of solution A and B are transferred to eppendorf tubes. For 1, 2, 5 and 10 μg standards use solution B and for 20, 30, 45, 60, 75, 90 and 105 μg standards use solution A. Benzene is then allowed to evaporate in the fume cupboard, with the dried standards remaining. Standards are stored at -20°C.

Method

This method has been standardised to use 20 μl of plasma. The procedure is done with the solvents in all tubes (essential for dissolving dried standards), although in the plasma samples it is best to add the solvents after bringing to volume (500 μl) to avoid turbidity. To the standards add 50 μl ethanol and vortex. Now add 15 μl of 1% triton and vortex. Add 500 μl saline. Plasma samples are made up to 500 μl with saline. Add 50 μl ethanol to all plasma samples and vortex. Add 15 μl of 1% triton to all plasma samples and vortex. Add 500 μl of reagent to all reaction vials, vortex and react at room temperature for 30 minutes. Measure the absorption at 500 nm (470-560 nm) within 2 hours. The cholesterol content is calculated from the calibration curve, usually linear regression is adequate ($r = 0.99$) for 2 to 90 μg. If the sample exceeds the range, dilute and repeat.

Intra-assay coefficient of variation was 4.2%.

Lipoprotein(a) analysis

General

Apolipoprotein(a) was measured in the plasma using the Mercodia Apo(a) ELISA, enzyme immunoassay (Mercodia AB, Seminariegatan 29, S-752 28 Uppsala, Sweden). Mercodia Apo(a) ELISA is a solid two phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the apolipoprotein molecule. During incubation, apo(a) in the sample reacts with peroxidase-conjugated anti-apolipoprotein(a) antibodies and anti-apolipoprotein(a) antibodies bound to the microtitration well. A simple washing step removes unbound enzyme labelled antibody. The bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine. The reaction is stopped by adding dilute acid to give a colorimetric endpoint (reference, package insert). The absorbance is read spectrophotometrically (450 nm) in an ELISA plate reader. The results are calculated from a standard curve.

Calibration

The kit is calibrated against a highly purified, fully validated, commercial Lp(a) preparation. The concentration of Apolipoprotein(a) is expressed in Units/L. It is not possible to express the concentration of Apo(a) in mass units as there are at least six different isoforms have been described, with molecular weights varying from approximately 300 kD to 900 kD (Uterman, 1989; MBewu and Durrington, 1990). Thus each patient sample will contain different proportions of the different isoforms. Therefore, no exact conversion factor can be given between Units of Apo(a) and milligrams of Apo(a), although 1 unit of Apo(a) is approximately equal to 0.7 mg Lp(a).

Intra-assay coefficient of variation was 3.5%.

The Oral Fat Tolerance Test

General

Lipid and lipoprotein analysis has traditionally been confined to the post-absorptive (overnight fasting) state, when triglyceride metabolism, and lipid transport in plasma has reached a state of equilibrium (Föger and Patsch, 1993). In the past decade, however, interest has been growing in analysing lipoproteins in the postprandial state, since it has been demonstrated that; (i) triglycerides measured in the postprandial state can serve as an independent predictor for CHD in multivariate analyses (Patsch *et al.*, 1992a), and (ii) an exaggerated postprandial triglyceridaemia can modify the other major lipoprotein classes in an atherogenic fashion (Austin *et al.*, 1988; Karpe *et al.*, 1993; Patsch *et al.*, 1983). The oral fat tolerance test describes the absorption and digestion in the gut as well as circulatory entrance and clearance of triglycerides. A standardised amount of fat is given by mouth and venous blood is sampled and measured for plasma triglyceride concentration. The oral fat tolerance test is contra-indicated in subjects with severe fat malabsorption and is also contra-indicated in subjects with severe hypertriglyceridaemia (>10 mmol/L) because it may precipitate pancreatitis.

Procedure for oral fat tolerance test

The oral fat tolerance test was performed as described by Föger and Patsch (1993). Subjects were asked to abstain from exercise and alcohol the day before the test, and from food and beverages (excluding water) 12 hours before the test. Subjects typically arrived in the laboratory at 06h45, at which time their mass and height were measured. Surface area was predicted from a nomogram using mass and height (Geigy Scientific Tables, 1984). Subjects were seated and fasted blood samples were drawn from an arm vein into plain Vacutainers and Vacutainers containing EDTA (ethylenediaminetetraacetic acid). The test meal was given to the subjects at 07h00 and was consumed within 3 minutes.

The content of the test meal was calculated per m^2 of body surface area, and prepared and mixed immediately before use; cream 175 ml (39.5% fat), powdered chocolate flavouring 15 ml, granulated sugar 2.5 ml, fat free milk powder 7.5 ml. This concoction delivers about 3050 J, of which 3% of energy content is derived from protein, 14% from carbohydrate and 83% (65 g) from fat. The PUFA/SFA ratio is 0.06 and the cholesterol content is 240 mg. No other food was permitted during the day, although subjects are allowed to consume water or energy free drinks.

The test meal was well tolerated, although some subjects complained of nausea during the first three to four hours, and all of hunger during the last three to four hours. Subjects were then allowed to leave the lab and continue with their normal daily activities (excluding exercise), and were asked to return to the lab at two hourly intervals for the next ten hours (up to 17h00), so that additional blood samples (5 ml) could be taken for assessment of triglyceride concentration. The total blood volume collected during the day is approximately 35 ml.

Interpretation of oral fat tolerance test

Triglyceride concentration was plotted against time. Postprandial triglyceridaemia (mmol/L.hr) was calculated by integration of the area under the curve of triglyceride concentration versus time. Postprandial triglyceride excursion was calculated by subtracting the minimal triglyceride concentration, multiplied by 10 hours, from the total area under the curve. Three essential patterns can emerge from the oral fat tolerance test. The "flat pattern" reveals no increase of triglyceride with time and is indicative of malabsorption, homozygous hypobetalipoproteinaemia, intestinal lymphagiectasia, or obstruction or leak from the cysterna chyli or thoracic duct. The "desirable pattern A" is for a rise from an ideal (normal) baseline triglyceride of about 0.5 to 1.5 mmol/L to a peak of about 3 mmol/L at which stage the plasma is faintly turbid. The 6 hour triglyceride concentration is already close to baseline and the 8 and 10 hour values have reached baseline. The "undesirable pattern B" may commence at a normal triglyceride concentration or slightly higher, has the same delay before rising to a peak at the same time as the desirable pattern, but there is a persistent hypertriglyceridaemia until 6 and even 8 to ten hours after the test. The area under the curve is much greater and can be used to discriminate changes due to an intervention. The LDL particle sizes are generally smaller in subjects with the undesirable (B) pattern, with lower HDLC concentrations. These subjects also have higher risk of coronary artery disease (about 3 to 5 fold) and the undesirable pattern is prominent in myocardial infarction survivors with apparently normal range cholesterol concentrations (Austin *et al.*, 1992; Campos *et al.*, 1992).

Non-Denaturing Gradient Gel Electrophoresis (GGE) for lipoproteins

General

This technique allows separation of lipoproteins by size. The lipoproteins are demonstrated by staining lipid with Sudan Black before the procedure. Separation of chylomicron and VLDL is difficult because of the size of these lipoproteins. The IDL range is also not so distinctly separated. Gels may be selected to demonstrate LpB series (LDL) and LpA series (HDL). A 2-16% gradient is usually recommended for LDL (Gambert *et al.*, 1990) and a 4-30% is usually recommended for HDL (Meyer *et al.*, 1989). The University of Cape Town Lipid lab uses a mini-gel of 2-8% acrylamide gradient for LpB, and a 4-18% for LpA. Fasted plasma samples should immediately be placed on ice and processed as soon as possible, although samples have been satisfactory for LDL characterisation for up to 1 week when stored at 4 °C. The samples can also be stored at -20 °C for a few weeks, and for several (>12) weeks at -50 °C.

Qualitative differences in LDL may be important. The first relation between smaller denser LDL and heart disease was shown by Austin *et al.* (1988), who recognised two types of LDL. Dormans *et al.* (1991) suggested that 3 LDL subtypes could be identified by either ultracentrifugation or GGE, although the latter method could on occasion identify 5 bands. Tashiro *et al.* (1993) found a "midband", which is probably Lp(a), that predicts heart disease in FH subjects. Similarly, HDL_3 and HDL_2 subtypes may influence atherosclerosis.

Materials

The lipid stain is Sudan Black, prepared by adding 1% sudan black to ethylene glycol. This solution is passed through filter paper to remove undissolved fragments. An additional stain solution containing bromophenol blue 0.2% and sucrose 60% is also prepared. The acrylamide monomer solution contains 30% acrylamide of which 2.7% is bis-acrylamide. A fresh acrylamide solution provides better clarity of separation. Gel buffer A is prepared by adding 250ml water to 68.1g Tris base. Once the Tris base has dissolved, 150ml (187.5g) glycerol is mixed into the solution. The pH is adjusted to 8.8 and the volume is brought up to 500ml. Gel buffer B is prepared in a similar manner to gel buffer A, except that glycerol is omitted from the solution. The stacking gel buffer is prepared by dissolving 8.4g Tris base, before adjusting the pH to 6.8 and bringing the solution to 500ml. The electrode buffer stock is prepared by dissolving Tris base 37.9g and glycine 180.1g, before making the solution up to 2.5L. This solution is five times (×5)

concentrated, and pH is adjusted to 8.3 after dilution. The required equipment is a minigel apparatus (Biorad) with 8 × 10 cm plates and a powerpack.

Procedure LDL Gel

Cast a 2-8% polyacrylamide gradient (running) gel. The 2% gel solution is prepared by mixing buffer B, and the 8% gel solution is prepared by mixing buffer A, with the appropriate volume of 30% acrylamide solution. Polymerisation of the gel solutions is initiated using ammonium persulfate (APS) 0.74% and TEMED 0.37%. The gradient is prepared using a Hoefer SG15 gradient maker. A 4% stacking gel is cast on top of the running gel. The stacking gel is prepared using the stacking gel buffer mixed with the appropriate volume of 30% acrylamide solution. Polymerisation of the stacking gel solution is initiated using APS 1.95% and TEMED 0.78%. Two gels are run in the system, with 15 lanes per gel. The gels are run in a cold room or in a refrigerator.

Plasma samples (100μl) are thawed at 4°C, mixed with 50μl of lipid stain (Sudan Black), incubated for a minimum of 1 hour at 4°C and spun for 20 minutes at 10 000G. An equal volume of supernatant (50μl) is mixed with a saturated sucrose/Bromophenol Blue solution and 12μl is loaded per well. The addition of Bromophenol Blue to the sample helps to improve the resolution of the leading edge of the LDL bands, and the saturated sucrose increases the density of the sample solution, facilitating sample loading into the wells.

Prefocus the gel at 20V for 30 minutes. The gel is then run at a voltage which keeps current flow below 60mA. As the gel resistance decreases, voltage can be increased up to a maximum of 130V. The gel is placed in a refrigerator (4°C) to minimise heating and is run for 16-17 hours (over night).

Interpretation of LpB (LDL) GGE

The gel is inspected without knowledge of clinical or biochemical detail. Some subject samples are rerun on subsequent gels to check the consistency of the resolution and evaluation of lipoprotein subspecies. Comment should be made about material in the stacking gel. The origin (top or least dense part) of the separation gel reflects the largest particles. The minigel is about 60mm long. In our system the stain reflecting lipoproteins in the LDL density range, is

from approximately the junction of the top 2/3 with the bottom 1/3. The chief LDL-related bands are in the bottom 1/6 of the stained area.

The gel is described by simple terminology avoiding terms inferring separation by density. Since the gel was developed to investigate LDL size, the zone between the origin and the LDL is referred to as "mid" and staining in this region is referred to as M, which was later modified to M-early, or M-late depending on whether the particles were larger or smaller. Subsequently it became clear that chylomicron-like particles tended to remain at the origin of the separation gel, and hence were termed O. At the "LDL" range, the largest particle is designated "A" while some intermediate "I" bands may be discerned or the "B" band as the smallest (densest) LDL which has been associated with a higher risk of CHD in familial combined hyperlipidaemia and with hypertriglyceridaemia. Subsequently it became clear that even further size distinctions can be made, now abbreviated to A-early, A/I, I/B and B-post. Sometimes a band between A and M-late can be made out and was then referred to as pre-A. This band was later identified to be due to Lp(a). A small letter is used to designate that the band stains faintly, and a capital letter is used to demonstrate dominant bands.

Describing particles from large to small on this gel would thus be: O, M-early, M-focus (in Me range usually), M-late, pre-A, A-early, A, A/I, I, I/B, B, B-post. Chylomicrons correspond to O, $VLDL_1$ (S_f 60-400) to Me, LpX to Mf, $VLDL_2$ (S_f 20-60) to M-late, pre-A is likely to be Lp(a), A to B-post are all species of LDL.

Calibration of LpB (LDL) GGE.

It has not been possible to calibrate the system accurately so that the diameter of the particles can be properly calculated. Currently we readily identify A and B patterns amongst the 15 lanes and use one of each LDL type to carry across from gel to gel to bring some standardisation about. Although gels that are prepared from day-to-day are not identical, it has been found that the classification of repeat samples in different gels is extremely reproducible.

Attempts to calibrate the system using commercially available latex beads with known diameters was unsuccessful, since these beads tended to clump and cause "ladders". We have generally accepted that LDL particles will have the typically published diameters and that there

is a broad size range for all other particles. This makes accurate diameter determinations less useful. The system has been standardised against ultracentrifugally prepared $VLDL_1$ (S_f 60-400), $VLDL_2$ (S_f 20-60), IDL (S_f 12-20) and LDL (S_f 0-12). Taking the B particle as Rs = 1.0, the following Rs values are typically found: V1 = 0.2-0.45; V2 = 0.45-0.7; IDL = 0.7-0.85; LDL A-B = 0.85-1.0, and B-post about 1.05.

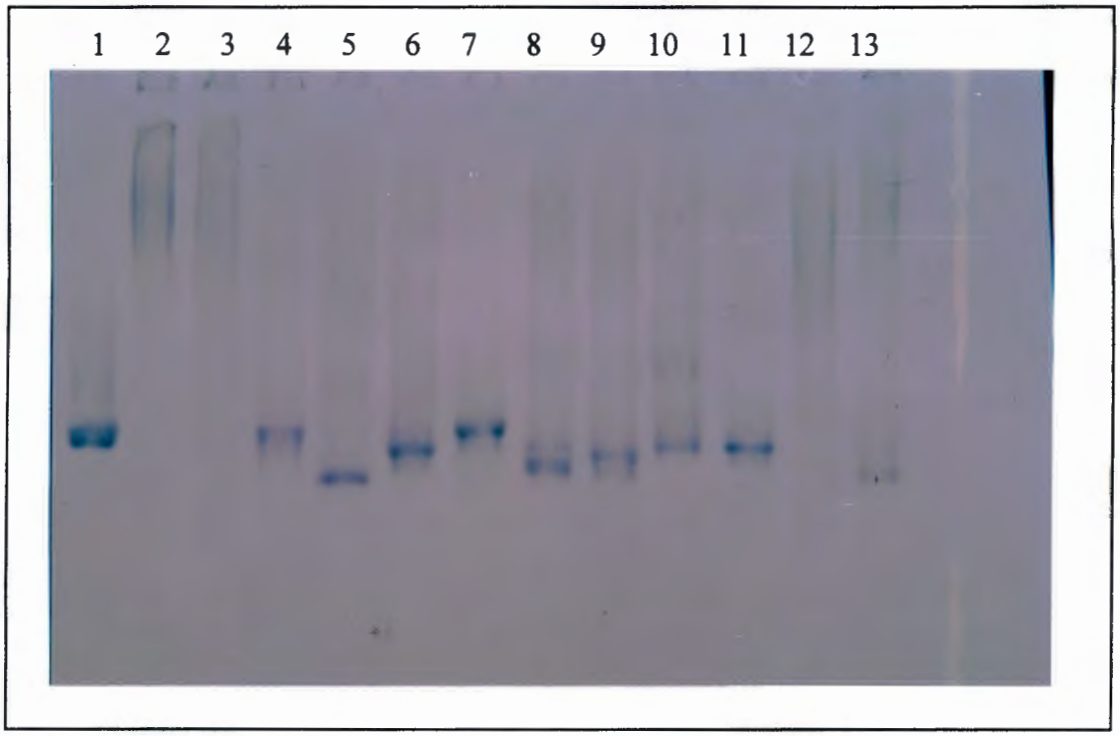

Figure 1. *A 2-8% non-denaturing polyacrylamide gradient gel, showing how LDL particle size and the presence of VLDL can differ between individuals. Lane: (1) preA, A/I; (2) M early; (3) M early, B post; (4) A/I; (5) M, PreA, B; (6) I; (7) A; (8) M, B; (9) M, I/B; (10) PreA, I; (11) I; (12) M late, B post; (13) M early, B.*

Procedure HDL Gel

Cast a 4-18% polyacrylamide (running) gradient gel. The 4% gel solution is prepared by mixing buffer B, and the 18% gel solution is prepared by mixing buffer A, with the appropriate volume of 30% acrylamide solution. Polymerisation of the gel solutions is initiated using ammonium persulfate (APS) 0.74% and TEMED 0.37%. The gradient is prepared using a Hoefer SG15 gradient maker. A 4% stacking gel is cast on top of the running gel. The stacking gel is prepared using the stacking gel buffer mixed with the appropriate volume of 30% acrylamide

solution. Polymerisation of the stacking gel solution is initiated using APS 1.95% and TEMED 0.78%. Two gels are run in the system, with 15 lanes per gel.

Plasma samples are thawed at 4°C, mixed with 50μl of lipid stain (Sudan Black) only, incubated for a minimum of 1 hour at 4°C and spun for 20 minutes at 10 000G; 16μl is loaded per well. Prefocus the gel at 20V for 30 minutes, run at 130V for 4 hours.

Interpretation of the LpA (HDL) GGE

The gel is inspected without knowledge of clinical or biochemical detail. Some subject samples are rerun on subsequent gels to check the consistency of the resolution and evaluation of lipoprotein subspecies. After selecting from the many lanes, likely equivalents of HDL_2 and HDL_3 species are identified. The bands tend to be poorly staining and broad, and only occasionally can speciation be distinguished in the two regions. The LDL bands at the top may be classifiable as A or B but are often inadequately separated for comment. Gels can also be scanned on a densitometric scanner (Hoefer Scientific Instruments GS300). The densitometric scan is used to confirm the visual analysis, and area under the curve can be calculated to assess the ratio of HDL_2 to HDL_3 subspecies.

Terminology of "HDL GEL".

Particles relating to HDL_2b and HDL_2a as well as HDL_3a, HDL_3b, HDL_3c are described in the review by Silverman and Pasternak (1993). In our system we find mostly 2 peaks, one with Mr of 135kD and another at about 165kD. The former appears to be HDL_3 and the latter HDL_2. In some patients there is a smaller size lipid-staining peak, at about 115kD. In some instances larger particles are seen in hyperalphalipoproteinaemia.

Current practice is to assume that the common species of smaller size is HDL_3, the larger is assumed to be HDL_2. The description is thus of the intensities being dominant in either one of the two bands or equivalent. This agrees remarkably well with the area under the curve and the peak intensities on our gel scanner. Occasionally there may be small species of lipoproteins, to which the label HDL_4 has been given, or larger species to which the label HDL_1 has been given. These can range to a size close to the LDL band. The HDL gels are usually described as follows: $2 > 3$ indicates a more prominent HDL_2 than HDL_3 band; $2 = 3$ indicates equivalent HDL_2 and

HDL_3 bands; 2 < 3 indicates less prominent HDL_2 than HDL_3 bands; 3 indicates that only particles in the HDL_3 size range are present (2 << 3 is intermediate between the latter two categories); flat indicates that HDL was not evident on the gel.

Standardisation of particle size.

This has not been satisfactorily performed yet. Usually the pattern can be described by comparison with the other 14 lanes on the same run. A subject, showing prominent HDL_2 and HDL_3 bands on a previous gel, is often run in parallel. Latex beads of defined diameter do not provide a single neat band on the LDL or HDL system, while on the HDL system the rainbow markers do not give neat bands when undenatured. Protein staining by Coomassie Blue on our system would be confusing especially in the HDL range as there are many proteins at these sizes.

