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# Quantitative Influence of Exogenous Androgens on Serum Lipid Profile and Endocrine Functions

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“Insanity is doing the same thing, over and over again, but expecting different results.”
*Narcotics Anonymous*

# ABSTRACT

Anabolic androgenic steroids (AAS) in doping have been a concern predominantly in sports. The focus has now switched to the doping in the society which is a significant problem for the public health. The abuse of AAS is associated with mental and somatic side effects and with the use of several other drugs including narcotics. This thesis focuses on the effects of AAS, particularly nandrolone and testosterone, on the serum lipid profile and endocrine functions.

We found a frequent co-abuse of AAS and narcotics among young people taken into custody for criminal activity. The two most common abused AAS were nandrolone and testosterone. We found a sustained suppression of LH and FSH for several months, sometimes 1 year. The suppression correlated significantly with the 19-norandrosterone (19-NA) metabolite of nandrolone in urine in individuals without co-abuse of narcotics. In healthy volunteers LH remained supressed up to 6 weeks after a dose of 500 mg and even suppressed below lower limit of reference range for two individuals. These results indicate that AAS have a more profound endocrine effect on the hypothalamic-pituitary-adrenal -axis than previously known. Altered blood-lipids profile was normalized within 6 months after cessation of AAS abuse. We found an early effect on the blood-lipid profile after a single dose of testosterone enanthate. Two days after testosterone injection, total cholesterol was increased and followed by a decrease in HDL and ApoA1 four and fourteen days after dose. The minimal dose for these alterations in the blood lipids and for increased serum testosterone concentrations was 250 mg. The impact on the cholesterol homeostasis may be mediated by an increase of the HMGCR expression.

There was a marked impact of the uridine glucuronosyl transferase 2B17 (UGT2B17) polymorphism on the T/E ratio in AAS abusers and some of the testosterone abusers did not test positive due to a genetic deletion polymorphism of the UGT2B17.

Increased knowledge and understanding of side-effects induced by AAS is important in order to find measures for treatment and care of these abusers.

# LIST OF PUBLICATIONS

I. Gårevik N, Rane A. Dual use of anabolic-androgenic steroids in Sweden. Drug Alcohol Depend.2010 Jun 1; 109 (1-3): 144-6

II. Gårevik N, Strahm E, Garle M, Lundmark J, Ståhle L, Ekström L, Rane A. Long term perturbation of endocrine parameters and cholesterol metabolism after discontinued abuse of anabolic androgenic steroids. J Steroid Biochem Mol Biol. 2011 Nov; 127(3-5):295-300

III. Gårevik N, Skogastierna C, Rane A, Ekström L.Single dose testosterone increases total cholesterol levels and induces the expression of HMG CoA reductase. Subst Abuse Treat Prev Policy. 2012 Mar 20;7:12

IV. Gårevik N, Ekström L, Rane A. Effects on gonadotropins and blood lipids of different doses of testosterone in healthy men. Manuscript

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AAS | anabolic androgenic steroids |
| hCG | human chorionic gonadotropin |
| LDL | low-density lipoprotein |
| Apo-B | apolipoprotein B |
| HDL | high-density lipoprotein |
| Apo-A1 | apolipoprotein A1 |
| Lp(a) | lipopoprotein (a) |
| HMGCR | 3-hydroxy-3-methyl-glutaryl-CoA reductase |
| FSH | follicle stimulating hormone |
| LH | luteinizing hormone |
| T/E | testosterone/epitestosterone |
| UGT | uridine diphospho-glucuronosyl transferase |
| 19- NA | 19-norandrosterone |