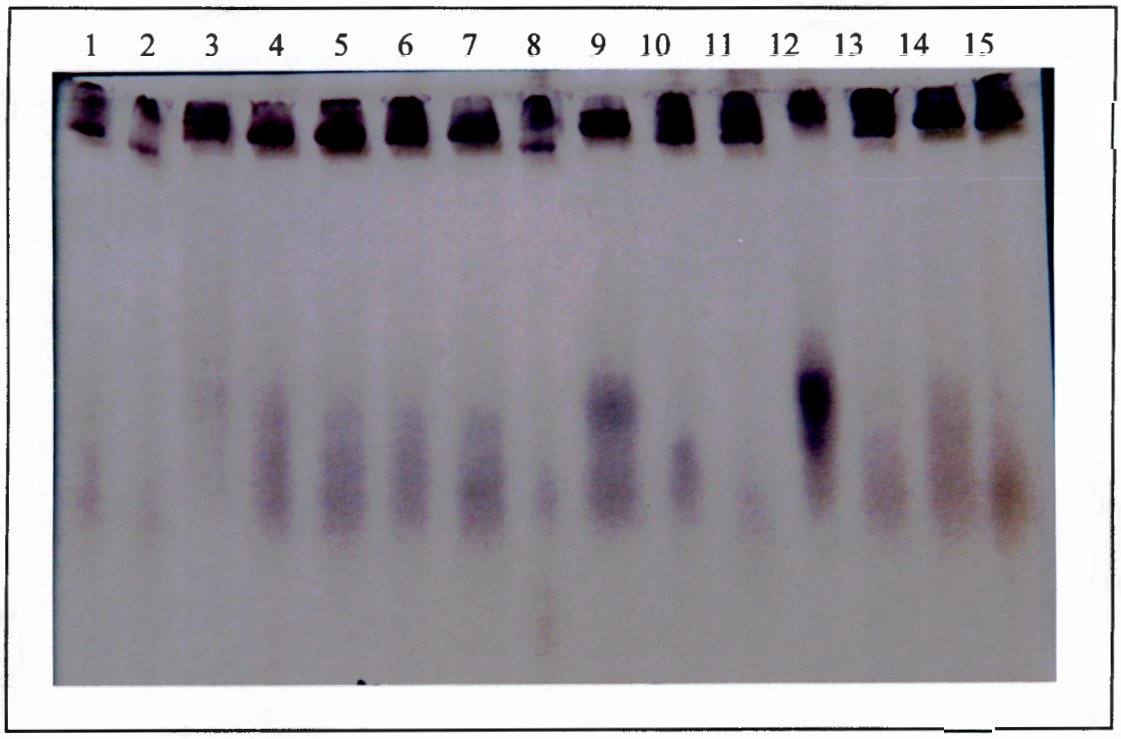

Figure 2. *A 4-18% non-denaturing polyacrylamide gradient gel, showing how the distribution of particles in the HDL size range differs between individuals. Lane: (1) 2 < 3; (2) 2 < 3; (3) 2 > 3 (? HDL 1), rare; (4) 2 < 3; (5) 2 < 3; (6) 2 < 3; (7) 2 < 3; (8) 2 < 3 (HDL 4), rare; (9) 2 > 3; (10) 2 < 3; (11) 2 < 3; (12) 2 > 3; (13) 2 < 3; (14) 2 = 3; (15) 2 < 3.*

Chapter 4: The effect of triptorelin, a testosterone lowering agent, on postprandial triglyceridaemia and lipoprotein profiles in normal men

Abstract

The aim of this study was to determine whether a reduction of endogenous testosterone concentration in healthy men and the consequent elevation of HDLC concentration is associated with changes in the magnitude of postprandial triglyceridaemia, LDL particle size and Lp(a) concentration. A reversible reduction of testosterone was induced using triptorelin (D-Trp-6-LHRH; Decapeptyl®), a powerful GnRH agonist. Lipoprotein profiles and postprandial triglyceridaemia were compared in normal men (T) (n=10) before triptorelin administration and five weeks later, when testosterone concentrations were minimal (23.9±4.9 vs 3.9±1.6 nmol/l, p=0.004). A control group (C) (n=8), which did not receive triptorelin, was assessed in parallel. Lipoproteins were assessed by non-denaturing gradient gel electrophoresis (GGE), lipoprotein concentrations by immuno and enzymatic assay, and postprandial triglyceridaemia by a standardised oral fat tolerance test (65g/m^2). HDLC concentration increased significantly (1.07±0.18 vs 1.41±0.28 mmol/L, p=0.002) in T and did not change in C. HDL profiles assessed by GGE showed a relative increase of larger HDL species in five T subjects and showed no change in C. Total cholesterol concentration increased significantly (4.77±0.80 vs 5.24±1.04 mmol/L, p=0.039) in T with a slight, but non significant increase in LDLC concentration. Neither total cholesterol nor LDLC concentration changed in the controls. LDL size assessed by GGE increased in four T subjects, whereas no increases occurred in C. Lp(a) concentration increased significantly (277.9±149.1 vs 376.5±222.2 U/L, p=0.004) in T, and was unchanged in the C group. Postprandial triglyceridaemia was similar for both T and C subjects. These results show that an acute reduction in endogenous testosterone concentration has no effect on the magnitude of postprandial triglyceridaemia. Thus, the elevated HDLC concentration is not likely to be due in part to a reduced postprandial triglyceridaemia. The results of this study suggest that endogenous testosterone has a suppressive effect on Lp(a) and HDLC concentration in normal men, without affecting LDLC and fasting triglyceride concentration or the magnitude of postprandial triglyceridaemia.

Introduction

Men are more likely to suffer from premature atherosclerosis than women (Godsland *et al.*, 1987). The onset of atherosclerotic disease usually begins 10-15 years later in women compared to men (Castelli, 1988). This difference could exist because males possess some factor which predisposes them to premature atherosclerosis, or because they lack some factor, inherent to females, which serves to protect from atherosclerosis. It has been hypothesised that the sex difference in the endogenous sex hormone concentrations may make a significant contribution to the sex difference in the onset of atherosclerosis (Kalin and Zumoff, 1990). Accordingly, the high testosterone concentration in men may aggravate atherosclerosis, whereas their low oestrogen concentration may render them less resistant to the atherosclerotic process than women. While good evidence exists to support a protective function for oestrogen against atherosclerosis in women (Knopp *et al.*, 1994), evidence that endogenous testosterone aggravates atherosclerosis is based largely on inferential evidence.

Endogenous testosterone is the major determinant of the lower HDLC concentration in men compared to women (Aascheman *et al.*, 1994; Bagatell *et al.*, 1992). In population studies, the risk for an atherosclerotic event is strongly and inversely related to HDLC concentration (Gordon *et al.*, 1989). Accordingly, it has been concluded that endogenous testosterone may contribute to the increased risk of cardiovascular disease in men (Bagatell and Bremner, 1995). However, it is difficult to determine if the suppressed HDLC concentration accounts for a significant portion of the sex difference in risk for premature atherosclerosis. Moreover, no direct evidence exists to indicate that an elevated HDLC concentration, induced by a suppression of testosterone, will reduce the risk of premature atherosclerosis in men. The possibility also exists that an equivalent HDLC concentration may not confer equivalent risk in men and women. Indeed it has been noted that an equivalent reduction of HDLC concentration is associated with a greater increase in risk for CHD in woman than in men (Gordon *et al.*, 1989), which suggests that HDLC concentration is a stronger predictor of atherosclerotic disease in women than in men (Knopp *et al.*, 1994). A recent review by Alexandersen *et al.* (1996) casts further uncertainty on the role of endogenous testosterone as a significant risk factor for premature atherosclerosis in men. These authors concluded that prospective and cross sectional studies which have examined

the relationship between endogenous testosterone and CHD, suggest either a neutral or a beneficial effect of testosterone on CHD in men.

A clinical trial for a GnRH agonist (triptorelin), which induces a reversible suppression of testosterone, provided the opportunity to investigate the effects of an acute reduction of endogenous testosterone in normal men on a phenotype termed 'atherogenic dyslipidaemia'. The phenotype is characterised by a low HDLC concentration, increased small dense LDL particles and an abnormal triglyceride metabolism which can manifest as an exaggerated postprandial triglyceridaemia or a mild fasting hypertriglyceridaemia. This phenotype has more recently been identified as a lipoprotein profile which frequently rivals hypercholesterolaemia as a risk factor for premature atherosclerosis (Grundy, 1996). Although it is known that men tend to have a greater prevalence of the small dense LDL particles than women (McNamara *et al.*, 1987), it is unclear how endogenous testosterone may influence LDL size. Moreover, there is a paucity of information about the effect of endogenous testosterone on the magnitude of postprandial triglyceridaemia and Lp(a) concentration in healthy men. It is also unclear whether an elevated HDLC concentration following testosterone suppression (Bagatell *et al.*, 1992; Goldberg *et al.*, 1985) may be caused, at least in part, by a reduced postprandial triglyceridaemia as proposed by Patsch *et al.* (1983). By doing this study it was hoped to gain new insight into the influence of endogenous testosterone on 'atherogenic dyslipidaemia', in so doing providing new insight into the role of testosterone as a risk factor for premature atherosclerosis in males.

Methods

Clinical trial

A clinical trial for a GnRH agonist, triptorelin (D-Trp-6-LHRH; Decapeptyl®) was conducted by Debiopharm, Lausanne Switzerland, at the Department of Physiology, University of Cape Town. The trial was approved by the Ethics and Research Committee of the University of Cape Town. The aim of the trial was to compare the efficacy and tolerance of two triptorelin sustained release formulations. Although the physiological role of GnRH is to stimulate the release of gonadotropins from the anterior pituitary gland, the chronic administration of GnRH results in a decrease of gonadotropin secretion, and an associated reduction in plasma testosterone

concentration. It provides a means of reversibly suppressing testosterone production, indicated in males for the treatment of prostate cancer. The study was an open, randomised, balanced, crossover design. The second leg of the study followed three months after the commencement of the first leg, once testosterone concentrations had returned to normal for several weeks.

Substudy protocol

A substudy was approved by the Ethics and Research Committee of the University of Cape Town, to allow investigation of some additional aspects of lipoprotein metabolism, and was conducted during the second leg of the crossover. Subjects were assessed both before triptorelin administration and five weeks later. Testosterone concentrations were known to have been minimal for the longest possible period at five weeks, from experience gained in the first leg of the study. Lipoprotein profiles, particle size and postprandial triglyceridaemia were therefore compared in the subjects before the administration of triptorelin and five weeks later.

Subjects

The substudy group consisted of ten healthy men who volunteered from the original clinical trial study group of twelve subjects. The ages of the subjects ranged from 23 to 55 years, and all had normal testosterone concentrations (inclusion criteria for clinical trial). A control group of eight men, which did not receive triptorelin, was matched with the trial group on the basis of age, socioeconomic status and relative physical activity. The control group was assessed in parallel with the trial group. All the subjects were fully informed about the protocol prior to the study and provided written informed consent prior to commencement. Medical histories were collected, and each subject underwent a physical examination, and liver function tests (assessed by pathologists Penman and partners, Loop St. Cape Town) on each test day. All the subjects were asked not to modify any of their lifestyle habits, exercise regimen and diet, throughout the duration of the study.

Anthropometry

Anthropometric measurement was performed by the same experienced individual, using standardised anthropometric landmarks to locate the measuring sites (Ross and Marfell-Jones, 1991). Calculation of body fat percentage requires the measurement of four skinfold sites: triceps, biceps, subscapular, and suprailiac. The log of the sum of these

measurements is substituted into the appropriate age and sex specific equation as described by (Durnin and Womersley, 1974). The calculation of muscle mass requires the measurement of stature, mid-thigh girth, mid-thigh skinfold, calf girth, calf skinfold, and forearm girth. The muscle mass is calculated by substitution into the appropriate equation as described by Martin *et al* (1990).

Dietary history

Subjects receiving triptorelin were asked to complete three day dietary records (covering one weekend day and the two preceding or following weekdays) as close to the test days as possible. Dietary analysis was performed using the Foodfundi® nutritional advice software (South African Medical Research Council) to determine mean daily energy, protein, carbohydrate, fat, cholesterol, saturated, monounsaturated and polyunsaturated fat intake.

Plasma lipids and lipoproteins

The concentration of total, LDL and HDL cholesterol was evaluated by pathologists Penman and partners (Loop St. Cape Town). These assays were done with commercially available enzyme kits on automated machines using conventional laboratory quality control techniques. Total cholesterol was confirmed in our laboratory using an enzymatic assay (Boehringer Mannheim kit no. 1442341). Apolipoprotein(a) was measured in the plasma using the Mercodia Apo(a) ELISA, enzyme immunoassay (Mercodia AB, Seminariegatan 29, S-752 28 Uppsala, Sweden). A detailed description of these methods can be found in the General Methodology chapter.

Oral fat tolerance test

The oral fat tolerance test was performed as described by Föger and Patsch (1993). Plasma triglyceride concentration was measured using a commercially available GPO PAP kit (Human, Germany, Kit no:10164.), and plotted against time. Postprandial triglyceridaemia (mmol/L.hr) was calculated by integration of the area under the curve. Postprandial triglyceride excursion was calculated by subtracting the fasting triglyceride concentration from the total area under the curve. A detailed description of this method can be found in the General Methodology chapter.

Non-Denaturing Gradient Gel Electrophoresis (GGE) for lipoproteins

The size of LDL and HDL particles were determined by polyacrylamide GGE using the Biorad minigel apparatus; 2-8% for LDL, and 4-18% for HDL. Samples from both the test days were run on the same gel. Visual interpretation of the gels was confirmed by densitometric scan (Hoeffer Scientific Instruments GS300). A detailed description of these methods can be found in the General Methodology chapter.

Statistical analysis

A statistical software package (Graphpad Instat) was used for data analysis. Data are expressed as a mean ($\bar{x}$) ± standard deviation (sd). Data were analysed using non-parametric methods, using the Wilcoxon matched pairs test. A value of $p < 0.05$ was accepted to define statistical significance.

Results

Subject characteristics and anthropometry

The mean age was 31±10 years, and 28±11 years for the T and C subjects respectively (Table 1). Anthropometric data are available for nine T subjects and seven C subjects. The other two subjects were not measured in error. Neither body mass, muscle mass, nor fat percentage changed between tests 1 and 2 in either the T or the C subjects. However, body mass in T subjects tended to be slightly greater than in C subjects; with the difference consisting mostly of fat mass.

Medical assessment

The subjects were all judged to be in good health following an examination by a medical practitioner. Although no significant side effects were reported, loss of libido, mild impotence and hot flushes were reported by some subjects. Liver function tests revealed no extreme derangements, i.e. no deviations three times greater than the reference range upper limit, for the test laboratory. The T group had a mean LDH concentration (351±99 U/L) which was elevated compared to the C group (257±54) and the upper reference range limit (290 U/L).

Table 1. Age and anthropometric values of triptorelin trial and control subjects

Subject	age (yr)	height (cm)	body mass (kg)		muscle mass (kg)		fat percentage (%)	
Triptorelin			Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1	23	175.1	77.2	77.3	42.4	40.2	15.9	15.5
2	55	186.2	95.5	95.3	45.3	42.9	27.8	27.8
3	26	166.0	70.5	70.5	35.9	38.3	20.1	19.4
4	39	179.6	95.6	96.0	52.1	52.7	23.2	23.0
5	27	181.0	76.0	81.5	-	-	-	-
6	30	180.4	72.2	71.1	37.4	37.9	18.6	17.7
7	37	173.5	94.5	96.8	45.2	49.4	28.8	29.7
8	29	187.0	86.5	87.0	52.5	50.7	11.3	10.4
9	20	166.0	59.4	58.3	36.5	35.5	10.2	10.0
10	25	164.6	80.7	80.4	42.2	43.7	25.3	25.2
mean	31	175.9	80.8	81.4	43.3	43.5	20.1	19.9
±sd	±10	±8.3	±12.2	±12.7	±6.1	±6.2	±6.8	±7.1
Control			NS		NS		NS	
11	25	185.7	85.7	84.5	49.4	49.6	8.5	8.1
12	20	182.3	59.7	58.5	33.1	32.9	9.1	7.8
13	21	189.1	74.0	73.5	-	-	-	-
14	29	180.5	63.3	63.5	38.5	38.3	10.5	8.1
15	33	173.5	88.1	92.5	47.9	46.0	26.4	27.0
16	24	177.5	86.0	86.0	46.2	46.1	18.9	19.4
17	21	180.5	79.6	79.5	40.9	40.0	17.1	18.5
18	52	168.5	72.6	72.6	38.0	39.9	26.2	25.4
mean	28	179.7	76.1	76.3	42.0	41.8	16.7	16.3
±sd	±11	±6.6	±10.7	±11.6	±6.0	±5.7	±7.7	±8.4
			NS		NS		NS	

Dietary analysis

Seven of the T group completed both dietary records (Table 2). There was no difference in diet between test days 1 and 2 in this group. Mean daily dietary consumption for the T group was; energy 25640±9320 kJ, protein 16±4%, carbohydrate 55±10%, fat 30±7% (saturated 23±10 g,

monounsaturated 23±10 g, polyunsaturated 13±6 g), cholesterol 810±340 mg. The C group were not asked to complete dietary records. A verbal interview indicated that these subjects made no changes to their dietary habits between the test days.

Table 2. Analysis of dietary recall in triptorelin subjects

Subject	energy (Kj)	protein (%)	CHO (%)	fat (%)	SF (g)	MUF (g)	PUF (g)	chol. (mg)
				Pre triptorelin				
1	36178	17.4	35.6	49.4	38.8	39.9	22.7	1162
2	31857	14.2	27.4	54.3	24.3	28.4	18.8	860
4	14255	15.2	27.7	58.7	11.4	13.6	7.8	428
6	17208	11.5	26.1	65.7	11.3	7.8	4.1	370
7	25321	18.4	45.4	37.1	30.7	41.5	15.1	779
8	35290	15.2	19.2	71.8	25.4	14.9	10.7	743
10	12466	22.3	40.2	42.6	12.4	16.9	8.2	406
mean	24654	16.3	31.7	54.2	22.0	23.3	12.5	678
±sd	±10086	±3.5	±9.1	±12.3	±10.8	±13.4	±6.6	±292
				Five weeks post triptorelin				
1	30449	12.3	30.0	59	31.1	28.8	15.4	723
2	38529	13.3	28.6	55.9	37.9	31.7	20.5	1160
4	19259	22.1	27.4	52.3	14.8	16.2	9.6	1374
6	21528	13.1	30.4	56.5	26.8	21.8	6.1	654
7	23717	16.1	34.6	51.1	20.2	25.3	19.9	1411
8	37859	14.3	20.8	70.0	26.6	19.5	9.4	555
10	15017	25.6	32.6	44.2	14.4	17.7	7.7	725
mean	26623	16.7	29.2	55.6	24.5	23.0	12.7	943
±sd	±9183	±5.1	±4.4	±8.0	±8.6	±5.8	±5.9	±361
				Total				
mean	25638	16.5	30.4	54.9	23.3	23.1	12.6	810.0
±sd	±9323	±4.2	±7.0	±10.0	±9.5	±9.9	±6.0	±344.2

CHO - carbohydrate; SF - saturated fat; MUF - monounsaturated fat; PUF - polyunsaturated fat; chol. - cholesterol

Testosterone

Testosterone concentration was similar in the T group (7.32±1.92 ng/ml) compared to the C group (7.30±2.08 ng/ml), at test 1, prior to triptorelin administration (Table 3). Testosterone concentration was significantly reduced by triptorelin administration in the T group, from 7.32±1.92 ng/ml at test1 to 1.15±0.57 ng/ml at test 2 after five weeks (p=0.002). Testosterone did not change in the C group at test1 (7.30±2.08 ng/ml) compared to test2 (6.50±2.18 ng/ml). A plot of testosterone concentration versus time in the T group (Figure 1), confirms that test 2, performed 37 to 41 days (5.3 - 5.8 weeks) after triptorelin administration, occurred when mean testosterone concentration had reached baseline and been suppressed for the maximum time period.

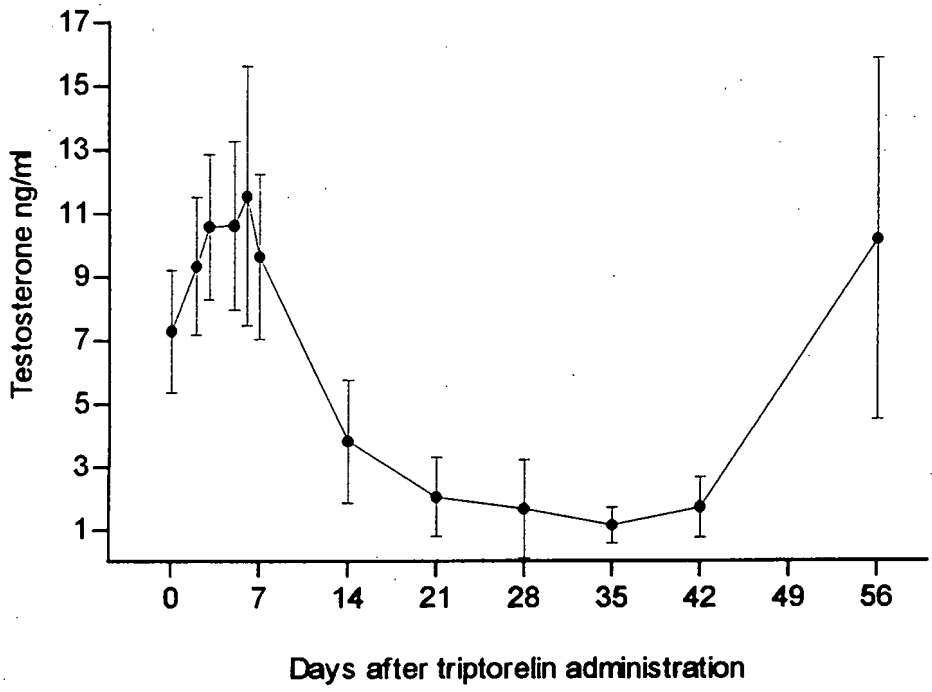

Figure 3. *Effect of triptorelin on testosterone concentration in normal men (n=10, $\bar{x} \pm sd$).*

Lipoprotein values

In the T group, mean total cholesterol was increased after five weeks (4.8±0.8 vs 5.2±1.0 mmol/L, p=0.04) (Table 3). Mean total cholesterol did not change in the C group (4.9±1.1 vs 4.9±0.9 mmol/L). Mean LDLC concentration was unchanged at test 1 compared to test 2 in both groups. In the T group, mean HDLC concentration increased significantly at test 2 from 1.1±0.2 to 1.4±0.3 mmol/L, p=0.002. HDLC concentration showed no change in the C group (1.2±0.2 vs 1.2±0.1 mmol/L). Lp(a) concentration increased after five weeks in the T group (277.9±149.1 vs 376.5±222.2 U/L, p=0.004) (median; 322.7 U/L test 1 and 397.6 U/L test 2). Lp(a) concentration did not increase in one individual. Lp(a) concentration showed no change in the C group (239.0±223.2 vs 235.9±298.9 U/L).