# 1 SETTING THE SCENE

Drug abuse has always concerned the health care, politicians and the public to varying degrees, for more than 100 years. The health problems and increased mortality rates associated with drug abuse have been recognized gradually during the 20th century. Drug abuse has been characterized by psychoactive effects and dependence. The medical use and use of psychoactive drugs for ritual or religious activity is a practice that dates back to prehistoric times (1). Anabolic androgenic steroids (AAS) have been discussed as agent without psychoactive effects and therefore not a topic in the public and political debate as a true drug abuse. There has been an understanding of AASs as mood alterating drugs which goes back to the 1930s when they were used in medical practice to treat depression (2). In recent years AAS have been mostly a concern in the sport field as a doping substance because of their anabolic effects and were banned by the International Olympic Committee in 1974. But the abuse of AAS substances in the society is today a larger and major problem for the public health. The abuse in society started during the late 1970s and 1980s, preferably among so called body builders and weight lifters. Some considered it as an abuse similar to that of opiates and alcohol (3-5). However, in one double-blind trial with testosterone, morphine and placebo, testosterone showed no immediate psychoactive effects. The study concluded that single doses of testosterone lacked the usual pharmacological effects that are associated with abuse (6). Moreover, there has been no scientific evidence that therapeutic use such as treatment of androgen deficiency syndromes may trigger abuse of AAS (7). Brower proposed 2002 a two-stage model of AAS dependence that incorporated the anabolic benefits early on, with physical dependence after prolonged use (8). It seemed that AAS abuse could be separated from other forms of drug abuse, that AAS abusers take the drugs in order to improve their esthetic character by the anabolic effects rather than an end in itself.

Beyond the discussion concerning AAS dependence there is a documented relationship between AAS use and several side effects.

The present background describes the characteristics of the abuse, common side-effects and the main focus of this thesis: the physiological and pharmacologic effects of AAS on serum lipid profile and endocrine functions. Finally in this background, the key points in research of AAS; the detection of

AAS is described with special focus on testosterone and nandrolone, which are the most commonly, abused AAS substances (39, www.wada-ama.org).

## 1.1 MOTIVES AND EFFECTS OF AAS ABUSE

All AAS are chemical modification of testosterone (9). Several hundred different types of anabolic androgenic steroids have been synthesized in unsuccessful attempts to maximize benefits and reduce side effects. Testosterone is one of the most frequent abused AAS in the society, along with nandrolone and stanozolol (10, 11). Testosterone itself, especially when combined with strength training, increases fat-free mass and muscle size and strength in normal men (12). A desire to achieve ideal body image suggested by western mass media seemed to be the main motivation for AAS abuse in the society (13, 14). In addition to that, there is a possibility that some side effects described in the literature might be wished by abusers (egoism, aggressiveness) with the aim to counteract insecurity, booster low self-esteem, become brave, or to prepare and commit crimes (15). Some of these motives are described as mental side effects of abuse such as: affective symptoms, loss of impulse control, aggression and even suicide. (16). A double-blind, placebo-controlled design study was unable to confirm an increased aggressive behavior in normal men in a controlled setting (17). However, other studies have shown increased ratings of manic symptoms in normal men after administration of testosterone. The symptoms were dose dependent and not uniform across individuals and might be more common in the natural context compared to a controlled setting (18).

## 1.2 RISK FACTORS FOR AAS ABUSE

Most of what was known about AAS abuse in the society was for a long time based on case-reports and anecdotes. The first studies to identify risk factors for onset of AAS abuse indicated that adolescent AAS users are significantly more likely to be males, Student athletes were also more likely than non-athletes to use AAS, and football players, wrestlers, weightlifters and bodybuilders had significantly higher prevalence rates than students not engaged in these activities (19).

Studies also reported poor body image as a risk factor for an onset of AAS abuse (20, 21). There were also studies reporting AAS abuse among non-athletes or men not performing any exercise at all (22, 23). One study found that AAS abusers are more satisfied with their bodies as compared to non-users the study did not tell how the body was perceived before onset of AAS abuse (24)