LDL gradient gel electrophoresis

LDL particle size increased in four triptorelin subjects, decreased in two subjects, and showed no change in four subjects (Table 4). LDL particle size decreased in three C subjects and showed no change in the other five C subjects.

HDL gradient gel electrophoresis

HDL profiles showed a relative increase of high density lipoprotein in the HDL_2 size range in five triptorelin subjects and showed no change in the other five T subjects (Table 4). HDL profile did not change in seven of the control subjects. One control subject showed a decrease of high density lipoprotein in the HDL_2 size range.

Triglycerides and postprandial triglyceridaemia

Fasting triglyceride concentrations were unchanged between test 1 and test 2 in both T and C subjects (Table 4). Postprandial triglyceridaemia, expressed as the total area under the curve, and as a triglyceride excursion were unchanged between test 1 and 2, in both T and C. Control subject 15 had an exaggerated postprandial triglyceridaemia at test 2. Subsequent inquiry revealed that his level of physical activity decreased following the first test. Data for control subject 15 were nevertheless included in the statistical analysis of the data for the control subjects and did not affect the statistical outcome.

Table 3. Lipoprotein and testosterone values of triptorelin trial and control subjects

Subject	Total Chol. (mmol/L)		LDLC (mmol/L)		HDLC (mmol/L)		Lp(a) (Units/L)		Testosterone (ng/ml)	
Triptorelin	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1	3.9	3.9	2.2	2.1	1.2	1.5	94.1	107.4	8.31	2.32
2	6.2	7.3	4.4	5.2	1.3	1.6	387.5	654.5	7.32	0.62
3	5.7	6.0	3.8	4.0	1.3	1.7	272.1	347.1	6.58	2.04
4	4.9	6.1	3.2	3.5	1.2	1.5	373.2	507.1	9.88	0.70
5	3.7	5.2	2.3	3.1	1.1	1.8	382.4	478.5	5.98	1.07
6	4.3	4.7	3.2	3.0	0.8	1.1	373.2	448.1	7.84	1.10
7	4.5	4.4	2.9	2.7	1.0	1.3	498.9	695.2	4.21	0.86
8	5.1	5.7	2.9	3.5	1.0	1.5	62.1	86.7	8.59	0.96
9	4.2	4.2	2.8	2.8	1.0	1.2	220.7	333.6	9.66	1.00
10	5.2	4.9	3.3	3.2	0.8	0.9	115.2	106.4	4.80	0.81
mean	4.8	5.2	3.1	3.3	1.1	1.4	277.9	376.5	7.32	1.15
±sd	±0.8	±1.0	±0.7	±0.8	±0.2	±0.3	±149.1	±222.0	±1.92	±0.57
Control	p=0.04		NS		p=0.002		p=0.004		p=0.002	
11	4.9	5.1	3.0	3.0	1.4	1.4	759.8	962.0	7.92	6.77
12	4.2	4.3	2.8	3.1	1.0	1.0	240.5	249.6	7.34	7.95
13	4.4	4.2	2.7	2.7	1.4	1.1	109.5	111.8	7.60	8.76
14	5.3	4.2	3.7	2.6	1.4	1.2	119.6	93.5	9.07	9.36
15	7.2	6.9	5.3	4.0	1.2	1.1	251.8	156.2	4.87	2.97
16	4.0	4.4	2.5	2.8	1.1	1.2	253.9	171.7	9.33	5.59
17	4.2	5.1	2.6	3.1	1.0	1.2	68.5	72.7	3.51	4.38
18	5.3	5.2	3.7	3.5	1.2	1.2	108.7	77.5	8.78	6.22
mean	4.9	4.9	3.3	3.1	1.2	1.2	239.0	236.9	7.30	6.50
±sd	±1.1	±0.9	±0.9	±0.5	±0.2	±0.1	±223.2	±298.9	±2.08	±2.18
	NS		NS		NS		NS		NS	

Table 4. Lipoprotein size by GGE and postprandial triglyceridaemia in triptorelin trial and control subjects

Subject	LDL GGE		HDL GGE		Fasting TG (mmol/L)		Area Under Curve (mmol/L.hr)		TG Excursion (mmol/L.hr)	
Triptorelin	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
1	I	A/I	2<3	2=3	1.1	0.6	13.6	9.5	6.5	4.3
2	A/I	A/I	2<3	2<3	0.9	1.3	13.4	17.1	5.6	8.9
3	I/B	I	2<3	2=3	1.2	0.7	21.2	15.0	13.7	8.4
4	I	I/B	2<3	2<3	1.5	2.6	13.4	25.2	4.3	11.8
5	I	I	2<3	2=3	1.2	0.7	16.7	16.1	6.6	9.3
6	I	I	2<3	2<3	1.3	1.7	14.6	16.0	9.1	7.9
7	I/B	I	2<3	2<3	1.2	0.8	19.5	18.8	8.1	12.1
8	I	I/B	2<3	2=3	0.8	1.5	12.9	18.0	5.8	10.8
9	I/B	I/B	2<3	2=3	1.0	0.6	17.1	10.5	10.0	4.9
10	I/B	I	2<3	2<3	1.3	2.0	23.8	20.7	15.1	9.1
mean					1.1	1.2	16.6	16.7	8.5	8.7
±sd					±0.2	±0.7	±3.8	±4.6	±3.5	±2.6
Control					NS		NS		NS	
11	I	I	2=3	2=3	1.9	1.5	21.6	18.1	10.7	9.0
12	I/B	I/B	2<3	2<3	0.8	0.6	11.8	11.2	6.6	5.1
13	I	I/B	2=3	2<3	0.6	0.8	7.6	13.9	3.1	8.6
14	A/I	A/I	2=3	2=3	0.6	0.7	9.3	13.9	5.9	8.1
15	I/B	B	2<3	2<3	1.4	3.9	17.3	53.5	6.7	24.5
16	I	I	2<3	2<3	0.9	1.0	13.2	18.7	7.0	9.5
17	I	I/B	2<3	2<3	1.1	1.6	12.1	23.2	5.3	9.0
18	I	I	2=3	2=3	0.9	1.1	11.4	18.9	5.4	11.9
mean					1.0	1.1	12.4	16.8	6.3	10.7
±sd					±0.5	±0.4	±4.5	±4.1	±2.2	±5.9
					NS		NS		NS	

Discussion

Plasma testosterone concentration was significantly reduced in a small group of normal men following the administration of triptorelin, a powerful GnRH agonist. It was found that after five weeks Lp(a) and HDLC concentration were significantly increased, whereas the concentrations of LDLC, triglyceride and the magnitude of postprandial triglyceridaemia were unchanged. The increased HDLC concentration was associated with a greater distribution of HDL particles in the HDL_2 size range in five of the triptorelin subjects. Four of the triptorelin subjects also showed an increase in LDL particle size. The results of this study suggest that endogenous testosterone has a suppressive effect on Lp(a) and HDLC concentration in normal men, without affecting LDLC and fasting triglyceride concentration or the magnitude of postprandial triglyceridaemia.

The significant increase in HDLC concentration associated with triptorelin induced hypoandrogenism, is consistent with other studies which have used either GnRH agonists or antagonists to induce hypoandrogenism (Bagatell *et al.*, 1992; Goldberg *et al.*, 1985). Although it is possible that triptorelin may have had a direct effect on HDL metabolism, Bagatell *et al.* (1992) and Goldberg *et al.* (1985) showed that HDLC concentration was restored following synthetic testosterone replacement, which led them to conclude that their respective GnRH agonist/antagonist had no intrinsic effect on plasma lipids. While this finding does not exclude an intrinsic effect unique to triptorelin, it is nevertheless unlikely. The significant reduction in HDLC concentration is also in keeping with the hypothesis that endogenous testosterone is the major determinant of sex differences in plasma HDLC concentration (Asscheman *et al.*, 1994; Goldberg *et al.*, 1985).

It should be noted that a suppression of testosterone in men also causes a significant secondary reduction in oestrogen, as much of the endogenous male oestrogen is derived from the aromatisation of testosterone (Bagatell *et al.*, 1992). Endogenous male oestrogen inhibits the suppressive effect of testosterone on HDLC and particularly HDL_2C concentration (Bagatell *et al.*, 1994a), apparently by counteracting the stimulatory effect of testosterone on HTGLa (Friedl *et al.*, 1990; Zmuda *et al.*, 1993). This in turn reduces the catabolic rate of HDL, and particularly HDL_2 (Tikkanen and Nikkilä, 1987). However, the stimulatory effect of oestrogen on HDLC and HDL_2C concentration does appear to be relatively minor compared to the powerful suppressive effect of testosterone. The reduced testosterone concentration induced

by triptorelin may cause a reduction in HTGLa, and increase HDLC concentration by reducing the catabolism of HDL. The increased HDLC concentration may also be caused by an increased synthesis of HDL. GGE studies indicate that a marked increase occurred in the HDL_2 size range in half of the T subjects. There are no previous studies which have described changes in the HDL profile in terms of particle size. This finding is, however, consistent with other studies which report a predominant increase in the cholesterol concentration in the HDL_2 rather than HDL_3 subfraction following changes in endogenous testosterone concentration (Asscheman *et al.*, 1994; Bagatell *et al.*, 1992).

An acute reversible suppression of testosterone was associated with a 26% increase in the mean Lp(a) concentration. This finding is consistent with a study by von Eckardstein *et al.* (1997) who reported a significant increase in Lp(a) concentration using the GnRH agonist cetrorelix. This finding is also consistent with a study by Berglund *et al.* (1996) who reported that orchidectomy in elderly men with prostatic carcinoma, resulted in a 20% increase in Lp(a) concentration, a change which was reversed following the replacement of testosterone. Moreover, synthetic testosterone supplementation significantly reduces Lp(a) concentration in both hypogonadal (Ozata *et al.*, 1996) and normal males (Marcovina *et al.*, 1996; Zmuda *et al.*, 1996). The reduction of testosterone concentration may influence Lp(a) concentration by permitting increased apo(a) synthesis or secretion, increased Lp(a) assembly, or reduced Lp(a) catabolism (Crook *et al.*, 1992). An elevated Lp(a) concentration is a risk factor for premature atherosclerosis, although the pathogenicity of Lp(a) does appear to be modulated by the concentration of LDLC (Armstrong *et al.*, 1986). The suppressive effect of testosterone on Lp(a) concentration in males may indicate an antiatherogenic effect of endogenous testosterone in males. It is, however, intriguing that there appears to be no sex difference in Lp(a) concentration (Jenner *et al.*, 1993).

The significant elevation in total cholesterol with triptorelin is accounted for mostly by the elevated HDLC concentration, and to a lesser extent by an elevated LDLC concentration in some individuals. The cause of the increased LDLC concentration in these subjects is unclear. Bagatell *et al.* (1992, 1994a) reported that suppression of both endogenous testosterone and oestrogen had no effect on LDLC concentration, whereas Goldberg *et al.* (1985) found that apoB was increased. GGE studies on LDL subfractions indicated a modest increase in the LDL particle

size in four triptorelin subjects, none of whom showed any remarkable changes in LDLC, HDLC or fasting triglyceride concentration compared to the other triptorelin subjects. The cause of the increased LDL particle size in these subjects is unclear, but may reflect a reduction in HTGLa, as HTGLa may be inversely associated with a preponderance of small, dense LDL (Watson *et al.*, 1994; Zambon *et al.*, 1993), and a lack of HTGL is accompanied by a massive triglyceride enrichment of LDL (Auwerx *et al.*, 1989; Breckenridge *et al.*, 1982). Endogenous testosterone may account in part for the tendency towards smaller, denser LDL in men compared to women (McNamara *et al.*, 1987), possibly through a stimulatory effect on HTGLa. The absence of a common pattern within the triptorelin group indicates that testosterone is probably not a major determinant of LDL size in men. This hypothesis is, however, not consistent with the finding that low concentrations of sex hormone-binding globulin and testosterone are associated with smaller, denser low density lipoprotein in normoglycemic men (Haffner *et al.*, 1996). The latter finding reflects the paradoxical observation that synthetic testosterone suppresses HDLC concentration, whereas endogenous testosterone is positively correlated with HDLC concentration (Bagatell and Bremner, 1995). It is thus possible that some factor which predisposes to small dense LDL also predisposes to a reduced testosterone concentration.

The fasting triglyceride concentration and the magnitude of postprandial triglyceridaemia were unaffected by an acute reduction in endogenous testosterone concentration. No previous study has assessed the effect of hypoandrogenism on postprandial triglyceridaemia. The results of the fat tolerance test conducted in the present study indicate that a possible decrease in HTGLa, caused by a reduction of testosterone, would be insufficient to significantly influence the clearance of triglyceride. The triptorelin induced hypoandrogenism does not appear to influence the other major lipolytic enzyme, LPL. Other studies which have assessed the effect of acute reversible hypoandrogenism on fasting triglyceride concentration in normal men have found no change (Bagatell *et al.*, 1992; Goldberg *et al.*, 1985). It is therefore unlikely that the elevated HDLC concentration, associated with a suppression of testosterone, is related to changes in postprandial triglyceridaemia as proposed by Patsch *et al.* (1983)

In summary, plasma testosterone concentration was significantly reduced after five weeks in normal men following the administration of triptorelin, a powerful GnRH agonist. The concentration of Lp(a) and HDLC were significantly increased, whereas the concentrations of

LDLC, fasting triglyceride and the magnitude of postprandial triglyceridaemia were unchanged, during the triptorelin induced hypogonadal state. The increased HDLC concentration was associated with a greater distribution of HDL particles in the HDL_2 size range in five triptorelin subjects. These results support the hypothesis that endogenous testosterone is the major determinant of the lower HDLC and particularly the lower HDL_2C concentration in men compared to women (Asscheman *et al.*, 1994). An increased LDL particle size in four triptorelin subjects indicates that endogenous testosterone may play a limited role in determining LDL size in men. Circulating testosterone does not appear to influence postprandial triglyceridaemia, indicating that the metabolic changes which permitted an increase in HDLC concentration are insufficient to influence the magnitude of postprandial triglyceridaemia, at least for the duration of the testosterone reduction obtained in this study. The suppressive effect of testosterone on Lp(a) concentration suggests an antiatherogenic effect of endogenous testosterone in men, although it is uncertain whether this effect will counteract the atherogenic effect of a reduced HDLC concentration.

Chapter 5: The effect of anabolic-androgenic steroids on postprandial triglyceridaemia and lipoprotein profiles in bodybuilders

Abstract

Although users of anabolic-androgenic steroid (AAS) are often regarded as having an increased risk of developing premature atherosclerosis, due to unfavourable plasma lipid changes, particularly in HDLC concentration, no direct link between AAS use and atherosclerosis has yet been made in humans. 'Atherogenic dyslipidaemia' is a phenotype characterised by increased postprandial triglyceridaemia, smaller, denser LDL particles and a low HDLC concentration, which is frequently associated with premature CHD. This study investigated how self administered AAS use in bodybuilders influences 'atherogenic dyslipidaemia', in an attempt to better describe their risk for premature atherosclerosis. The magnitude of postprandial triglyceridaemia, LDL and HDL particle size, and LDLC, HDLC and Lp(a) concentration were compared in male (n=9) and female (n=3) subjects after self administration of AAS for 5-6 weeks (on-cycle) and again after a 4-6 week ($\bar{x}$ = 5.5 weeks) 'washout' period. AAS use was confirmed by urinalysis. Lipoproteins were assessed by non-denaturing gradient gel electrophoresis (GGE), lipoprotein concentrations by immuno and enzymatic assay, and postprandial triglyceridaemia by a standardised oral fat tolerance test (65g/m^2). HDLC concentration decreased in male subjects (0.94±0.30 vs 0.70±0.27 mmol/L, p=0.004; $\bar{x}$ ± SD) and female subjects (1.3±0.5 vs 0.8±0.2 mmol/L) after using AAS. GGE studies suggested that mostly HDL_2 was reduced. No significant reduction in LDL particle size was found in subjects using AAS, and two subjects had slightly larger species when using AAS. Total cholesterol, triglyceride and LDLC concentration were unchanged when the subjects were using AAS. Lp(a) concentration decreased significantly in male subjects (124.7±128.0 to 69.3±73.3 U/L, p=0.008), decreased in two female subjects, and increased in the third subject while they were using AAS. The magnitude of postprandial triglyceridaemia was unchanged in females; when corrected for fasting triglyceride concentration, the reduction of postprandial triglyceridaemia was significant in males (p=0.027). These results suggest that postprandial triglyceridaemia and smaller, denser LDL are not increased in AAS users. Thus, a low HDLC concentration associated with AAS use is not likely to be due, in part, to an increased postprandial triglyceridaemia. Decreased Lp(a) concentration and decreased small, dense LDL particles and postprandial triglyceridaemia, in individuals who are predisposed to 'atherogenic dyslipidaemia', may be antiatherogenic effects of AAS.

Introduction

The use of anabolic-androgenic steroids (AAS) is usually associated with a marked reduction in HDLC concentration and frequently with an increase in LDLC concentration (Glazer, 1991). In population studies, the risk for an atherosclerotic event is strongly and inversely related to HDLC concentration (Gordon *et al.*, 1989). Moreover, among the risk factors for premature atherosclerosis, the LDLC/HDLC ratio is considered to be the most predictive (McGill, 1996). Accordingly, it has been concluded that AAS users have a significantly increased risk for premature atherosclerosis (Glazer 1991). However, no prospective or retrospective studies in humans are available to show that AAS users have an increased risk of premature atherosclerosis compared to matched controls. Studies in female animals indicate that AAS may induce premature atherosclerosis (Adams *et al.*, 1995; Obasanjo *et al.*, 1996), whereas studies on male animals are equivocal (Fogelberg *et al.*, 1990; Larsen *et al.*, 1993; Toda *et al.*, 1984). Unfortunately, none of these studies attempted to simulate the cyclical pattern of AAS use typically used by athletes and bodybuilders. Furthermore, it remains uncertain whether data from animal atherosclerosis models can be extrapolated to humans.

To date, there have been nineteen case reports documenting adverse cardiovascular events associated with the use of AAS in twenty-one men, most of whom were bodybuilders (Akter *et al.*, 1994; Appleby *et al.*, 1994; Bowman *et al.*, 1989; Bowman, 1990; Dickerman *et al.*, 1995; Ferenchick *et al.*, 1991; Ferenchick *et al.*, 1992; Fisher *et al.*, 1996; Frankle *et al.*, 1988; Huie, 1994; Jaillard *et al.*, 1994; Kennedy, 1993; Kennedy *et al.*, 1993a; Laroche, 1990; Luke *et al.*, 1990; Lyngberg, 1991; McNutt *et al.*, 1988; Mewis *et al.*, 1996; Mochizuki and Richter 1988; Siekierzynska-Czarnecka *et al.*, 1990). However, it is difficult to determine from these reports if AAS were responsible for the inferred atherosclerosis. Given that it is estimated that there are more than one million current or former AAS users in the United States (Yesalis *et al.*, 1993), and that AAS were first used by athletes as early as the 1950's (Strauss and Yesalis, 1991), it is perhaps surprising that the prevalence of complications due to premature atherosclerosis are not more common. Although it has been argued that individuals may be reluctant to admit AAS use, and the identification of AAS as a causative factor in

cardiovascular events is difficult, it is likely that the intensity of medical interest in reporting assumed cases may compensate for under reporting (Rockhold, 1993).

It has more recently become evident that a phenotype termed 'atherogenic dyslipidaemia' frequently rivals hypercholesterolaemia as a risk factor for premature atherosclerosis (Grundy, 1996). This phenotype is characterised by a low HDLC concentration, a predominance of small dense LDL particles and an abnormal triglyceride metabolism which can manifest as an exaggerated postprandial triglyceridaemia or a mild fasting hypertriglyceridaemia. Very little information exists about the possible effect of AAS on 'atherogenic dyslipidaemia'. Accordingly, the present study was undertaken to investigate the effect of AAS on the magnitude of postprandial triglyceridaemia and LDL particle size, so as to provide new insight into the effect of AAS on this phenotype. By combining this information with the effect of AAS on LDLC, HDLC and Lp(a) concentration it is hoped that the present study will better describe risk for premature atherosclerosis in AAS users.

Methods

Subjects

This study was approved by the Ethics and Research Committee of the University of Cape Town, Medical School. Since (i) AAS are widely believed to have potentially serious side effects, (ii) the use of AAS without prescription is unlawful in South Africa, and (iii) the recruitment of subjects for the study might be viewed as encouragement of the use of AAS, the Ethics Committee stipulated that all subjects be informed of the potential side effects of AAS, and be discouraged from using AAS by an impartial person, not directly associated with the study. The study was publicised at regional bodybuilding competitions and at local gymnasiums. Twelve subjects, nine men, and three women, volunteered to participate in the study, providing written informed consent prior to commencement. They had been practising body building for approximately five years (range 2 - 8 years) and trained between four and six times a week. Training sessions typically comprised 1-1½ hours of heavy resistance training and ½ hour of 'aerobic' training. All the subjects were in the 'bulking' phase of their training, apart from two female subjects (10 and 12) who were 'cutting' (dieting to reduce fat mass so as to improve

muscle definition for competition). Medical histories were collected, and each subject underwent a physical examination by a physician, electrocardiogram, and liver function tests (assessed by pathologists Penman and partners, Loop St. Cape Town) which included assessment of lactate dehydrogenase (LDH), bilirubin (total and conjugate), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamic transaminase (GGT), alkaline phosphatase (AP) total protein, total globulin and albumin concentration on each test day.

Anabolic-androgenic steroids

AAS used by participants were self-obtained and self-administered (Table 5). Subjects used a combination of both oral and parenteral AAS. All subjects, apart from one, (subject 6), had used AAS previously. Subjects 1,2,9 had used AAS for 5 years, subjects 4,7 for 4 years, subjects 11,12 for 3 years, and subjects 3, 5, 8, 10 for ≤ 1 year. The AAS users cycled on (ON) and off (OFF) use, with the OFF cycle, being regarded as a 'washout period', necessary to allow recuperation from any adverse effect of the ON cycle period. At the time of testing, most of the subjects had been OFF cycle for ≥ 6 weeks. Subjects 2 and 5 had chosen a 5 week OFF cycle, and subjects 7 and 9 had elected to spend only 4 weeks OFF cycle. The mean ON cycle period was 6.9 ± 1.8 weeks. The investigators were prohibited from giving subjects any advice regarding cycle lengths and AAS dosage. The two test days therefore occurred when subjects chose to finish each cycle. Seven of the subjects had their first test day at the end of their OFF cycle and five at the end of their ON cycle so as to prevent analytical bias due to test order. Subjects acted as their own controls.

Subjects agreed to use no AAS during their OFF cycle period. Urine samples were collected for urinalysis at the University of the Orange Free State doping control laboratory, an IOC accredited laboratory, to confirm the use and type of AAS both ON and OFF cycle (Table 8). The procedure for detection of AAS involved gas chromatography with a mass selective detector after deconjugation and derivitisation of the urine extracts (Van der Merwe and Kruger, 1992). Although the assay can only identify AAS type, in some cases a relative estimate of concentration could also be made.

Table 5. Bodybuilding and AAS use history, length of OFF and ON cycle, and cycle at first test

Subject	Bodybuilding history (years)	AAS use (years)	OFF cycle length (weeks)	ON cycle length (weeks)	Cycle at first test
1	6	5	6	6	ON
2	8	5	5	10	ON
3	2	0.5	8	7	ON
4	5	4	≥6	8	OFF
5	3	0.5	5	6	ON
6	3	-	-	7	OFF
7	5	4	4	5	OFF
8	6	1	6	6	ON
9	5	5	4	10	OFF
10	4	1	≥6	8	OFF
11	4	3	≥6	6	OFF
12	5	2	≥6	4	OFF

Subjects 10, 11 and 12 are female

Anthropometry

Anthropometric measurement was performed by the same experienced individual, using standardised anthropometric landmarks to locate the measuring sites (Ross and Marfell-Jones, 1991). Calculation of body fat percentage requires the measurement of four skinfold sites: triceps, biceps, subscapular, and suprailiac. The log of the sum of these measurements is substituted into the appropriate age and sex specific equation as described by Durnin and Womersley (1974). The calculation of muscle mass requires the measurement of stature, mid-thigh girth, mid-thigh skinfold, calf girth, calf skinfold, and forearm girth. The muscle mass is calculated by substitution into the appropriate equation as described by Martin *et al.* (1990).

Dietary and exercise record

Subjects were asked to complete three day dietary records (covering one weekend day and the two preceding or following weekdays) as close to the ON and OFF cycle test days as possible. Dietary analysis was performed using the Foodfundi® nutritional advice software (South African

Medical Research Council) to determine mean daily energy, protein, carbohydrate, fat, cholesterol, saturated, monounsaturated and polyunsaturated fat intake. Subjects were also asked to complete seven day physical activity diaries.

Plasma lipids and lipoproteins

The concentration of total, LDL and HDL cholesterol was evaluated by pathologists Penman and partners (Loop Street, Cape Town). These assays were done with commercially available enzyme kits on automated machines using conventional laboratory quality control techniques. Total cholesterol was confirmed in our laboratory using an enzymatic assay (Boehringer Mannheim kit no. 1442341). Apolipoprotein(a) was measured in the plasma using the Mercodia Apo(a) ELISA, enzyme immunoassay (Mercodia AB, Seminariegatan 29, S-752 28 Uppsala, Sweden). A detailed description of these methods can be found in the General Methodology chapter.

Oral fat tolerance test

The oral fat tolerance test was performed as described by Föger and Patsch (1993). Plasma triglyceride concentration was measured using a commercially available GPO PAP kit (Human, Germany, Kit no:10164.), and plotted against time. A detailed description of these methods can be found in the General Methodology chapter. Postprandial triglyceridaemia (mmol/L.hr) was calculated by integration of the area under the curve. Postprandial triglyceride excursion was corrected by subtracting the area attributable to the fasting triglyceride concentration from the total area under the curve. Postprandial triglyceride excursion was also calculated by subtracting the mean fasting and ten hour triglyceride concentrations (in subjects who were deemed to have returned to fasting concentration) from the total area under the curve, and found to produce the same results.

Non-Denaturing Gradient Gel Electrophoresis (GGE) for lipoproteins

The size of LDL and HDL particles were determined by polyacrylamide GGE using the Biorad minigel apparatus; 2-8% for LDL, and 4-18% for HDL. Samples from both the ON and OFF cycle test days were run on the same gel. Visual interpretation of the gels was confirmed by densitometric scan (Hoeffer Scientific Instruments GS300). A relative comparison of the amount of HDL at the OFF and ON cycle test could also be made by calculating the area under the curve

derived from the densitometric scan. A detailed description of these methods can be found in the General Methodology chapter.

Statistical analysis

A statistical software package (Graphpad Instat) was used for data analysis. Data are expressed as a mean ($\bar{x}$) ± standard deviation (sd). Data were analysed using non-parametric methods, using the Wilcoxon matched pairs test. A value of $p < 0.05$ was accepted to define statistical significance.

Results

Subject characteristics and anthropometry

The mean age was 25±2 years for the male, and 31±7 years for the female subjects (Table 6). Body mass of the male subjects increased significantly ON cycle from 96.2±11.1 to 99.7±11.1 kg ($p=0.004$). The range in the increase of body mass was from 1.2 to 9.3 kg, or 1.2% to 9.8% of starting body mass. This could be partially attributed to a significant increase in muscle mass from 60.8±7.6 to 62.5±7.5 kg ($p=0.03$), since there was no increase in fat mass ON cycle. Female subjects 10 and 11 had increased muscle mass ON cycle, whereas the other female subject had a decreased muscle mass. Body and fat mass decreased in subjects 10 and 12 and increased in subject 11.

Medical assessment

The subjects were all judged to be in good health following an examination by a medical practitioner. No clinically significant adverse effects of AAS use were detected by general medical examination or by ECG. Resting blood pressure was within the normal range for all subjects, and did not change with one cycle of AAS use (Table 7). Abdominal examination revealed no hepatomegaly in subjects who had used AAS previously.

Liver function tests revealed no extreme derangements, i.e. no deviations three times greater than the upper limit of the reference range (Table 7). In addition, there were no significant

differences between the mean OFF and ON cycle concentrations for any of the liver function tests. Subjects 2 and 8 had slight elevations (≤2 μmol/L above reference range) in total bilirubin concentration. The mean lactate dehydrogenase (LDH) activity, both OFF (325±79 U/L) and ON cycle (311±80 U/L) were greater than the reference range upper limit of 290 U/L. The mean OFF cycle aspartate aminotransferase (AST) activity (32±9 U/L) was greater than the reference range upper limit of 25 U/L. The mean ON cycle AST activity (35±16 U/L) was greater than OFF cycle, although not significantly. The mean alanine aminotransferase (ALT) activity, both OFF (42±12 U/L) and ON cycle (40±15 U/L) were greater than the reference range upper limit of 29 U/L. Alkaline phosphatase (AP) was elevated both OFF (227 U/L) and ON cycle (252 U/L) in subject 5, compared to the reference range upper limit of 207 U/L.

Table 6. Age and anthropometric values of AAS using bodybuilder subjects

Subject	age (yr)	height (cm)	body mass (kg)		muscle mass (kg)		fat mass (kg)	
			OFF cycle	ON cycle	OFF cycle	ON cycle	OFF cycle	ON cycle
1	27	183.0	109.0	110.6	71.1	74.4	11.5	11.9
2	26	178.5	107.2	111.7	64.8	66.0	14.7	15.1
3	22	170.3	77.3	81.8	48.4	53.6	9.7	9.8
4	27	170.5	88.7	90.9	56.3	57.6	11.1	13.7
5	21	182.9	93.2	97.3	56.2	55.9	20.0	17.1
6	22	183.3	95.1	104.4	61.3	63.8	15.1	17.0
7	27	183.0	104.0	105.2	67.7	66.5	10.6	10.1
8	26	178.0	85.0	86.4	53.8	54.3	9.5	9.6
9	25	185.1	106.1	108.9	67.5	70.3	13.9	12.4
mean	25	179.4	96.2	99.7	60.8	62.5	12.9	13.0
±sd	±2	±5.6	±11.1	±11.1	±7.6	±7.5	±3.4	±3.0
			p=0.004		p=0.03		NS	
10	25	172.2	69.5	66.9	41.5	42.4	9.5	7.8
11	30	172.2	75.5	77.7	41.2	43.7	14.4	16.0
12	39	170.3	66.7	64.0	36.9	33.1	13.1	11.1
mean	31	171.6	70.6	69.5	39.9	39.7	12.3	11.6
±sd	±7	±1.1	±4.5	±7.2	±2.6	±5.8	±2.5	±4.1

Subjects 10, 11 and 12 are female

NS - not statistically significant

Table 7. Blood pressure and liver function test data of AAS using bodybuilder subjects

Subjects		1	2	3	4	5	6	7	8	9	10	11	12	mean	±SD
BP	OFF	-	-	145/80	150/95	160/80	150/80	150/90	120/70	120/70	110/70	-	120/80		
mmHg	ON	-	140/90	135/75	145/95	130/75	140/70	140/80	140/70	130/80	130/80	100/70	130/90		
Bilir. total	OFF	13	13	10	10	12	14	20	*22	13	12	17	13	14	±4
	ON	12	*23	11	17	16	10	18	12	11	20	14	8	14	±4
Bilir. conj.	OFF	2	0	1	1	2	3	5	2	2	1	2	1	2	±1
	ON	1	5	1	2	4	1	2	0	5	3	1	0	2	±2
LDH	OFF	256	245	257	257	*311	*511	*415	*320	*357	*342	*355	268	325	±79
	ON	247	*361	247	243	*474	*401	*297	273	261	*412	272	247	311	±80
AST	OFF	22	*30	*31	*28	25	*35	*42	*28	*29	*34	*54	20	32	±9
	ON	*30	*59	*27	*28	*40	*74	*30	*27	*29	*40	24	16	35	±16
ALT	OFF	*30	*31	*53	*44	*40	28	*62	*49	*48	*35	*59	*30	42	±12
	ON	*33	*51	26	*33	*36	*62	*56	*41	*61	*44	22	18	40	±15
GGT	OFF	8	12	12	33	37	12	17	19	26	14	23	14	19	±9
	ON	8	13	13	21	35	12	11	12	16	15	19	13	16	±7
AP	OFF	112	142	114	154	*227	147	141	141	152	91	123	96	137	±36
	ON	109	127	178	129	*252	122	92	126	102	89	85	85	125	±48
Prot. total	OFF	70	74	66	77	74	75	78	68	72	76	73	63	72	±5
	ON	70	74	68	72	73	69	68	67	70	78	62	65	70	±4
Alb.	OFF	43	45	43	47	45	45	43	45	47	49	44	40	45	±2
	ON	44	48	44	44	43	42	42	43	41	50	40	43	44	±3
Glob. total	OFF	27	29	23	30	29	30	35	23	25	27	29	23	28	±4
	ON	26	26	24	28	30	27	26	24	29	28	22	22	26	±3

Reference ranges

Bilirubin (total)	8 - 21 μmol/L	Bilirubin (conjugate)	0 - 8 μmol/L	LDH	120 - 290 U/L
AST	0 - 25 U/L	ALT	0 - 29 U/L	GGT	8 - 38 U/L
AP	73 - 207 U/L	Protein total	60 - 85 g/L	Albumin	35 - 55 g/L
Globulin total	20 - 35 g/L				

* indicates values which fall outside the reference range

Subjects 10, 11 and 12 are female

Anabolic-androgenic steroid use

Some AAS reported by subjects were not detected by urinalysis (Table 8) including: Metonolone enanthate (subject 2), Oxymetholone (subjects 3,9), Methandrostenolone (subject 4), and Mesterolone (subjects 8,10). Mesterolone was detected in subject 12, although its use was not reported by the subject. Nandrolone was detected at the OFF cycle test in all subjects who had used AAS previously, excepting subjects 8, 11, 12 who had no detectable AAS. Nandrolone detected in subjects 4 and 9 OFF cycle could be attributed to use of this agent in a previous cycle. Testosterone and stanozolol were detected in subject 7 at the OFF cycle test, the first test day for this subject, and can be attributed to use of these agents in a previous cycle. Stanozolol was detected nine weeks later in the same subject, at the ON cycle test, although not reported by the subject for the ON cycle AAS regimen. Subjects 4 and 10 reported that their use of AAS was supplemented with growth hormone i.m. 25 iu's/wk for seven weeks.

Dietary and physical activity record

Seven male subjects each completed one dietary record, and only two subjects completed the second dietary record (Table 9). The other five subjects were not prepared to complete the second dietary record, claiming that their diets had not changed since the first test. Two male subjects failed to return their dietary records at any stage in the study. All three female subjects returned their first dietary record, two subjects returned the second dietary record, with the third subject claiming that her diet had not changed since the first dietary record. Three subjects completed activity records for both test days, and nine subjects completed either one only or neither. Some of the physical activity records were completed incorrectly, indicating that close supervision is required in order to obtain an accurate assessment of physical activity. Analysis of these data are thus not included.

Table 8. AAS use reported by bodybuilder subjects and as detected by urinalysis

Subj.	reported by subject ON cycle	mean dose /wk × n weeks	detected ON cycle	comments	detected OFF cycle	comments
1	Nandrolone decanoate Testosterone cypionate	200mg/wk.6 200mg/wk.6	Nandrolone Testosterone	very high medium	Nandrolone	~5%‡
2	Metonolone enanthate Testosterone propionate Testosterone cypionate Testosterone undecanoate	700mg/wk.8 1000mg/wk.10 200mg/wk.10 200mg/wk.10	Nandrolone Testosterone	medium high	Nandrolone	~70%
3	Nandrolone decanoate Testosterone fenylpropionate Oxymetholone	84mg/wk.7 315mg/wk.7 350mg/wk.7	Nandrolone Testosterone	high high	Nandrolone	~20%
4*	Testosterone cypionate Testosterone proprionate Methandrostenolone	550mg/wk.8 1200mg/wk.3 125mg/wk.1	Nandrolone Testosterone	very low medium	Nandrolone	very low
5	Nandrolone decanoate Testosterone cypionate	50mg/wk.6 200mg/wk.6	Nandrolone Testosterone	very high high	Nandrolone	~20%
6†	Nandrolone laurate Testosterone cypionate	215mg/wk.7 285mg/wk.7	Nandrolone Testosterone	-	none	-
7*	Nandrolone Testosterone cypionate	200mg/wk.6 200mg/wk.6	Nandrolone Testosterone Stanozolol	very high ~60% ~60%	Nandrolone Testosterone Stanozolol	~25% very high very high
8	Testosterone cypionate Testosterone proprionate Mesterolone	300mg/wk.2 1400mg/wk.6 1400mg/wk.3	Testosterone	medium	none	-
9*	Testosterone enanthate Testosterone fenylpropionate Testosterone proprionate Oxymetholone Stanozolol	800mg/wk.5 50mg/wk.5 1025mg/wk.8 625mg/wk.8 400mg/wk.10	Nandrolone Testosterone Stanozolol	~10% very high high	Nandrolone	medium
10*	Nandrolone laurate Mesterolone	75mg/wk.8 350mg/wk.8	Nandrolone	medium	Nandrolone	~20%
11*	Metenolone acetate Methandrostenolone	150mg/wk.6 15mg/wk.4	Metenolone Methandro-stenolone	-	none	-
12*	Metenolone acetate Boldenone	210mg/wk.4 125mg/wk.4	Metenolone Mesterolone Boldenone	-	none	-

Subjects 10, 11 and 12 are female

* - indicates that subject was OFF cycle prior to the first test

† - indicates subject with no prior AAS use

‡ - indicates approximately x% of amount detected in the other sample of the same subject.

Table 9. Analysis of dietary recall in AAS using bodybuilder subjects

Subject	energy (Kj)	protein (%)	CHO (%)	fat (%)	SF (g)	MUF (g)	PUF (g)	chol. (mg)
1 OFF	13791	27.6	50.5	21.9	22.1	25.5	18.5	1083
1 ON	15677	28.7	49.9	21.4	24.3	27.6	24.7	1009
3	10756	28.8	47.9	23.3	18.5	15.0	27.5	272
4	21840	27.5	59.6	12.9	11.2	29.4	16.7	428
5 OFF	8396	21.3	63.0	18.4	13.6	15.4	7.9	181
5 ON	16329	28.4	50.5	21.1	27.4	25.9	16.2	490
6	17710	31.7	51.6	16.7	19.4	21.7	27.9	420
8	10504	31.4	48.8	19.8	13.9	19.6	15.4	457
9	21806	20.5	55.5	24.0	50.1	44.2	28.8	551
mean	15201	27.3	53.0	19.9	22.3	24.9	20.4	543
±sd	±4817	±3.9	±5.2	±3.5	±11.7	±8.9	±7.2	±306
10	5014	48.3	24.7	27.0	5.9	22.1	5.0	122
11 OFF	7618	27.4	57.8	14.8	8.1	9.6	9.0	229
11 ON	7984	28.9	58.3	12.8	6.9	8.9	7.9	247
12 OFF	7909	34.7	54.4	10.9	5.5	7.2	6.6	325
12 ON	6909	36.4	55.5	8.1	3.0	4.4	5.7	252

Subjects 10, 11 and 12 are female

CHO - carbohydrate; SF - saturated fat; MUF - monounsaturated fat; PUF - polyunsaturated fat; chol. - cholesterol

Lipoprotein values

In the male subjects, mean total cholesterol and mean LDLC concentration tended to be lower ON cycle, although the trend was not statistically significant (Table 10). Total cholesterol decreased in two female subjects, and increased in the third female subject when ON cycle. LDLC concentration decreased in one female subject, showed no change in one and increased in the other. In the male subjects, mean HDLC concentration decreased consistently when subjects were ON cycle from 0.9±0.3 to 0.7±0.3 mmol/L (p=0.004). HDLC concentration decreased by 50% in two female subjects, and showed no change in the third. In the male subjects, Lp(a) concentration decreased significantly when subjects were ON cycle from 124.7±128.0 to 69.3±73.3 U/L, p=0.008 (median; 80.0 U/L OFF and 45.5 U/L ON cycle). The

Lp(a) concentration does not appear to decrease as a fixed percentage of the initial concentration. Lp(a) concentration decreased ON cycle in two female subjects, and increased in the third. Female subject 11 was characterised by a particularly high Lp(a) concentration.