It was previously understood that AAS abusers were less likely to abuse other illicit drugs based on the assumption that AAS users look upon themselves as healthy persons and not as abusers (25, 26). Other studies showed the opposite, a statistically significant association between AAS use and abuse of illicit drugs (27-29). Few studies have reported whether the illicit drug abuse was a gateway for AAS abuse or the contrary. One study suggested AAS abuse may serve as a "gateway" to opioid abuse in some cases (30). In paper I, individuals (n=56) suspected of infringement of the narcotic laws in Sweden we observed that the use of AAS was preceded by the use of narcotic agents in 55% of subjects. The most commonly co-abused substance was cannabis. Studies on "gateway" substances for AAS abuse have mostly concerned performance enhancing supplements (generally denoted as "dietary supplements") suggesting a gateway effect of these products for AAS abuse (31,32). A study was performed in recent years which compared social background between individuals with an ongoing, regular AAS abuse, individuals with earlier sporadic AAS abuse, and gym visitors with no history of AAS abuse. It showed that abusers of AAS often come from severely disadvantaged family backgrounds and that they also live their adult lives in difficult social situations (33). However, confounders such as educational level, socioeconomic status, ethnicity and age are incompletely considered and require further investigations.

### 1.3 ABUSE PATTERN

AAS are generally administrated by intramuscular injection, sometimes orally (8). The doses are most often supraphysiologic. The supposed physiological basis for abusing several types of AAS is to maximize AR (androgen receptor) binding and to activate multiple steroid receptor sites. However, no research has shown that these effects occur (34). The periods are called "cycles" and last typically for 8–16 weeks, separated by drug-free intervals for months or years (35, 36). The reason for this abuse pattern is expectations to reduce the suppression on the hypothalamic–pituitary–testicular axis, which leads to decreased endogenous testosterone production in men (37). These cycles usually end up with a so called "post-cycle treatment" which includes human chorionic gonadotrophin (hCG) and/or antiestrogens in order to avoid AAS-induced deterioration in spermatogenesis and/or gynecomastia (38). There are individual variations in frequency and intensity of the side-effects. (39). Other commonly co-abused drugs are human growth hormone, thyroid hormones, insulin, caffeine, ephedrine, and clenbuterol (40, 41).

## 1.4 CO-ABUSE OF NARCOTICS

Studies have reported that the likelihood of abusing AAS together with the use of several other drugs including marijuana, cocaine, stimulants, relaxants, heroin and alcohol is associated (26, 41, 49). Autopsies involving human AAS abusers commonly reveal mixed substance abuse (42). Kanayama et al (43) present in a review the hypothesis that "AAS users appear particularly prone to opioid use. There may well be a biological basis for this association, since both human and animal data suggest that AAS and opioids may share similar brain mechanisms". This hypothesis has been supported by other studies (44-46) However, Wood suggests in her ground breaking article "Reinforcing aspects of androgens" that androgen reinforcement is mediated by the brain and it appears to act through the mesolimbic dopamine system, but they are not comparable to that of cocaine or heroin. Instead, testosterone resembles other mild reinforces, such as caffeine, nicotine, or benzodiazepines (47). Even if the co-abuse of AAS and narcotics is not fully understood, there is a clear a relationship between these two forms of drug abuse, which is further discussed in paper I.

## 1.5 SIDE EFFECTS OF AAS ABUSE

### 1.5.1 PSYCHIC SIDE EFFECTS

The abuse of AAS is associated with mental side effects such as affective symptoms, loss of impulse control and higher level of aggression, body dysmorphic disorder, suicide and even violent acts (48-52). The interindividual variation seems to be high for these side effects. Moreover, a very important confounder is the association with concomitant abuse of narcotics; AAS seem to be strongly synergistic in precipitating impulsive violent behavior (51, 53).