Table 10. Lipid and lipoprotein values of fasted AAS using bodybuilder subjects

Subject	Total Chol. (mmol/L)		LDLC (mmol/L)		HDLC (mmol/L)		Lp(a) (Units/L)		Triglyceride (mmol/L)	
	OFF cycle	ON cycle	OFF cycle	ON cycle	OFF cycle	ON cycle	OFF cycle	ON cycle	OFF cycle	ON cycle
1	4.3	4.7	2.5	3.3	1.4	1.1	95.2	71.6	1.1	0.7
2	8.6	5.4	7.1	4.2	1.2	0.9	222.4	120.5	1.6	0.6
3	4.4	4.6	3.2	3.6	1.0	0.6	80.0	22.7	0.6	0.8
4	5.7	4.2	3.5	3.0	0.9	0.6	0.8	1.7	1.7	1.5
5	6.2	5.3	4.5	3.8	0.9	0.8	53.9	43.8	1.6	1.4
6	2.8	2.6	1.8	1.6	0.8	0.6	426.2	242.6	0.7	0.8
7	8.3	6.1	7.3	5.4	0.4	0.3	60.7	49.7	1.1	1.0
8	4.4	4.1	2.9	2.5	1.2	1.0	115.4	25.3	0.7	1.1
9	3.9	6.5	2.9	5.5	0.7	0.4	68.2	45.5	0.7	1.3
mean	5.4	4.8	4.0	3.7	0.9	0.7	124.7	69.3	1.1	1.0
±sd	±2.0	±1.2	±2.0	±1.3	±0.3	±0.3	±128.0	±73.3	±0.5	±0.3
	NS		NS		p=0.004		p=0.008		NS	
10	3.6	4.6	2.3	3.4	0.9	0.9	53.0	77.6	0.9	0.7
11	4.6	3.8	2.9	2.9	1.2	0.6	1556.5	1027.7	1.1	0.7
12	5.5	3.4	3.2	2.2	1.8	0.9	72.3	45.3	1.1	0.9
mean	4.6	3.9	2.8	2.8	1.3	0.8			1.0	0.8
±sd	±0.9	±0.6	±0.5	±0.6	±0.5	±0.2			±0.1	±0.1

Subjects 10, 11 and 12 are female

LDL gradient gel electrophoresis

Six of the male subjects showed no change in LDL size between OFF and ON cycle (Table 11); one subject had a decrease in LDL size from an A/I to an I pattern when ON cycle. The two male subjects showing the smaller B and I/B LDL size when OFF cycle, showed an increase in LDL particle size into the 'intermediate' size range when they were ON cycle. Subjects 4,5,6, had mid bands. Female subjects 10 and 11 showed no change in LDL size ON cycle; LDL in subject 12 decreased in size ON cycle from A to A/I.

HDL gradient gel electrophoresis

Five of the male subjects showed a reduction in HDL particles in the HDL_2 size range (Table 11). The other four male subjects showed no change in HDL profile ON cycle. HDL was not detectable by GGE in subject 9 when ON cycle and in subject 7 both OFF and ON cycle. Two of the female subjects showed a similar reduction in HDL size ON cycle; the third female subject showed no alteration in HDL profile ON cycle. The absence of a change in HDL GGE in female subject 10 was consistent with the finding that her HDLC concentration did not change ON cycle. Densitometric scans of HDL GGE in subjects 3 and 8 are provided in figures 1.1 - 1.4, to illustrate the reduction of HDL in the HDL_2 size range, OFF compared to ON cycle.

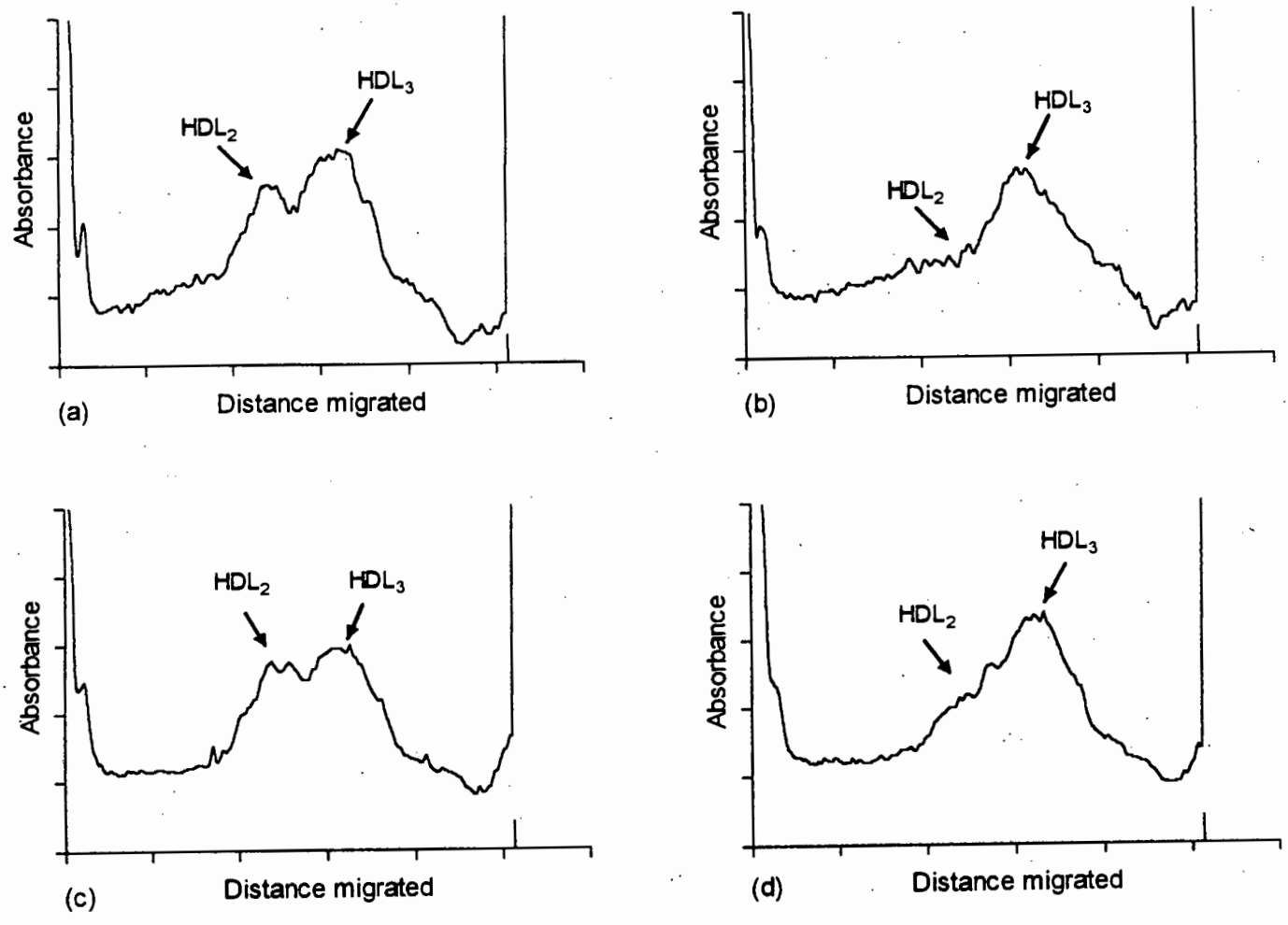

Figure 4. *Densitometric scans of HDL GGE for (a) subject 3 OFF cycle (2=3); (b) subject 3 ON cycle (2<3); (c) subject 8 OFF cycle (2>3); (d) subject 8 ON cycle (2<3). The high absorbance at the beginning of the scan indicates the line drawn on the GGE plates to show the start of the separating gel, to allow exactly reproducible comparisons of migration distance. The first small peak near the origin indicates LDL. The high absorbance at the end of the scan indicates the edge of the gel (units are arbitrary).*

Triglycerides and postprandial triglyceridaemia

Fasting triglyceride concentrations were unchanged when subjects were ON cycle (Table 6). Postprandial triglyceridaemia, expressed as the total area under the curve, tended to be reduced ON cycle, although not significantly (p=0.055) (Table 11). However, when corrected for fasting

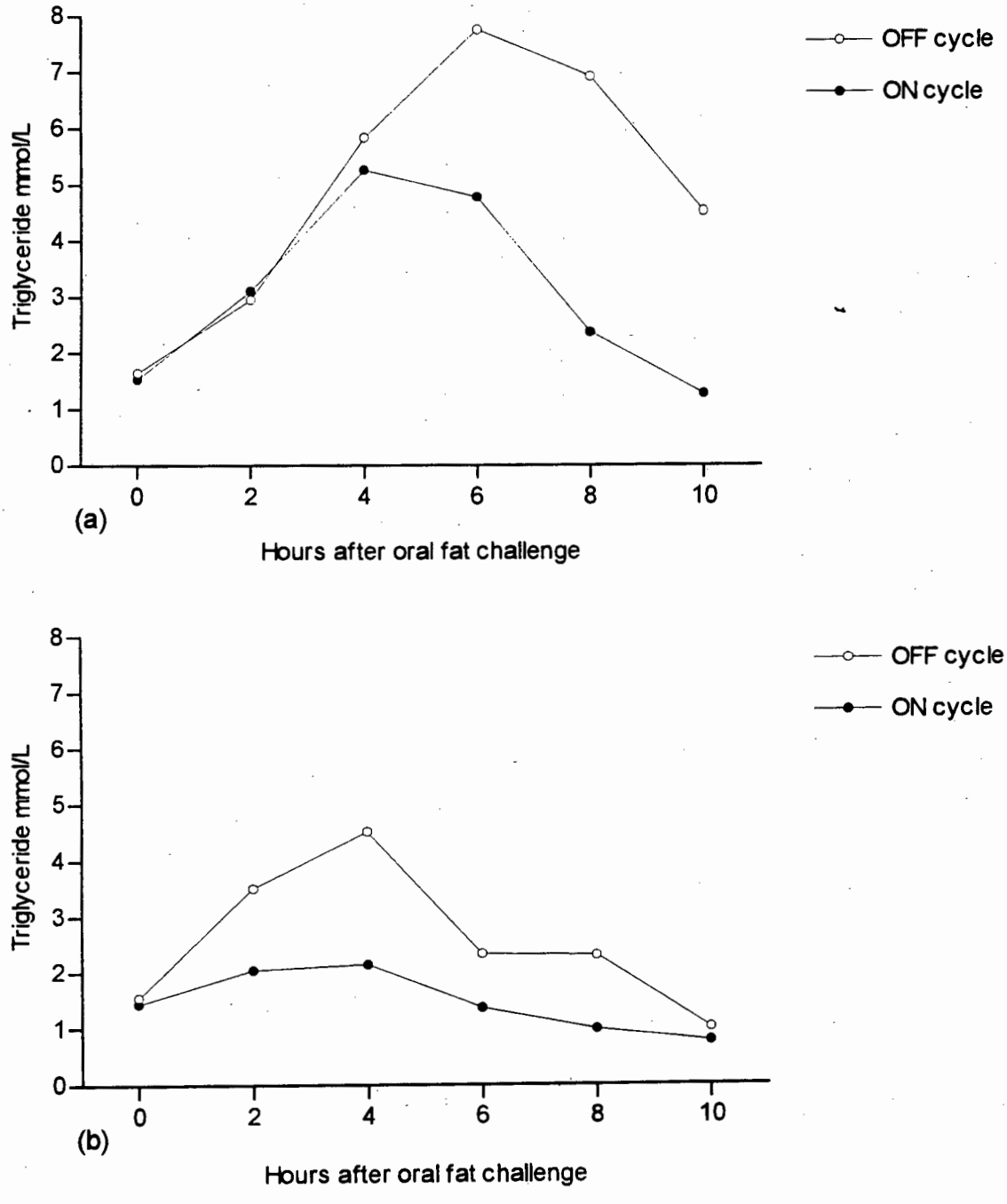

Figure 5. *Effect of a 65 g/m² oral fat challenge on plasma triglyceride concentration in (a) subject 4 OFF and ON AAS cycle; (b) subject 5 OFF and ON AAS cycle.*

triglyceride concentration, and expressed as a triglyceride excursion above fasting concentration, postprandial triglyceridaemia was significantly decreased ON cycle (p=0.027). Moreover, peak postprandial triglyceride concentration was also significantly reduced ON cycle (p=0.027). The two subjects who had type B and I/B LDL profiles OFF cycle showed 42 and 58% reductions respectively in postprandial triglyceridaemia when ON cycle (Figure 2.1, 2.2).

Table 11. Lipoprotein size by GGE and postprandial triglyceridaemia in AAS using bodybuilder subjects

Subject	LDL GGE		HDL GGE		Peak TG conc. (mmol/L)		Area Under Curve (mmol/L.hr)		Triglyceride excursion (mmol/L.hr)	
	OFF cycle	ON cycle	OFF cycle	ON cycle	OFF cycle	ON cycle	OFF cycle	ON cycle	OFF cycle	ON cycle
1	A/I	A/I	2<3	2<3	2.0	1.7	12.3	12.1	4.5	6.3
2	A/I	I	2=3	2=3	2.1	1.0	15.4	9.4	8.1	3.2
3	A	A	2=3	2<3	1.9	1.6	12.5	12.4	6.4	5.0
4	m,B	m,I	2<3	3	7.8	5.3	52.8	33.9	36.3	21.2
5	m,I/B	m,I	3	3	4.5	2.2	27.9	15.3	17.9	7.6
6	m,I	m,I	2<3	2<<3	1.6	1.5	10.8	10.8	5.9	5.2
7	pA,A	pA,A	flat	flat	2.5	2.0	18.6	11.9	7.2	4.3
8	A	A	2>3	2<3	2.2	2.5	16.4	14.3	9.1	8.8
9	I	I	2<3	flat	2.5	2.6	16.3	19.0	9.0	6.2
mean					3.0	2.3	20.1	15.7	11.6	7.5
±sd					±2.0	±1.2	±13.3	±7.5	±10.0	±5.4
					p=0.027		NS		p=0.027	
10	A/I	A/I	2<3	2<3	1.4	1.3	11.7	10.3	3.0	3.5
11	pA,A	pA,A	2=3	2<3	2.1	2.6	15.9	17.8	4.7	6.9
12	A	A/I	2=3	2<3	2.4	1.8	16.7	13.9	7.2	6.6
mean					2.0	1.9	14.8	14.0	5.0	5.7
±sd					±0.5	±0.7	±2.7	±3.8	±2.1	±1.9

Subjects 10, 11 and 12 are female

Discussion

Postprandial triglyceridaemia and lipoprotein profiles were assessed in a small group of bodybuilders before and after one cycle of self-administered, high dose AAS. The results of this study suggest that postprandial triglyceridaemia and smaller, denser LDL are not increased in individuals who self-administer AAS in a cyclical pattern. Thus, a low HDLC concentration associated with AAS use is unlikely to be due in part to an increased postprandial triglyceridaemia. Decreased Lp(a) concentration may be an antiatherogenic effect of AAS use. An increase in LDL size and a reduced postprandial triglyceridaemia may be additional antiatherogenic effects of AAS use in individuals who are predisposed to 'atherogenic dyslipidaemia'.

Urinalysis confirmed that all of the subjects were using AAS when ON cycle. Notably, AAS were still detectable after the OFF cycle period in the majority of the subjects. In most cases this was identified as nandrolone, a parenteral AAS. Nandrolone has a longer half life than the other AAS because the steroid nucleus is altered to slow the rate of inactivation (Wilson, 1988). One subject still had high concentrations of testosterone and stanozolol at the OFF cycle test, indicating that the short OFF cycle that he elected to take had not been sufficient to allow for significant AAS clearance from his previous heavy cycle. Although it is possible that this subject did not actually go OFF cycle, discussion with the subject indicated that this was not the case. The ON cycle urinalysis also revealed that some of the AAS used by the subjects were either counterfeit, or a different agent to that which they believed they were using, as was also found by Cohen *et al.* (1996). This did not influence the study adversely since the use of multiple AAS in these subjects provided alternatives.

Subject compliance with dietary recall analysis was poor, and nutritional data is thus incomplete. Nevertheless, some useful information was obtained. Total energy and cholesterol intake in the bodybuilding subjects was not excessive by 'Western standards', but tended rather to be lower than that typically seen in their sedentary counterparts (dietary analysis, Triptorelin study subjects, chapter 4), and lower than in another study on bodybuilders (Kleiner *et al.*, 1989). Total energy and cholesterol intake certainly did not reflect the widely held opinion that all bodybuilders eat 'enormous', cholesterol rich diets. A cholesterol intake of greater than 500 mg per day is nevertheless excessive relative to recommendations for a prudent diet

(Chait *et al.*, 1993). The percentage of total energy intake as fat was low, and the proportioning is roughly appropriate for prudent recommendations, but the absolute intake of saturated fat was a bit high. The fat intake of the bodybuilding subjects in this study appear to be lower than reported previously for bodybuilders (Kleiner *et al.*, 1989). Total dietary energy intake in subject 5 was double at the ON compared to the OFF cycle test five weeks later. An increase in energy intake for this duration is not expected to affect the magnitude of postprandial triglyceridaemia or other lipoprotein concentrations. However, the increase in dietary cholesterol intake in the range seen in this subject when ON cycle, would be expected to cause an increase in LDLC concentration. It is thus interesting that LDLC concentration decreased in this subject ON cycle despite an increased dietary cholesterol intake. While it is not possible to conclude that the other subjects in the present study did not alter their diet between the two test days, anecdotal accounts indicate that these subjects are acutely diet conscious, and tend to be regimental and habitual about their daily caloric intake, and were thus unlikely to have modified their dietary habits significantly.

Anthropometric measurement indicates that muscle mass was increased ON cycle in most of the subjects, a finding which is consistent with the view that AAS use with resistance training does increase muscle mass (Haupt and Rovere, 1984). The significant increase in mean body mass ON cycle can be attributed to a combination of increased muscle and fat mass in most subjects. The discrepancy evident in some subjects, and particularly subject 5, is frequently attributed to increased water retention with AAS use (Bagatell and Bremner, 1996).

Medical examination did not reveal elevated blood pressure or irregularities in hepatic function, sometimes reported with AAS use (Lenders *et al.*, 1988). Although AST, ALT and LDH activities were elevated, abnormalities in these liver function tests are also associated with weight training in the absence of AAS, and may not be good indicators of hepatic damage (Haupt and Rovere, 1984). Alkaline phosphatase activity was elevated in subject 5 only, although the magnitude of the elevation is not thought to be clinically significant. The slight elevations in bilirubin concentrations in subjects 2 and 12 are also not thought to be clinically significant.

Mean total cholesterol and LDLC concentration were not elevated by one cycle of AAS use, as is typically reported for studies of this type (Baldo-Enzi *et al.*, 1990; Faber *et al.*, 1986;

Hurley *et al.*, 1984; Kleiner *et al.*, 1989; Lajarin *et al.*, 1996; Lenders *et al.*, 1988; Webb *et al.*, 1984), but tended rather to be slightly reduced. The response of total cholesterol and LDLC concentration to one cycle of AAS use varied widely interindividually. The cause of the dramatic ON cycle reduction of total cholesterol and LDLC concentration in subjects 2 and 7, is unknown in subject 2, and was attributed to an acute phase reaction due to influenza at the beginning of the ON cycle of subject 7. Only one subject showed a marked increase in LDLC concentration ON cycle. The majority of subjects had LDLC concentrations less than 4.9 mmol/L, which are not considered to be clinically significant, especially since hypertension, diabetes and smoking were absent.

A significantly reduced HDLC concentration is consistently reported with AAS use (Alen and Rahkila, 1984; Alen *et al.*, 1985; Baldo-Enzi *et al.*, 1990; Faber *et al.*, 1986; Hurley *et al.*, 1984; Kleiner *et al.*, 1989; Kuipers *et al.*, 1991; Lajarin *et al.*, 1996; Lenders *et al.*, 1988; Peterson and Fahey, 1984; Webb *et al.*, 1984; Zuliani *et al.*, 1988). The low OFF cycle HDLC concentration in subjects 7 and 9 is compatible with the urinalysis finding that these subjects still had high and medium concentrations of plasma AAS activity respectively, and illustrates the sensitivity of HDLC concentration to the presence of AAS. The low HDLC concentration in female subject 10 is also likely to be due to an effect of residual plasma AAS. Reductions in HDLC concentration associated with the use of AAS, occur predominantly in the HDL_2 subfraction (Alen *et al.*, 1985; Baldo-Enzi *et al.*, 1990; Cohen *et al.*, 1986; Kantor *et al.*, 1985; Kleiner *et al.*, 1989; Hurley *et al.*, 1984; McKillop and Ballantyne, 1987). Changes in HDL subfractions have not previously been assessed by GGE studies of particle size. Results of these studies confirm that the reduction in HDL is in the HDL_2 subfraction in most cases. Exceptions include two subjects who had no detectable HDL_2 by GGE at the OFF cycle test, and two subjects in whom the reduction in HDL appeared to be equivalent in both subfractions. An increased lipolytic degradation due to an increased HTGLa is believed to be the principal cause of the reduced HDLC and particularly HDL_2C concentration in AAS users (Applebaum-Bowden, 1984; Baldo-Enzi *et al.*, 1990; Crook *et al.*, 1992; Haffner *et al.*, 1983).

The significant reduction in Lp(a) concentration with AAS use is consistent with findings in other studies (Albers *et al.*, 1984; Cohen *et al.*, 1996; Crook *et al.*, 1992; Farish *et al.*, 1995; Lajarin *et al.*, 1996). The wide variability in Lp(a) concentration in the general population is

reflected in the results obtained for the subjects in this study (Lawn, 1992). It is unknown why there is such a wide variability in the percentage reduction of Lp(a) in different individuals. AAS may affect Lp(a) concentration by reducing apo(a) synthesis or secretion, by impairing Lp(a) assembly, or by affecting Lp(a) catabolism (Crook *et al.*, 1992). Anabolic steroids modify the action of various enzyme systems, including hepatic hydrolytic enzymes, which could lead to an effect on synthesis or processing of Lp(a) (Crook *et al.*, 1992). It remains unknown whether reduction of Lp(a) concentration will reduce the incidence of atherosclerosis and CHD, as no suitable Lp(a) lowering therapy is currently available (Lawn and Scanu, 1996). A reduction in Lp(a) concentration is expected to reduce the risk of premature atherosclerosis and CHD, since a high concentration is associated with an increased risk of myocardial infarction (Armstrong *et al.*, 1986; Kostner *et al.*, 1981; Schaefer *et al.*, 1994), and Lp(a) shows proatherogenic and prothrombotic characteristics *in vitro* (Maher and Brown, 1995).

The size of LDL particles has not been described previously in the context of AAS use. A predominance of small, dense LDL in plasma is thought to be particularly atherogenic, and has been associated with up to a three-fold increase in risk of acute myocardial infarction (Austin *et al.*, 1988). Most of the subjects had average to good LDL profiles as judged by GGE, and showed no change in LDL size ON cycle. Interestingly, the two male subjects who displayed what is regarded as the more atherogenic B and I/B LDL size when OFF cycle, showed an increase in LDL particle size into the 'intermediate' size range when they were ON cycle. The use of AAS does not appear to be associated with any significant reduction in LDL particle size which might be viewed as atherogenic, but may be associated with an improvement in LDL size in individuals characterised by the smaller denser LDL.

The postprandial triglyceride excursion, as well as peak postprandial triglyceride concentration, were significantly reduced ON cycle. Two subjects had an exaggerated postprandial triglyceridaemia in the absence of an elevated fasting triglyceride concentration confirming, that in some individuals, an impaired triglyceride clearance is only revealed following a fat challenge (Patsch *et al.*, 1983). Both of these subjects showed marked reductions in postprandial triglyceridaemia ON cycle. Moreover, these subjects showed the small, dense LDL pattern OFF cycle, and the reduction in postprandial triglyceridaemia ON cycle was associated with an increase in the LDL particle size. This observation is consistent with the

hypothesis that an impaired triglyceride clearance, and consequent increased residence time of TGRL, is an important determinant of a reduced LDL particle size (Karpe *et al.*, 1993). Two studies have investigated the relationship between AAS and intravenous fat tolerance. Thompson *et al.* (1989) reported a non-significant increase in the capacity to clear intravenous fat with AAS treatment. Their data indicate that stanozolol, a 17α-alkylated (oral) AAS may have induced a greater fat clearance rate than testosterone enanthate, a 17β-esterified (parenteral) AAS. Olsson *et al.* (1974) reported a decrease in fasting triglyceride concentration, and a significant increase in the rate of intravenous fat clearance with oxandrolone treatment (7.5 mg/d) in patients with 'hyperlipoproteinaemia'.

It is unclear how AAS induce a reduction in triglyceridaemia following a fat challenge, although an increase in the activity of the lipase enzymes is the likely cause. While increases in LPLa associated with AAS are inconsistent, with some investigators reporting increases (Sorva *et al.*, 1988 ; Thompson *et al.*, 1989; Zmuda *et al.*, 1993), and others finding no change (Hazzard *et al.*, 1984; Haffner *et al.*, 1983; Lenders *et al.*, 1988; Taggart *et al.*, 1982), HTGL activity is consistently and dramatically increased with the use of 17α-alkylated (oral) AAS but not with 17β-esterified (parenteral) AAS (Applebaum-Bowden *et al.*, 1987; Friedl *et al.*, 1990; Haffner *et al.*, 1983; Taggart *et al.*, 1982; Thompson *et al.*, 1989). The latter finding may account for the difference in the rate of fat clearance between stanozolol and testosterone in the study by Thompson *et al.* (1989). The reduction in postprandial triglyceride excursion seen in this study may have been influenced by a reduced exercise intensity OFF compared ON to cycle, as an interruption of exercise training has been found to affect postprandial triglyceridaemia (Mankowitz *et al.*, 1992). However, none of the subjects indicated that they had changed their training regimen in any way between the two test days. Moreover, anecdotal accounts indicate that the study subjects are regimental and habitual about their daily exercise regimen. The reduction in the postprandial triglyceride excursion ON cycle is thus unlikely to have been influenced by a change in exercise intensity between the two tests.

In summary, postprandial triglyceridaemia and lipoprotein profiles were assessed in bodybuilders before and after one cycle of self-administered, high dose AAS. The results of this study suggest that postprandial triglyceridaemia and smaller, denser LDL are not increased in individuals who self-administer AAS in a cyclical pattern. Thus, a low HDLC concentration

associated with AAS use is unlikely to be due to an increased postprandial triglyceridaemia, as has previously been found in some other individuals with a low HDLC concentration (Patsch *et al.*, 1983). GGE studies indicate a predominant decrease of HDL particles in the HDL_2 size range. Mean LDLC concentration was not increased with AAS use. Decreased Lp(a) concentration may be an antiatherogenic effect of AAS use. An increase in LDL size and a reduced postprandial triglyceridaemia 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.

General Conclusions

The aim of the experimental work described in this thesis was to better describe the effect of endogenous testosterone and AAS on lipid and lipoprotein risk factors for premature atherosclerosis in humans, but was not designed to unravel the underlying mechanisms for the observations. Although it is widely believed that endogenous testosterone and AAS may be atherogenic in humans, a literature review reveals that there are few studies which have sought a direct association between these hormones, and premature atherosclerosis. Moreover, data from the studies that have been done is limited, and difficult to interpret. The slow progression of research in this field may be because ethical and practical constraints have limited the feasibility of conducting studies of sufficient scope and duration to conclusively show a difference in the initiation or progression of atherosclerosis. As a consequence of this, current opinion is dependent largely on the interpretation of inferential evidence.

Evidence to provide a direct link between endogenous and synthetic testosterone, and premature atherosclerosis, in otherwise normal men, is weak. The principal atherogenic effect of testosterone appears to be a suppression of HDLC concentration. While a lower HDLC concentration may be associated with a diminished reserve in reverse cholesterol transport and a reduction in the other protective effects of HDL, it remains unknown whether this change will in fact manifest as premature atherosclerosis, or whether testosterone has other protective effects which may mitigate the impact of a reduced HDLC concentration. The present triptorelin study did not reveal any additional atherogenic effects of endogenous testosterone. While testosterone may tend to reduce the size of LDL in some individuals, testosterone does not appear to play a major role in the regulation of LDL particle size. Moreover, testosterone does not appear to influence LDLC concentration or postprandial triglyceridaemia in an atherogenic manner. A suppressive effect on Lp(a) concentration may, however, represent an antiatherogenic effect of endogenous testosterone in men. Although the duration of the testosterone reduction obtained in the present triptorelin study was short, the results of this study do not favour the hypothesis that endogenous testosterone is a significant risk factor in men. The metabolic mechanism for the differences between individuals in lipoprotein particle size changes and Lp(a) concentration changes with testosterone suppression require further study.

Resolution of the true atherogenic potential of endogenous testosterone in human males will require a long term follow up of atherosclerotic progression in hypogonadal compared to eugonadal males. Needless to say, ethical and practical constraints make such a study unlikely. This issue could, however, be resolved with a fair degree of certainty by conducting further equivalent male animal studies, although this data will have to be extrapolated to humans with caution. Renewed interest in the therapeutic use of synthetic testosterone as a male contraceptive, and for hormone replacement therapy in elderly men, has recently led to a dramatic increase in the number of clinical trials which have supplemented synthetic testosterone in normal men. Data from equivalent but more prolonged trials may provide new insight into the potential atherogenicity of endogenous testosterone.

A lack of controlled trials into the effects of AAS in bodybuilders has hindered efforts to resolve whether these agents do in fact predispose users to premature atherosclerosis. Although the use of AAS causes a significant increase in the LDLC/HDLC ratio, no epidemiological data has been forthcoming to prove that AAS use is associated with premature atherosclerosis. Indeed, given the apparent atherogenicity of AAS, and the fact that AAS have been used since the 1950's, by more than one-million athletes and bodybuilders in the United States alone, it is surprising that there has not been an epidemic of AAS related atherosclerotic complications. It has been argued that the relative paucity of AAS related atherosclerotic complications may be because individuals are reluctant to admit AAS use, since non-therapeutic use of these agents is illegal in many countries (including South Africa), and because the identification of AAS as a causative factor in cardiovascular events is difficult. However, the intensity of medical interest in reporting assumed cases is likely to compensate for the afore mentioned problems (Rockhold, 1993). It is also possible that specific characteristics of athletes and bodybuilders, such as an increased consciousness about lean body mass, exercise and diet, may reduce atherosclerotic risk attributed to their AAS use.

Results from the present study do not reveal any further adverse effects of AAS. Rather, these results indicate that AAS use may have two potentially antiatherogenic effects; (i) by reducing postprandial triglyceridaemia and increasing LDL particle size in individuals who are predisposed to 'atherogenic dyslipidaemia', and (ii) by causing a marked suppression of Lp(a) concentration. Anecdotal accounts indicate that AAS users are not convinced that premature

atherosclerosis will be a likely consequence of their drug use. Controlled clinical trials comparing atherosclerotic progression (possibly using non-invasive techniques) in AAS using versus matched volunteers are urgently required to conclusively prove whether AAS use is in fact associated with premature atherosclerosis. In such studies, close attention will have to be paid to the possible thrombogenic effects of AAS, and also to the possibility that adverse effects of AAS use may differ in males compared to females.

References

Adams M.R., Williams J.K., Kaplan J.R. 1995 Effects of androgens on coronary artery atherosclerosis and atherosclerosis-related impairment of vascular responsiveness. *Arterioscler. Thromb. Vasc. Biol.* 15, 562-570

Akhter J., Hyder S., Ahmed M. 1994 Cerebrovascular accident associated with anabolic steroid use in a young man. *Neurology* 44, Dec, 2405-2406

Albers J.J., Taggart H.McA., Applebaum-Bowden D., Haffner S., Chestnut C.H. III, Hazzard W.R. 1984 Reduction of lecithin-cholesterol acyltransferase, apolipoprotein D and the Lp(a) lipoprotein with the anabolic steroid Stanozolol. *Biochimica et Biophysica Acta* 795, 293-296

Alén M., Rahkila P. 1984 Reduced high-density lipoprotein-cholesterol in power athletes: use of male sex hormone derivates, an atherogenic factor. *Int. J. Sports Med.* 5, 341-342

Alén M., Rahkila P., Marniemi J. 1985 Serum lipids in power athletes self-administering testosterone and anabolic steroids. *Int. J. Sports Med.* 6, 139-144

Alexandersen P., Haarbo J., Christiansen C. 1996 The relationship of natural androgens to coronary heart disease. *Atherosclerosis* 125, 1-13

Anderson R.A., Wallace E.M., Wu F.C. 1995 Effect of testosterone enanthate on serum lipoproteins in man. *Contraception* 52(2), 115-9

Applebaum-Bowden D. 1984 Sex steroid effects on HDL_2 and lipases. *Clin. Chem.* 30(6), 1081

Applebaum-Bowden D., Haffner S.M., Hazzard W.R. 1987 The dyslipoproteinemia of anabolic steroid therapy: increase in hepatic triglyceride lipase precedes the decrease in high density $lipoprotein_2$ cholesterol. *Metabolism* 36(10), 949-952

Appleby M., Fisher M., Martin M. 1994 Myocardial infarction, hyperkalaemia and ventricular tachycardia in a young male body-builder. *Int. J. Cardiology* 44, 171-174

Armstrong V.W., Cremer P., Eberle E., Manke A., Schulze F., Wieland H. 1986 The association between serum Lp(a) concentrations and angiographically assessed coronary atherosclerosis. Dependence on serum LDL levels. *Atherosclerosis*, 62: 249-257

Asscheman H., Gooren L.J.G., Megens J.A.J., Nauta J., Kloosterboer H.J., Eikelboom F. 1994 Serum testosterone level is the major determinant of the male-female differences in serum levels of high-density lipoprotein (HDL) cholesterol and HDL_2 cholesterol. *Metabolism* 43(8), 935-939

Assmann G., Schulte H. 1992 Relation of high-density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease (the PROCAM experience). *Am. J. Cardiol.* 70, 733-737

Austin M.A., Breslow J.L., Hennekens C.H., Burling J.E., Willett W.C., Krauss R.M. 1988 Low density lipoprotein subclass patterns and the risk of myocardial infarction. *J. Am. Med. Assoc.*, 260, 1917

Austin M.A., Hokanson J.E. 1996 Emerging evidence for triglyceride as a risk factor for coronary artery disease: a review of the epidemiologic data. *In* Drugs Affecting Lipid Metabolism: Risk Factors and Future Directions. Gotto A.M. Jr., Paoletti R., Smith L.C., Catapano A.L., Jackson A.S., editors. Kluwer Academic Publishers, Dordrecht, ISBN 0-7923-4167-8. 187-194

Auwerx J.H., Marzetta C.A., Hokanson J.E., Brunzell J.D. 1989 Large buoyant LDL-like particles in hepatic lipase deficiency. *Arteriosclerosis* 9(3), 319-325

Bagatell C.J., Knopp R.H., Vale W.W., Rivier J.E., Bremner W.J. 1992 Physiologic testosterone levels in normal men suppress high-density lipoprotein cholesterol levels. *Annals Int. Med.* 116(12:1), 967-973

Bagatell C.J., Heiman J.R., Matsumoto A.M., Rivier J.E., Bremner W.J. 1994 Metabolic and behavioural effects of high-dose, exogenous testosterone in healthy men. *J. Clin. Endocrinol. Metab.* 79, 561-567

Bagatell C.J., Knopp R.H., Rivier J.E., Bremner W.J. 1994a Physiological levels of estradiol stimulate plasma high density lipoprotein$_2$ cholesterol levels in normal men. *J. Clin. Endocrinol. Metab.* 78, 855-861

Bagatell C.J., Bremner W.J. 1995 Androgen and progestagen effects on plasma lipids. *Prog. in Cardiovasc. Diseases* 38(3), 255-271

Bagatell C.J., Bremner W.J. 1996 Androgens in men - uses and abuses. *New Engl. J. Med.* 334(11), 707-714

Baldo-Enzi G., Giada F., Zuliani G., Baroni L., Vitale E., Enzi G., Magnanini P., Fellin R. 1990 Lipid and apoprotein modifications in body builders during and after self-administration of anabolic steroids. *Metabolism* 39(2), 203-208

Baumstark M., Berg A., Frey I., Rokitzki L., Keul J. 1988 Analphalipoproteinemia in body-builders induced by anabolic steroids. *Int. J. Sports Med.* 9, 400

Berglund L., Carlström K., Stege R., Gottlieb C., Eriksson M., Angelin B., Henriksson P. 1996 Hormonal regulation of serum lipoprotein (a) levels: effects of parenteral administration of estrogen or testosterone in males. *J. Clin. Endocrinol. Metab.* 81(7), 2633-2637

Bhasin S., Storer T.W., Berman N., Callegari C., Clevenger B., Phillips J., Bunnell T.J., Tricker R., Shirazi A., Casaburi R. 1996 The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. *N. Engl. J. Med.* 335(1), 1-7

Blades B., Vega G.L., Grundy S.M. 1993 Activities of lipoprotein lipase and hepatic triglyceride lipase in postheparin plasma of patients with low concentrations of HDL cholesterol. *Arterioscler. Thromb.* 13, 1227-1235

Blankenhorn D.M., Nessim S.A., Johnson R.L., Sanmarco M.E., Azen S.P., Cashin-Hemphill L. 1987 Beneficial effects of combined colestipol-niacin therapy on coronary atherosclerosis and coronary venous bypass grafts. *JAMA* 257(23), 3233-3240

Bowman S., Tanna S., Fernando S., Ayodeji A., Weatherstone R.M. 1989 Anabolic steroids and infarction. *Br. Med. J.* 299, Sept 2, 632

Bowman S. 1990 Anabolic steroids and infarction. *Br. Med. J.* 300, Mar 17, 750

Bradley W.A., Gianturco S.H. 1994 Triglyceride-rich lipoproteins and atherosclerosis: pathophysiological considerations. *J. Intern. Med.* 236(S736), 33-39

Breckenridge W.C., Little J.A., Alaupovic P., Wang C.S., Kuksis A., Kakis G., Lindgren F., Gardiner G. 1982 Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. *Atherosclerosis* 45(2), 161-179

Brodsky I.G., Balagopal P., Nair K.S. 1996 Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men - a clinical research center study. *J. Clin. Endocrinol. Metab.* 81(10), 3469-75

Brown G., Albers J.J., Fisher L.D., Schaefer S.M., Lin J.T., Kaplan C., Zhao X.Q., Bisson B.D., Fitzpatrick V.F., Dodge H.T. 1990 Regression of coronary artery disease as a result of intensive lipid-lowering therapy in men with high levels of apolipoprotein B. *N. Engl. J. Med.* 323(19), 1289-1298

Campos H., Genest J.J. Jr., Blijlevens E., McNamara J.R., Jenner J.L., Ordovas J.M., Wilson P.W.F., Schaefer E.J. 1992 Low density lipoprotein particle size and coronary artery disease. *Arterioscler. Thromb.* 12(2), 187-195

Carey M.C., Small D.M., Bliss C.M. 1983 Lipid digestion and absorption. *Ann. Rev. Physiol.* 45, 651-677

Casassus P., Fontbonne A., Thibult N., Ducimetiere P., Richard J.L., Claude J.R., Warnet J.M., Rosselin G., Eschwege E. 1992 Upper body fat distribution: a hyperinsulinaemia-independent predictor of coronary heart disease mortality. The Paris Prospective Study. *Arterioscler. Thromb.* 12(12), 1387-1392

Castelli W.P. 1988 Cardiovascular disease in women. *Am. J. Obstet. Gynecol.* 158 (6), 1553-1560

Chait A., Brazg R.L., Tribble D.L., Krauss R.M. 1993 Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. *Am. J. Med.*, 94(4), 350-356

Chait A., Brunzell J.D., Denke M.A., Eisenberg D., Ernst N., Franklin F.A. Jr., Ginsberg H., Kotchen T.A., Kuller L., Mullis R.M., Nichaman M.Z., Nicolosi R.J., Schaefer E.J., Stone N.J., Weiman W.H. 1993 Rationale of the Diet-Heart Statement of the American Heart Association: report of the nutrition committee. *Circulation* 88(6), 3008-3029

Cheung M.C., Albers J.J., Wahl P.W., Hazzard W.R. 1980 High density lipoproteins during hypolipidemic therapy. A comparative study of four drugs. *Atherosclerosis* 35(3), 215-228

Chisolm G.M. 1991 Antioxidants and atherosclerosis: a current assessment. *Clin. Cardiol.* 14, 125-130

Chou T.M., Sudhir K., Hutchison S.J., Ko E., Amidon T.M., Collins P., Chatterjee K. 1996 Testosterone induces dilation of canine coronary conductance and resistance arteries in vivo. *Circulation* 94, 2614-2619

Chung B.H., Segrest J.P., Smith K., Griffin F.M., Brouillette C.G. 1989 Lipolytic surface remnants of triglyceride-rich lipoproteins are cytotoxic to macrophages but not in the presence of high density lipoprotein. A possible mechanism of atherogenesis? *J. Clin. Invest.*, 83(4), 1363-1374

Cohen J.C., Faber M., Benadé A.J.S., Noakes T.D. 1986 Altered serum lipoprotein profiles in male and female power lifters ingesting anabolic steroids. *Phys. Sportsmed.* 14(6), 131-136

Cohen J.C., Noakes T.D., Benadé A.J.S. 1988 Hypercholesterolemia in male power lifters using anabolic-androgenic steroids. *Phys. Sportsmed.* 16(8), 49-56

Cohen J.C., Stray-Gundersen J., Grundy S.M. 1991 Dissociation between postprandial lipemia and high density lipoprotein cholesterol concentrations in endurance-trained men. *Arterioscl. Thromb.* 11, 838-843

Cohen J.C., Grundy S.M. 1992 Normal postprandial lipemia in men with low plasma HDL concentrations. *Arterioscler. Thromb.* 12, 972-975

Cohen J.C., Wang Z., Grundy S.M., Regier Stoesz M., Guerra R. 1994 Variation at the hepatic lipase and apolipoprotein AI/CIII/AIV loci is a major cause of genetically determined variation in plasma HDL cholesterol levels. *J. Clin. Invest.* 94, 2377-2384

Cohen L.I., Hartford C.G., Rogers G.G. 1996 Lipoprotein (a) and cholesterol in body builders using anabolic androgenic steroids. *Med. Sci. Sports Exerc.* 28(2), 176-179

Cohn J.S., McNamara J.R., Krasinski S.D., Russell R.M., Schaefer E.J. 1989 Role of triglyceride-rich lipoproteins from the liver and intestine in the etiology of postprandial peaks in plasma triglyceride concentration. *Metabolism* 38(5), 484-490

Connelly P.W., Maguire G.F., Lee M., Little J.A. 1990 Plasma lipoproteins in familial hepatic lipase deficiency. *Arteriosclerosis* 10, 40-48

Costill D.L., Pearson D.R., Fink W.J. 1984 Anabolic steroid use among athletes: changes in HDL-c levels. *Phys. Sportsmed.* 12(6), 113-117