## 1.6 SOMATIC SIDE EFFECTS

There are several somatic side effects and risks associated with AAS abuse, many of which are potentially serious. They may be considered under the following headings:

Cosmetic: AAS abuse leads to hypertrophy of sebaceous glands together with increased sebum excretion, increased production of skin surface lipids and increased acne, particularly on the back (54). Androgens can suppress hair growth and cause hair loss (55), abuse of AAS increases the risk for male baldness

(reported to the Anti-Doping Hotline). Abuse of AAS is associated with gynecomastia caused by conversion of androgens to estradiol and estrone. (56). Fluid retention and strieae are also common side effects (57, 58). Hepatic: liver function disturbances and diseases have been described in treated patients, as well as in AAS abusers especially after oral administration. Subcellular changes in hepatocytes, impaired excretory functions, cholestasis, peliosis hepatitis and carcinomas are often hepatic complications of AAS abuse (59). Infections: There is no evidence of a higher prevalence of HIV, hepatitis B or C in AAS abusers. However complications from the injection techniques such as: nonsterile injection and reuse of needles has been described. Infections reported include bacterial abscesses, septic arthritis, and septic shock. Other injection complications arise from frequent repeated injection into the same site which may result in inflammation and intramuscular fibrosis (60), also reported to the Anti-Doping Hotline.

### 1.6.1 DIRECT CARDIOVASCULAR RISKS

The direct risks include sudden cardiac death due to focal myocardial fibrosis without any history of coronary heart disease (61-63). Left ventricular hypertrophy has also been reported in AAS abusers elicited via androgen receptors in cardiac muscle cells (64-66). Moreover, abuse of AAS appears to increase the risk of life-threatening arrhythmia leading to sudden death, although the underlying mechanisms are still far from being clarified (67). There is a lack of long-term prospective studies of AAS abuse. Case studies have linked AAS abuse to sudden death (68-70). A 12 –year follow up study of a cohort of 62 power lifters, suspected of AAS abuse, were compared with controls. Premature death and mortality was higher in the abusers (12.9%) than in the control population (3.1%) (74). Supportive evidence for this was also published by Thiblin et al in a study of 34 autopsies performed on AAS abusers of which 12 had cardiac changes (42). However, the co-abuse of other drugs and narcotic substances makes it difficult to prove the causality with AAS abuse. In clinical practice AAS abuse may not be considered at all and AAS abusers show little trust in physicians' knowledge about AAS. Often they do not disclose their AAS abuse to physicians (71). Moreover, adverse side effects of AAS abuse such as reduced HDL and increased LDL concentrations may increase the risk of atherosclerosis later in life. As the abuse in society took place during the

late 1970s and 1980s (3-5) the first generation of AAS abusers may possibly suffer from atherosclerosis today as a consequence of the abuse.

### 1.6.2 INDIRECT CARDIOVASCULAR RISKS

Many studies have shown that abuse of AAS can cause dyslipidemia by increasing low-density lipoprotein (LDL) and apolipoprotein B (Apo-B) and decreasing high-density lipoprotein (HDL) and apolipoprotein A1 (Apo-A1) in blood (72,73). This increases the risk of atherosclerosis although it seems that these changes are reversible. In our study group (paper 2) the AAS abusers had an HDL level below 1.0 mmol/L at visit 1, whereas six months after AAS cessation they had reached an HDL level of 1.1 mmol/L. According to the Framingham data individuals with HDL below 1.0 mmol/L had a fourfold increase in risk of coronary heart disease compared to those with concentrations of 1.03–1.27 mmol/L (75).

The mechanism leading to dyslipidemia in AAS abusers is unclear. Hepatic triglyceride lipase is a strong candidate to mediate the changes in the lipid profile (76). In one study an increase in hepatic triglyceride lipase activity occurred prior to the change in HDL, after AAS administration in healthy controls. This timing of the increase in hepatic triglyceride lipase indicated that the enzyme may be necessary for the catabolism of HDL (77). Furthermore, hepatic triglyceride lipase activity can convert HDL to particles that are smaller in size and increased in density which can be taken up by the liver. In addition, hepatic triglyceride lipase by the same mechanism converts large, buoyant LDL to small, dense LDL (78,79). Increased hepatic triglyceride lipase activity is associated with a decrease in HDL after administration of supraphysiological amounts of testosterone in eugonadal, obese, elderly men (81). Moreover, a novel mechanism sugged by Morikawa et al, consisted of delayed removal of chylomicrons remnants in AAS abusers. AAS abuse impaired the removal from plasma of cholesteryl esters which in turn indicates that chylomicrons remnants accumulate in the circulation in AAS abusers (80). Studies have shown that total cholesterol concentrations remain unchanged following AAS abuse (82). However, we have demonstrated significantly increased concentrations in total cholesterol two days after a testosterone injection in healthy volunteers (paper III).