Cowart V.S. 1989 Support lags for research on steroid effects. *JAMA* 262(18), 2500-2502

Criqui M.H., Wallace R.B., Heiss G., Mishkel M., Schonfeld G., Jones G.T.L. 1980 Cigarette smoking and plasma high-density lipoprotein cholesterol. *Circulation* 62(IV), 70-76

Criqui M.H. 1996 Triglycerides as a risk factor for coronary heart disease: epidemiologic studies and clinical trials. *In* Drugs Affecting Lipid Metabolism: Risk Factors and Future Directions. Gotto A.M. Jr., Paoletti R., Smith L.C., Catapano A.L., Jackson A.S., editors. Kluwer Academic Publishers, Dordrecht, ISBN 0-7923-4167-8. 203-210

Crist D.M., Peake G.T., Stackpole P.J. 1986 Lipemic and lipoproteinemic effects of natural and synthetic androgens in humans. *Clin. Exp. Pharm. Phys.* 13, 513-518

Crook D., Sidhu M., Seed M., O'Donnell M., Stevenson J.C. 1992 Lipoprotein Lp(a) levels are reduced by danazol, an anabolic steroid. *Atherosclerosis* 92, 41-47

Dahlgren E., Janson P.O., Johansson S., Lapidus L., Oden A. 1992 Polycystic ovary syndrome and risk for myocardial infarction. Evaluated from a risk factor model based on a prospective population study of women. *Acta. Obstet. Gynecol. Scand.* 71(8), 599-604

De Stefano V., Leone G., Teofili L., Ferelli R., Pollari G. 1988 Transient ischemic attack in a patient with congenital protein-C deficiency during treatment with stanozalol. *Am. J. Hematol.* 29, 120-121

Dickerman R.D., Schaller F., Prather I., McConathy W.J. 1995 Sudden cardiac death in a 20-year-old bodybuilder using anabolic steroids. *Cardiology* 86, 172-173

Dionyssiou-Asteriou A., Katimertzi M. 1993 Endogenous testosterone and serum apolipoprotein(a) levels. *Atherosclerosis* 100, 123-126

Dormans T.P.J., Swinkels D., de Graaf J., Hendriks J.C.M., Stalenhoef A.F.H., Demacker P.N.M. 1991 Single spin density ultracentrifugation vs gradient gel electrophoresis: two methods for detecting low density lipoprotein heterogeneity compared. *Clin. Chim.* 37, 853-858

Drexel H., Muntwyler J., Beran J., Schmid H., Gasser T., Amann F.W. 1996 Triglycerides are independently predictive of the extent of coronary atherosclerosis. *In* Drugs Affecting Lipid Metabolism: Risk Factors and Future Directions. Gotto A.M. Jr., Paoletti R., Smith L.C., Catapano A.L., Jackson A.S., editors. Kluwer Academic Publishers, Dordrecht, ISBN 0-7923-4167-8. 195-202

Durnin J.V.G.A., Womersley J. 1974 Body fat assessed from the total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Brit. J. Nutr.* 32: 77-97

Ebenbichler C.F., Kirchmair R., Egger C., Patsch J.R. 1995 Postprandial state and atherosclerosis. *Curr. Opin. Lipidol.* 6(5), 286-290

Eisenberg S. 1984 High density lipoprotein metabolism. *J. Lipid Res.* 25, 1017-1058

Ernst N., Fisher M., Smith W., Gordon T., Rifkind B.M., Little J.A., Mishkel M.A., Williams O.D. 1980 The association of plasma high-density lipoprotein cholesterol with dietary intake and alcohol consumption. *Circulation* 62(IV), 41-52

Faber M., Benadé A.J.S., van Eck M. 1986 Dietary intake, anthropometric measurements, and blood lipid values in weight training athletes (body builders). *Int. J. Sports Med.* 7, 342-346

Farish E., Barnes J.F., Rolton H.A., Spowart K., Fletcher C.D., Hart D.M. 1995 Effects of tibolone on lipoprotein(a) and HDL subfractions. *Maturitas* 20, 215-219

Ferenchick G.S. 1990 Are androgenic steroids thrombogenic? *N. Engl. J. Med.* 322, 476

Ferenchick G.S. 1991 Anabolic/androgenic steroid abuse and thrombosis: is there a connection? *Medical Hypotheses* 35, 27-31

Ferenchick G.S., Kirlin P., Potts R 1991 Steroids and cardiomyopathy: how strong a connection? *Phys. Sportsmed.* 19(9), 107-110

Ferenchick G.S., Adelman S. 1992 Myocardial infarction associated with anabolic steroid use in a previously healthy 37-year-old weight lifter. *American Heart Journal* 124(2), 507-508

Ferenchick G.S., Schwartz D., Ball M., Schwartz K. 1992 Androgenic-anabolic steroid abuse and platelet aggregation: a pilot study in weight lifters. *Am. J. Med. Sci.* 303(2), 78-82

Fielding C.J., Fielding P.E. 1995 Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* 36, 211-228

Fisher M., Appleby M., Rittoo D., Cotter L. 1996 Myocardial infarction with extensive intracoronary thrombus induced by anabolic steroids. *Brit. J. Clin. Practice* 50(4), 222-223

Fogelberg M., Björkhem I., Diczfalusky U., Henriksson P. 1990 Stanozolol and experimental atherosclerosis: atherosclerotic development and blood lipids during anabolic steroid therapy in New Zealand White rabbits. *Scand. J. Clin. Lab. Invest.* 50, 693-696

Föger B., Patsch J.R. 1993 Strategies and methods for the assessment of disturbed postprandial lipid metabolism. *Curr. Opin. Lipidol.* 4, 428-433

Franceschini G, Sirtori CR, Capurso A, Weisgraber KH, Mahley RW.1980 AI_{Milano} apoprotein: Decreased high density lipoprotein cholesterol levels with significant lipoprotein modifications and without clinical atherosclerosis in an Italian family. *J Clin Invest* 66, 892-900

Frankle M.A., Eichberg R., Zachariah S.B. 1988 Anabolic androgenic steroids and a stroke in an athlete: case report. *Arch. Phys. Med. Rehabil.* 69, 632-633

Friedl K.E., Hannan C.J., Jones R.E., Plymate S.R. 1990 High-density lipoprotein cholesterol is not decreased if an aromatizable androgen is administered. *Metabolism* 39(1), 69-74

Gambert P., Bouzerand-Gambert C., Athias A., Farnier M., Lallemant C. 1990 Human low density lipoprotein subfractions separated by gradient gel electrophoresis: composition, distribution, and alterations induced by cholesteryl ester transfer protein. *J. Lipid Res.* 31(7), 1199-1210.

Gaubatz J.W., Heideman C., Gotto A.M. Jr., Morrisett J.D., Dahlen G.H. 1983 Human plasma lipoprotein (a): structural properties. *J. Biol. Chem.* 258, 4582-4589

Geigy Scientific Tables, Volume 3, 1984 Ciba-Geigy Ltd. Basle, ISBN 0-914168-52-5. 329

Glazer G. 1991 Atherogenic effects of anabolic steroids on serum lipid levels. *Arch. Intern. Med.* 151, 1925-1933

Glazer G., Suchman A.L. 1994 Lack of demonstrated effect of nandrolone on serum lipids. *Metabolism* 43(2), 204-210

Glueck C.J., Taylor H.L., Jacobs D., Morrison J.A., Beaglehole R., Williams O.D. 1980 Plasma high-density lipoprotein cholesterol: association with measurements of body mass. *Circulation* 62(IV), 62-69

Godsland I.F., Wynn V., Crook D., Miller N.E. 1987 Sex, plasma lipoproteins, and atherosclerosis: prevailing assumptions and outstanding questions. *Am. Heart J.* 114, 1467-1503

Goh H.H., Loke D.F., Ratnam S.S. 1995 The impact of long-term testosterone replacement therapy on lipid and lipoprotein profiles in women. *Maturitas* 21(1), 65-70

Goldberg R.B., Rabin D., Alexander A.N., Doelle G.C., Getz G.S. 1985 Suppression of plasma testosterone leads to an increase in serum total and high density lipoprotein cholesterol and apoproteins A-I and B. *J. Clin. Endocrinol. Metab.* 60(1), 203-207

Gordon T., Kannel W.B., Castelli W.P., Dawber T.R., 1981 Lipoproteins, cardiovascular disease, and death. The Framingham Study. *Arch. Intern. Med.*, 141(9), 1128-1131

Gordon D.J., Probstfield J.L., Garrison R.J., Neaton J.D., Castelli W.P., Knoke J.D., Jacobs D.R. Jr., Bangdiwala S., Tyroler A. 1989 High-density lipoprotein cholesterol and cardiovascular disease - four prospective American studies. *Circulation* 79, 8-15

Groot P.H.E., van Stiphout W.A.H.J., Krauss X.H., Jansen H., van Tol A., van Ramshorst E., Chin-On S., Hofman A., Cresswell S.R., Havekes L. 1991 Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. *Arterioscler. Thromb.* 11, 653-662

Grundy S.M. 1996 Lipids, Nutrition, and Coronary Heart Disease. *In* Atherosclerosis and Coronary Artery Disease. Fuster V., Ross R., Topol E.J., editors. Lippincott-Raven Publishers, Philadelphia, ISBN 0-7817-0266-6. 45-68

Guyton A.C. 1986 Digestion and Absorption in the Gastrointestinal Tract. *In* Textbook of Medical Physiology, seventh edition, Igaku-Shoin / Saunders International Edition, ISBN 4-7557-0113-9. 787-796

Haffner S.M., Kushwaha R.S., Foster D.M., Applebaum-Bowden D., Hazzard W.R. 1983 Studies on the metabolic mechanism of reduced high density lipoproteins during anabolic steroid therapy. *Metabolism* 32(4), 413-420

Haffner S.M., Mykkänen L., Gruber K.K., Rainwater D.L., Laakso M. 1994 Lack of association between sex hormones and Lp(a) concentrations in American and Finnish men. *Arterioscler. Thromb.* 14, 19-24

Haffner S.M., Laakso M., Miettinen H., Mykkänen L., Karhapää P., Rainwater D.L. 1996 Low levels of sex hormone-binding globulin and testosterone are associated with smaller, denser low density lipoprotein in normoglycemic men. *J. Clin. Endocrinol. Metab.* 81, 3697-3701

Handa K., Ishii H., Kono S., Shinchi K., Imanishi K., Mihara H., Tanaka K. 1997 Behavioural correlates of plasma sex hormones and their relationships with plasma lipids and lipoproteins in Japanese men. *Atherosclerosis* 130, 37-44

Haupt H.A., Rovere G.D. 1984 Anabolic steroids: a review of the literature. *Am. J. Sports Med.* 12(6), 469-484

Hazzard W.R., Haffner S.M., Kushwaha R.S., Applebaum-Bowden D., Foster D.M. 1984 Preliminary report: Kinetic studies on the modulation of high-density lipoprotein , apolipoprotein, and subfraction metabolism by sex steroids in a postmenopausal woman. *Metabolism* 33(9), 779-784

Heiss G., Johnson N.J., Reiland S., Davis C.E., Tyroler H.A. 1980 The epidemiology of plasma high-density lipoprotein cholesterol levels. *Circulation* 62(IV), 116-136

Hessler J.R., Robertson A.L. Jr., Chisolm G.M. 1979 LDL-induced cytotoxicity and endothelial cells in culture. *Arteriosclerosis* 32, 213-229

Hickson R.C., Ball K.L., Falduto M.T. 1989 Adverse effects of anabolic steroids. *Med. Toxicol. Adverse Drug Exp.* 4(4), 254-271

Hirano K., Yamashita S., Kuga Y., Sakai N., Nozaki S., Kihara S., Arai T., Yanagi K., Takami S., Menju M., Ishigami M., Yoshida Y., Kameda-Takemura K., Hayashi K., Matsuzawa Y. 1995 Atherosclerotic disease in marked hyperalphalipoproteinemia. Combined reduction of cholesterol transfer protein and hepatic triglyceride lipase. *Arterioscler. Thromb. Vasc. Biol.* 15, 1849-1856

Huie M.J. 1994 An acute myocardial infarction occurring in an anabolic steroid user. *Med. Sci. Sports Exerc.* 26(4), 408-413

Hurley B.F., Seals D.R., Hagberg J.M., Goldberg A.C., Ostrove S.M., Holloszy J.O., Weist W.G., Goldberg A.P. 1984 High-density-lipoprotein cholesterol in bodybuilders v powerlifters. *JAMA* 252(4), 507-513

Jaillard A.S., Hommel M., Mallaret M. 1994 Venous sinus thrombosis associated with androgens in a healthy young man. *Stroke* 25, 212-213

Jenner J.L., Ordovas J.M., Lamon-Fava S., Schaefer M.M., Wilson P.W.F., Castelli W.P., Schaefer E.J. 1993 Effects of age, sex, and menopausal status on plasma lipoprotein(a) levels. *Circulation* 87, 1135-1141

Jones D.B., Higgins B., Billet J.S., Price W.H., Edwards C.R.W., Beastall G.H., Shepherd J., Sweeting V.M., Horn D.B., Wenham P.R. 1989 The effect of testosterone replacement on plasma lipids and apolipoproteins. *Eur. J. Clin. Invest.* 19, 438-441

Kalin M.F., Zumoff B. 1990 Sex hormones and coronary disease: a review of the clinical studies. *Steroids* 55, 330-352

Kantor M.A., Bianchini A., Bernier D., Sady S.P., Thompson P.D. 1985 Androgens reduce HDL2-cholesterol and increase hepatic triglyceride lipase activity. *Med. Sci. Sports Exerc.* 17(4), 462-465

Karpe F., Steiner G., Olivecrona T., Carlson L.A., Hamsten A. 1993 Metabolism of triglyceride-rich lipoproteins during alimentary lipemia. *J. Clin. Invest.* 91, 748-758

Karpe F., Tornvall P., Olivecrona T., Steiner G., Carlson L.A., Hamsten A. 1993a Composition of human low density lipoprotein: effects of postprandial triglyceride-rich lipoproteins, lipoprotein lipase, hepatic lipase and cholesterol ester transfer protein. *Atherosclerosis* 98, 33-49

Karpe F., Steiner G., Uffelman K., Olivecrona T., Hamsten A. 1994 Postprandial lipoproteins and progression of coronary atherosclerosis. *Atherosclerosis* 106, 83-97

Kennedy C. 1993 Myocardial infarction in association with misuse of anabolic steroids. *The Ulster Med. J.* 63(2), 174-176

Kennedy M.C., Corrigan A.B., Pilbeam S.T.H.H. 1993a Myocardial infarction and cerebral haemorrhage in a young body builder taking anabolic steroids. *Aust. N.Z. J. Med.* 23, 713

Kiraly C.L. 1988 Androgenic-anabolic steroid effects on serum and skin surface lipids, on red cells, and on liver enzymes. *Int. J. Sports Med.* 9(4), 249-252

Kirkland R.T., Keenan B.S., Probstfield J.L., Patsch W., Lin T-L., Clayton G.W., Insull W. 1987 Decrease in plasma high-density lipoprotein cholesterol levels at puberty in boys with delayed adolescence. *JAMA* 257(4), 502-507

Kleiner S.M., Calabrese L.H., Fielder K.M., Naito H.K., Skibinski C.I. 1989 Dietary influences on cardiovascular disease risk in anabolic steroid-using and nonusing bodybuilders. *J. Amer. Coll. Nutr.* 8(2), 109-119

Knopp R.H., Zhu X., Bonet B. 1994 Effects of estrogens on lipoprotein metabolism and cardiovascular disease in women. *Atherosclerosis* 110, S83-S91

Kostner G.M., Avagaro P., Zazzolato G., Marth E., Bittolo-Bon G., Quinci G.B. 1981 Lipoprotein Lp(a) and the risk for myocardial infarction. *Arteriosclerosis* 38: 51-61

Kouri E.M., Pope H.G., Oliva P.S. 1996 Changes in lipoprotein-lipid levels in normal men following administration of increasing doses of testosterone cypionate. *Clin. J. Sport Med.* 6(3), 152-157

Krauss D.J., Taub H.A., Lantinga L.J., Dunsky M.H., Kelly C.M. 1991 Risks of blood volume changes in hypogonadal men treated with testosterone enanthate for erectile impotence. *J. Urol.* 146, 1566-1570

Kuipers H., Wijnen J.A.G., Hartgens F., Willems S.M.M. 1991 Influence of anabolic steroids on body composition, blood pressure, lipid profile and liver functions in body builders. *Int. J. Sports Med.* 12, 413-418

Kunitake S.T., Jarvis M.R., Hamilton R.L., Kane J.P. 1992 Binding of transition metals by apolipoprotein A-I containing plasma lipoprotein: Inhibition of oxidation of low density lipoproteins. *Proc. Natl. Acad. Sci. USA* 89, 6993-6997

Li H., Radix R.L., Maeda N. 1993 Lack of apo A-I is not associated with increased susceptibility to atherosclerosis in mice. *Arterioscler. Thromb.* 13, 1814-1821

Lipid Research Clinics Program. 1984 The Lipid Research Clinics coronary primary prevention trial results: I. Reduction in the incidence of coronary heart disease. *JAMA* 251(3), 351-364

Lipid Research Clinics Program. 1984a The Lipid Research Clinics coronary primary prevention trial results: II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *JAMA* 251(3), 365-374

Longcope C., Pratt J.H., Schneider S.H., Fineberg S.E. 1978 Aromatization of androgens by muscle and adipose tissue in vivo. *J. Clin. Endocrinol. Metab.* 46, 146-152

Lukas S.E. 1993 Current perspectives on anabolic-androgenic steroid abuse. *Trends Pharm. Sci.* 14, 61-68

Luke J.L., Farb A., Virmani R., Sample R.H.B. 1990 Sudden cardiac death during exercise in a weight lifter using anabolic androgenic steroids: pathological and toxicological findings. *J. Forensic Sci.* 35(6), 1441-1447

Lyngberg K.H. 1991 A case of fatal myocardial infarction in a body builder treated with anabolic steroids. *Ugeskr. Laeger* 153, 587-588 (In Danish)

Mackness M.I., Arrol S., Abbott C., Durrington P.N. 1993 Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis* 104, 129-135

Mackness M.I., Durrington P.N. 1995 HDL, its enzymes and its potential to influence lipid peroxidation. *Atherosclerosis* 115, 243-253

Maher V.M.G., Brown B.G. 1995 Lipoprotein(a) and coronary heart disease. *Curr. Opin. Lipidol.* 6(4), 229-235

Mahley R.W., Hussain M.M. 1991 Chylomicron and chylomicron remnant catabolism. *Curr. Opin. Lipidol.* 2(3), 170-176

Mankowitz K., Seip R., Semenkovich C.F., Daugherty A., Schonfeld G. 1992 Short-term interruption of training affects both fasting and post-prandial lipoproteins. *Atherosclerosis* 95, 181-189

Marcovina S.M., Lippi G., Bagatell C.J., Bremner W.J. 1996 Testosterone-induced suppression of lipoprotein(a) in normal men; relation to basal lipoprotein(a) level. *Atherosclerosis* 122, 89-95

Mårin P., Odén B., Björntorp P. 1995 Assimilation and mobilization of triglycerides in subcutaneous abdominal and femoral adipose tissue in vivo in men: effects of androgens. *J. Clin. Endocrinol. Metab.* 80, 239-243

Martin A.D., Spenst L.F., Drinkwater D.T., Clarys J.P. 1990 Anthropometric estimation of muscle mass in men. *Med. Sci. Sports Ex.* 22: 729-733

Matsumoto A.M. 1990 Effects of chronic testosterone administration in normal men: safety and efficacy of high dosage testosterone and parallel dose-dependent suppression of leutanizing hormone, follicle-stimulating hormone, and sperm production. *J. Clin. Endocrinol. Metab.* 70, 282-287

Matsunaga T., Hiasa Y., Yanagi Y., Maeda T., Hattori N., Yamakawa K., Yamanouchi Y., Tanaka I., Obara T., Hamaguchi H. 1991 Apolipoprotein A-I deficiency due to a codon 84 nonsense mutation of the apolipoprotein A-I gene. *Proc. Natl. Acad. Sci. USA* 88, 2793-2797

MBewu A.D., Durrington P.N. 1990 Lipoprotein(a): structure, properties and possible involvement in thrombogenesis and atherogenesis. *Atherosclerosis* 85, 1-14

McGill 1996 Overview. *In* Atherosclerosis and Coronary Artery Disease. Fuster V., Ross R., Topol E.J., editors. Lippincott-Raven Publishers, Philadelphia, ISBN 0-7817-0266-6. 25-41

McKillop G. , Ballantyne D. 1987 Lipoprotein analysis in bodybuilders. *Int. J. Cardiol.* 17, 281-286

McNamara J.R., Campos H., Ordovas J.M., Peterson J., Wilson P.W.F., Schaefer E.J. 1987 Effect of gender, age, and lipid status on low density lipoprotein subfraction distribution. *Arteriosclerosis* 7: 483-490

McNutt R.A., Ferenchick G.S., Kirlin P.C., Hamlin N.J. 1988 Acute myocardial infarction in a 22-year-old world class weight lifter using anabolic steroids. *Am. J. Cardiol.* 62(1), 164

Melchert R.B., Welder A.A. 1995 Cardiovascular effects of androgenic-anabolic steroids. *Med. Sci. Sports Exerc.* 27(9), 1252-1262

Meriggiola M.C., Marcovina S., Paulsen C.A., Bremner W.J. 1995 Testosterone enanthate at a dose of 200mg/week decreases HDL-cholesterol levels in healthy men. *Int. J. Andrology* 18, 237-242

Mewis C., Spyridopoulos I., Kühlkamp V., Seipel L. 1996 Manifestation of severe coronary heart disease after anabolic drug abuse. *Clinical Cardiology* 19(2), 153-155

Meyer B.J., Ha Y.C., Barter P.J. 1989 Effects of experimental hypothyroidism on the distribution of lipids and lipoproteins in the plasma of rats. *Biochim. Biophys. Acta.* 1004(1), 73-79.