### 1.6.3 SERUM LIPID PROFILE

### 1.6.4 CHOLESTEROL

Cholesterol has several important biological functions as key constituent of cellular membranes and precursor of steroid hormones. Cholesterol homeostasis is regulated by a balance between endogenous synthesis, biliary excretion and dietary uptake in the intestine. The liver is the major site of cholesterol synthesis. Thus, cholesterol is recycled; the liver excretes it in a non-esterified form (via bile) into the digestive tract (83). Cholesterol is only slightly soluble in water; it can travel in the bloodstream at exceedingly small concentrations. Since cholesterol is insoluble in blood, it is transported in lipoproteins. Lipoproteins can be classified from their densities into five major classes: chylomicrons- the largest lipoprotein particle, very low density lipoproteins (VLDL) intermediate density lipoproteins (IDL), LDL and HDL. Chylomicrons and VLDL carry almost all of the triglycerides in the bloodstream while cholesterol is mainly transported in LDL and HDL. Concentrations of the blood lipids are often measured in: total cholesterol, LDL, HDL, triglycerides. The apoproteins: apoB and apoA1 are also often analyzed and the ratio ApoB/ApoA1 calculated.

### 1.6.5 LIPOPROTEIN PARTICLES

LDL delivers cholesterol to extrahepatic tissues and the half life of the LDL particle is 2-3 days. LDL particles interact with the endothelium and might cause oxidation and aggregation (84). LDL particles can vary in size and density, and studies have shown that small dense LDL particles are associated with an increased risk for coronary heart disease than less dense LDL particles. This is because the smaller particles more easily penetrate the endothelium (85). ApoB is the major component of the LDL particle and reflects the number of LDL particles in fasting state. HDL particles transport cholesterol from peripheral tissues back to the liver and this reverse transport is considered protective for developing coronary heart disease. High HDL concentrations are also correlated with cardiovascular health. The best known antiatherogenic function of HDL particles relates to their ability to promote reverse cholesterol transport from peripheral cells (86). HDL also acquires antioxidant, anti-inflammatory, and antithrombotic effects (87). Apo A1 is the major component of the HDL particle. ApoB/ApoA1 has proven to be a strong predictor for the risk of coronary heart disease (88).

A decrease in p-HDL and in p-ApoA1 a few days up to two weeks after one single injection (500mg and 250mg) of testosterone is shown in paper IV. The effect seems to be dose dependent as there was no significant change at 125 mg testosterone administration in the p-ApoA1 and p-HDL concentrations.
Triglycerides are esters derived from glycerol and three fatty acids.
Triglycerides represent an important biomarker of coronary heart disease risk because of its association with atherogenic remnant particles (89). Studies show that the abuse of AAS either elevates triglyceride (90) concentrations or does not (91).
In contrast with their unfavorable effects on lipids, AASs may favorably lower lipopoprotein (a) (Lp (a) concentrations (92,93). The concentration of Lp(a) is an independent risk indicator for the development of vascular disease. The fat composition of Lp(a) is comparable to that of LDL-C while in addition to ApoB in the LDL the apoproteins (a) is also attached to Lp(a) particle. The serum concentration of Lp(a) seems to be genetically determined and, when raised, cannot be lowered by alterations in food intake or cholesterol lowering drugs (Statins or HMG-CoA reductase inhibitors) (94).