Miesenböck G., Patsch J.R. 1992 Postprandial hyperlipidemia: the search for the atherogenic lipoprotein. *Curr. Opin. Lipidol.* 3(3), 196-201

Miller G.J., Miller N.E. 1975 Plasma high density lipoprotein concentration and development of ischaemic heart disease. *Lancet* I, 16-19

Mochizuki R.M., Richter K.J. 1988 Cardiomyopathy and cerebrovascular accident associated with anabolic-androgenic steroid use. *Phys. Sportsmed.* 16(11), 109-114

Moffat R.J. , Wallace M.B., Sady S.P. 1990 Effects of anabolic steroids on lipoprotein profiles of female weight lifters. *Phys. Sportsmed.* 18(9), 106-115

Morley J.E., Perry H.M. 3d, Kaiser F.E., Kraenzle D., Jensen J., Houston K., Mattammal M., Perry H.M. Jr. 1993 Effects of testosterone replacement therapy in old hypogonadal males: a preliminary study. *J. Am. Geriatr. Soc.* 41(2), 149-152

Mowri H., Patsch J.R., Ritsch A., Föger F., Brown S., Patsch W. 1994 High density lipoproteins with differing apolipoproteins: relationships to postprandial lipemia, cholesterol ester transfer protein, and activities of lipoprotein lipase, hepatic lipase, and lecithin: cholesterol acyl transferase. *J. Lipid Res.* 35, 291-300

Nagelberg S.B., Laue L., Loriaux D.L., Liu L., Sherins R.J., 1986 Cerebrovascular accident associated with testosterone therapy in a 21-year-old hypogonadal man. *N. Engl. J. Med.* 314(10), 649-650

Navab M., Imes S.S., Hama S.Y., Hough G.P., Ross L.A., Bork R.W., Valente A.J., Berliner J.A., Drinkwater D.C., Laks H., Fogelman A.M. 1991 Monocyte transmigration induced by modification of low-density lipoprotein in co-cultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis, and is abolished by high density lipoprotein. *J. Clin. Invest.* 88, 2039-2046

Navab M., Hama-Levy S., Van Lenten B.J., Fonarow G.C., Cardinez C.J., Castellani L.W., Brennan M-L., Lusis A.J., Fogelman A.M. 1997 Mildly oxidized LDL induces an increased apolipoprotein J/paraoxonase ratio. *J. Clin. Invest.* 99, 2005-2019

Ng DS., Leiter L.A., Vezina C., Connelly P.W., Hegele R.A. 1994 Apolipoprotein A-I Q[-2]X causing isolated apolipoprotein A-I deficiency in a family with analphalipoproteinemia. *J. Clin. Invest.* 93, 223-229

Norum KR, Gjone E, Glomset JA. Familial lecithin: cholesterol acyltransferase deficiency including fish eye disease. *In* The Metabolic Basis of Inherited Disease. Scriver CR, Beaudet AL, Sly WS, Valle D, editors. McGraw-Hill, Inc., New York. 1181-1194

Norum R.A., Lakier J.B., Goldstein S., Angela A., Goldberg R.B., Block W.D., Noffze D.K., Dolphin P.J., Edelglass J., Bogorad D.D., Alaupovic P. 1982 Familial deficiency of apolipoproteins A-I and C-III precocious coronary-artery disease. *N. Engl. J. Med.* 306, 1513-1519

Obasanjo I.O., Clarkson T.B., Weaver D.S. 1996 Effects of anabolic steroid nandrolone decanoate on plasma lipids and coronary arteries of female cynomolgus macaques. *Metabolism* 45(4), 463-468

Olivecrona T., Bengtsson-Olivecrona G. 1990 Lipases involved in lipoprotein metabolism. *Curr. Opin. Lipidol.* 1(2), 116-121

Olivecrona T., Bengtsson-Olivecrona G. 1990a Lipoprotein lipase and hepatic lipase. *Curr. Opin. Lipidol.* 1(3), 220-230

Olsson A.G., Orö L., Rössner S. 1974 Effects of oxandrolone on plasma lipoproteins and the intravenous fat tolerance in man. *Atherosclerosis* 19, 337-346

Ozata M., Yildirimkaya M., Bulur M., Yilmaz K., Bolu E., Corakci A., Gundogan M.A. 1996 Effects of gonadotropin and testosterone treatments on lipoprotein(a), high density lipoprotein particles, and other lipoprotein levels in male hypogonadism. *J. Clin. Endocrinol. Metab.* 81(9), 3372-3378

Packard C.J., Munro A., Lorimer A.R., Gotto A.M., Shepherd J. 1984 Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoproteins of normal and hypertriglyceridaemic subjects. *J. Clin. Invest.* 74, 2178-2192

Parthasarathy S., Barnett J., Fong L.G. 1990 High-density lipoprotein inhibits the oxidative modification of low-density lipoprotein. *Biochimica et Biophysica Acta* 1044, 275-283

Patsch J.R., Karlin J.B., Scott L.W., Smith L.C., Gotto A.M. Jr. 1983 Inverse relationship between blood levels of high density lipoprotein subfraction 2 and magnitude of postprandial lipemia. *Proc. Natl. Acad. Sci.* 80, 1449-1453

Patsch J.R., Miesenböck G., Hopferwieser T., Mühlberger V., Knapp E., Dunn J.K., Gotto A.M. Jr., Patsch W. 1992a Relation of triglyceride metabolism and coronary artery disease: Studies in the postprandial state. *Arterioscler. Thromb.* 12, 1336-1345

Patsch J.R. 1994 Triglyceride-rich lipoproteins and atherosclerosis. *Atherosclerosis* 110, S23-S26

Patsch W., Sharrett A.R., Sorlie P.D., Davis C.E., Brown S.A. 1992 The relation of high density lipoprotein cholesterol and its subfractions to apolipoprotein A-I and fasting triglycerides: the role of environmental factors. *Am. J. Epidemiol.* 136, 546-557

Peterson G.E., Fahey T.D. 1984 HDL-C in five elite athletes using anabolic-androgenic steroids. *Phys. Sportsmed.* 12(6), 120-130

Plymate S.R., Swerdloff R.S. 1992 Androgens, lipids, and cardiovascular risk. *Annals of Internal Medicine* 117(10) 871-872

Rapp J.H., Lespine A., Hamilton R.L., Colyvas N., Chaumeton A.H., Tweedie-Hardman J., Kotite L., Kunitake S.T., Havel R.J., Kane J.P. 1994 Triglyceride-rich lipoproteins isolated by selected-affinity anti-apolipoprotein B immunosorption from human atherosclerotic plaque. *Arterioscler. Thromb.* 14, 1767-1774

Reeves R.D., Morrison M.D., Barbour G.L. 1976 Hyperlipidemia due to oxymetholone therapy. *J. Am. Med. Assoc.* 236, 469-472

Rockhold R.W. 1993 Cardiovascular toxicity of anabolic steroids. *Annu. Rev. Pharmacol. Toxicol.* 33, 497-520

Ross R. 1993 The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362, 801-809

Ross WD, Marfell-Jones MJ 1991 Kinanthropometry. *In* Physiological Testing of the High-Performance Athlete, (2nd ed) MacDougall JD, Wenger HA, Green, HJ. Human Kinetics Books, Champaign, IL, USA

Rubin E.M., Krauss R.M., Spangler A.E., Verstuyft J.G., Clift S.M. 1993 Inhibition of early atherogenesis in transgenic mice by human apolipoprotein A-I. *Nature* 365, 265-267

Ryu J.E., Howard G., Craven T.E., Bond M.G., Hagaman A.P., Crouse J.R. III 1992 Postprandial triglyceridemia and carotid atherosclerosis in middle-aged subjects. *Stroke* 23, 823-828

Sacks F.M., Gerhard M., Walsh B.W. 1995 Sex hormones, lipoproteins, and vascular reactivity. *Curr. Opin. Lipidology* 6, 161-166

Schaefer E.J., Lamon-Fava S., Jenner J.L., McNamara J.R., Ordovas J.M., Davis C.E. 1994 Lipoprotein(a) levels and risk of coronary heart disease in men: the Lipid Research Clinics Coronary Primary Prevention Trial. *JAMA* 271: 999-1003

Schmitz G., Assmann G., Robenek H., Brennhausen B. 1985 Tangier disease: A disorder of intracellular membrane traffic. *Proc. Natl. Acad. Sci. USA* 82, 6305-6309

Shah A., Roberts T., McQueen I., Graham J.G., Walker K. 1987 Danazol and benign intracranial hypertension. *Br. Med. J.* 294, 1323

Shiozawa Z., Yamada H., Mabuchi C., Hotta T., Saito M., Sobue I., Huang Y.P. 1982 Superior sagittal sinus thrombosis associated with androgen therapy for hypoplastic anemia. *Ann. Neurol.* 12, 578-580

Shiozawa Z., Tsunoda S., Noda A., Saito M., Yamada H. 1986 Cerebral hemorrhagic infarction associated with anabolic steroid therapy for hypoplastic anemia. *Angiology* 37, 725-730

Siekierzynska-Czarnecka A., Polowiec Z., Kulawinska M., Rowinska-Zakrzewska E. 1990 Death caused by pulmonary embolism in a bodybuilder taking anabolic steroids (metanabol). *Wiad. Lek.* 43, 972-975 (In Polish)

Silverman D.I., Pasternak R.C. 1993 High-density lipoprotein subfractions. *Am. J. Med.* 94, 636-645.

Sorva R., Kuusi T., Taskinen M-R., Perheentupa J., Nikkilä E.A. 1988 Testosterone substitution increases the activity of lipoprotein lipase and hepatic lipase in hypogonadal males. *Atherosclerosis* 69, 191-197

Stamler J., Wentworth D., Neaton J.D. 1986 Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *JAMA* 256(20), 2823-2828

Strauss R.H., Yesalis C.E. 1991 Anabolic steroids in the athlete. *Annu. Rev. Med.* 42, 449-457

Street C., Antonio J., Cudlipp D. 1996 Androgen use by athletes: a reevaluation of the health risks. *Can. J. Appl. Physiol.* 21(6), 421-440

Stryer L. 1995 Biosynthesis of Membrane Lipids and Steroids. *In* Biochemistry 4th edition, W.H. Freeman and Co., New York, ISBN 0-7167-2009-4. 685-712

Swerdloff R.S., Wang C. 1993 Androgens and ageing in men. *Exp. Gerontol.* 28(4-5), 435-46

Taggart H. McA., Applebaum-Bowden D., Haffner S., Warnick G.R., Cheung M.C., Albers J.J., Chestnut III C.H., Hazzard W.R. 1982 Reduction in high density lipoproteins by anabolic steroid (Stanozolol) therapy for postmenopausal osteoporosis. *Metabolism* 31(11), 1147-1152

Tall A.R. 1996 Plasma High-Density Lipoproteins and Atherogenesis. *In* Atherosclerosis and Coronary Artery Disease. Fuster V., Ross R., Topol E.J., editors. Lippincott-Raven Publishers, Philadelphia, ISBN 0-7817-0266-6. 105-128

Tashiro J., Nishide T., Shinomiya M., Shirai K., Saito Y., Yoshida S., Yamashita M., Ohshima H., Murayama H. 1993 The 'midband' lipoprotein is a coronary risk factor in Japanese patients with familial hypercholesterolaemia. *Scand. J. Clin. Lab. Invest.* 53, 335-338.

Tenkanen L., Mänttäri M., Pietilä K., Manninen V. 1996 Triglycerides as a signal of increased coronary heart disease risk due to adverse lifestyle. *In* Drugs Affecting Lipid Metabolism: Risk Factors and Future Directions. Gotto A.M. Jr., Paoletti R., Smith L.C., Catapano A.L., Jackson A.S., editors. Kluwer Academic Publishers, Dordrecht, ISBN 0-7923-4167-8. 211-218

Tenover J.S. 1992 Effects of testosterone supplementation in the aging male. *J. Clin. Endocrinol. Metab.* 75(4), 1092-1098

Thompson G.R. 1994 A Handbook of Hyperlipidaemia. Current Science Ltd., London, ISBN 1-85922-140-8. 258pp.

Thompson P.D., Cullinane E.M., Sady S.P., Chenevert C., Saritelli A.L., Sady M.A., Herbert P.N. 1989 Contrasting effects of testosterone and Stanozolol on serum lipoprotein levels. *JAMA* 261, 1165-1168

Tikkanen M.J., Nikkilä E.A. 1987 Regulation of hepatic lipase and serum lipoproteins by sex steroids. *Am. Heart J.* 113, 562-567

Toda T., Toda Y., Cho B.H., Kummerow F.A. 1984 Ultrastructural changes in the comb and aorta of chicks fed excess testosterone. *Atherosclerosis* 51(1), 47-57

Tully M.P., Cooper R.G., Jayson M.I.V. 1990 Intracranial hypertension associated with stanozalol. *DICP Ann. Pharm.* 24, 1234

Uiterwaal C.S.P.M., Grobbee D.E., Witteman J.C.M., van Stiphout W-A.H.J., Krauss X.H., Havekes L.M., de Bruijn A.M., van Tol A., Hofman A. 1994 Postprandial triglyceride response in young adult men and familial risk for coronary atherosclerosis. *Ann. Inter. Med.* 121, 576-583

Urban R.J., Bodenburg Y.H., Gilkison C., Foxworth J., Coggan A.R., Wolfe R.R., Ferrando A. 1995 Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. *Am. J. Physiol.*, 269 (5), E820-6

Uterman G. 1989 The mysteries of lipoprotein(a). *Science*, 17 Nov, 904-910

van der Merwe P.J., Kruger H.S.L. 1992 Drugs in sport - results of the past 6 years of dope testing in South Africa. *S. Afr. Med. J.*, 82(3), 151-153

Vega G.L., Grundy S.M. 1996 Hypoalphalipoproteinemia (low high density lipoprotein) as a risk factor for coronary heart disease. *Curr. Opin. Lipidol.* 7(4), 209-216

von Eckardstein A., von Eckardstein S., Kliesch S., Assmann G., Nieschlag E., Behre H.M. 1997 Relationship between endogenous testosterone and cardiovascular risk factors in men and women. 9th International Dresden Symposium on Lipoproteins and Atherosclerosis. Abstract, 20

Wang C., Eyre D.R., Clark R., Kleinberg D., Newman C., Iranmanesh A., Veldhuis J., Dudley R.E., Berman N., Davidson T., Barstow T.J., Sinow R., Alexander G., Swerdloff R.S. 1996 Sublingual testosterone replacement improves muscle mass and strength, decreases bone resorption, and increases bone formation markers in hypogonadal men - a clinical research center study. *J. Clin. Endocrinol. Metab.*, 81(10), 3654-62

Wang C., Alexander G., Berman N., Salehian B., Davidson T., McDonald V., Steiner B., Hull L., Callegari C., Swerdloff R.S. 1996a Testosterone replacement therapy improves mood in hypogonadal men - a clinical research center study. *J. Clin. Endocrinol. Metab.* 81(10), 3578-83

Warden C.H., Hedrick C.C., Qiao J-H, Castellani L.W., Lusis A.J. 1993 Atherosclerosis in transgenic mice overexpressing apolipoprotein A-II. *Science* 261, 469-472

Watson A.D., Navab M., Hama S.Y., Sevanian A., Prescott S.M., Stafforini D.M., McIntyre T.M., La Du B.N., Fogelman A.M., Berliner J.A. 1995 Effect of platelet activating factor-acetylhydrolase on the formation and action of minimally oxidised low density lipoprotein. *J. Clin. Invest.* 95, 774-782

Watson A.D., Berliner J.A., Hama S.Y., La Du B.N., Faull K.F., Fogelman A.M., Navab M. 1995a Protective effect of high density lipoprotein associated paraoxonase: inhibition of the biological activity of minimally oxidised low density lipoprotein *J. Clin. Invest.* 96, 2882-2891

Watson T.D., Caslake M.J., Freeman D.J., Griffin B.A., Hinnie J., Packard C.J., Shepherd J. 1994 Determinants of LDL subfraction distribution and concentrations in young normolipidaemic subjects. *Arterioscler. Thromb.* 14(6), 902-910

Webb O.L., Laskarzewski P.M., Gleuck C.J. 1984 Severe depression of high-density lipoprotein cholesterol levels in weight lifters and body builders by self administered exogenous testosterone and anabolic-androgenic steroids. *Metabolism* 33(11), 971-975

Welder A.A., Melchert R.B. 1993 Cardiotoxic effects of cocaine and anabolic-androgenic steroids in the athlete. *J. Pharm. Toxicol. Methods* 29(2), 61-68

Wilson J.D. 1988 Androgen abuse by athletes. *Endocrine Reviews* 9(2), 181-199

Windsor R.E., Dumitru D. 1988 Anabolic steroid use by athletes - how serious are the health hazards? *Postgraduate Medicine* 84(4), 37-49

Witztum J.L., Steinberg D. 1991 Role of oxidised low density lipoprotein in atherogenesis *J. Clin. Invest.* 88, 1785-1792

Witztum J.L. 1994 The oxidation hypothesis of atherosclerosis. *Lancet* 344, 793-795

Wojcicki J., Tustanowski S., Samochowiec L. 1989 Endocrine functions in atherosclerotic rabbits. *Pol. J. Pharmacol. Pharm.* 41(2), 109-113

World Health Organisation. 1993 Drug use and sport: Current issues and implications for public health *Programme on Substance Abuse* 93.3

Wu S.Z., Weng X.Z. 1993 Therapeutic effects of an androgenic preparation on myocardial ischemia and cardiac function in 62 elderly male coronary heart disease patients. *Chin. Med. J.* 106(6), 415-418

Yeater R., Reed C., Ullrich I., Morise A., Borsch M. 1996 Resistance trained athletes using or not using anabolic steroids compared to runners: effects on cardiorespiratory variables, body composition, and plasma lipids. *Br. J. Sports Med.* 30, 11-14

Yesalis C.E., Kennedy N.K., Kopstein A.N., Bahrke M.S. 1993 Anabolic-androgenic steroid use in the United States. *JAMA* 270, 1217-1221

Yue P., Chatterjee K., Beale C., Poole-Wilson P.A., Collins P. 1995 Testosterone relaxes rabbit coronary arteries and aorta. *Circulation* 91, 1154-1160

Zambon A., Austin M.A., Brown B.G., Hokanson J.E., Brunzell J.D. 1993 Effect of hepatic lipase on LDL in normal men and those with coronary artery disease. *Arterioscler. Thromb.* 13(2), 147-153

Zgliczynski S., Ossowski M., Slowinska-Srzednicka J., Brzezinska A., Zgliczynski W., Soszynski P., Chotkowska E., Srzednicki M., Sadowski Z 1996 Effect of testosterone replacement therapy on lipids and lipoproteins in hypogonadal and elderly men. *Atherosclerosis* 121, 35-43

Zilversmit D.B. 1979 Atherogenesis: a postprandial phenomenon. *Circulation* 60(3), 473-485

Zmuda J.M., Fahrenbach M.C., Younkin B.T., Bausserman L.L., Terry R.B., Catlin D.H., Thompson P.D. 1993 The effect of testosterone aromatisation on high-density lipoprotein cholesterol level and postheparin lipolytic activity. *Metabolism* 42(4), 446-450

Zmuda J.M., Thompson P.D., Dickenson R., Bausserman L.L. 1996 Testosterone decreases lipoprotein(a) in men. *Am. J. Cardiol.* 77(14), 1244-7

Zuliani U., Bernardini B., Catapano A., Cerioli G., Spattini M. 1988 Effects of anabolic steroids, testosterone, and HGH on blood lipids and echocardiographic parameters in bodybuilders. *Int. J. Sports Med.* 10(1), 62-66

Appendix I

Protein composition of lipoproteins in fasting plasma				
	Chylomicrons	VLDL	LDL	HDL
Total protein (mg/dl plasma)	—	6	80	190
Apoproteins (% of total protein)				
ApoA-I	Trace	Trace	Trace	66
ApoA-II	Trace	Trace	Trace	20
ApoB	5-20	37	97	—
ApoC-I	15	3	Trace	3
ApoC-II	15	7	Trace	Trace
ApoC-III	40-50	40	2	4
ApoD	—	—	—	5
ApoE	4	13	1	1

From: Thompson G.R. 1994 A Handbook of Hyperlipidaemia. Current Science Ltd., London, ISBN 1-85922-140-8. 258pp.