### 1.6.6 HMGCR

HMGCR (3-hydroxy-3-methyl-glutaryl-CoA reductase or HMG-CoA reductase) is the rate-controlling enzyme in the mevalonate pathway, the metabolic pathway that produces cholesterol. Most of our cells can synthesize cholesterol; the liver and the intestine contribute with the major part.
Competitive inhibitors (statins) of the HMGCR induce the expression of LDL receptors in the liver and increase the catabolism of plasma LDL and lower the plasma concentration of cholesterol, an important risk factor for atherosclerosis (95-96). Since the synthesis of cholesterol is dependent on the activity of HMGCR, we investigated if testosterone could affect the expression of this enzyme. In paper III we showed that a supra-physiological dose of testosterone induces the expression of HMGCR *in vivo* in healthy volunteers.

### 1.6.7 EFFECTS ON ENDOCRINE REPRODUCTIVE FUNCTIONS

The reproductive hormonal axis in men consists of three main components: the hypothalamus, the pituitary gland, and the testes. This axis normally functions in a closely regulated way to produce concentrations of circulating steroids required for normal male sexual development, sexual function and fertility by a neuroendocrine negative feedback pathway. Spermatogenesis is regulated by the pulsatile release of gonadotropin-releasing hormone from the arcuate nucleus of the hypothalamus, which stimulates the anterior pituitary to episodically release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). LH stimulates the Leydig cells to produce testosterone. FSH exerts its effect directly on the Sertoli cells to promote spermatogenesis (Fig 1). Exogenous testosterone suppresses the secretion of LH and FSH in healthy men and induces reversible azoospermia. AAS efficiency as a male contraceptive has been accepted by the WHO, (97, 98). These effects result from the negative feedback of androgens on the hypothalamic-pituitary gonadal axis, and possibly from local suppressive effects of exogenous androgens on the testes (99). An unexpected long lasting effect in healthy volunteers with a significant decline in s-LH-concentrations 6-8 weeks after a single dose of 500 mg testosterone is shown in paper (IV).

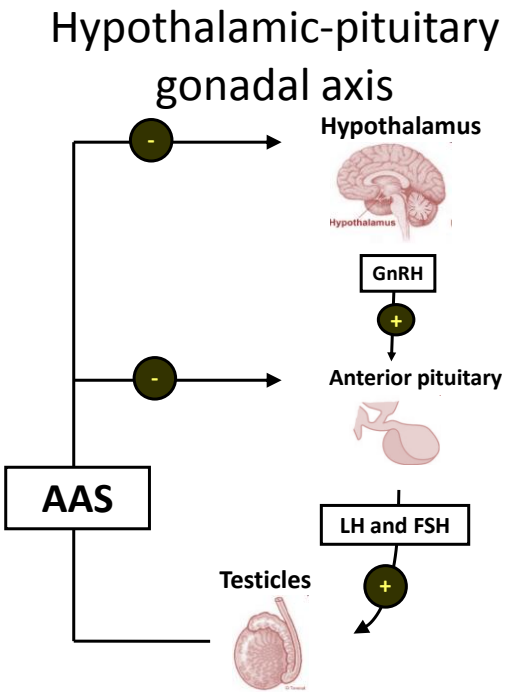



**Fig 1)** The hypothalamus secretes gonadotropin releasing-hormone which stimulates synthesis and secretion of the gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

AASs induced hypogonadism is associated with lower serum testosterone concentrations, testicular atrophy, impaired spermatogenesis and potency problems (39, 100, 101). However there are few studies published on steroid-induced infertility during a steroid cycle and following the cessation of abuse. Karila et al (38) reported, in accordance with Torres-Calleja et al (102), that an abuse of AAS does not only reduce the numbers of sperm, but also impairs the percentage of morphologically normal spermatozoa.

## 1.7 TESTOSTERONE CONCENTRATIONS

Testosterone circulates in the body both as free fraction (2%) and bound to albumin (54%) and sex hormone-binding globulin (44%). Free and albumin-bound testosterone comprises bioavailable testosterone (103). In addition to AASs induced hypogonadism the diagnosis of hypogonadism has been associated with expression as andropause or male menopause. Even though testosterone concentrations decline slowly with age, hypogonadism is a clinical condition marked by low concentrations of serum testosterone combined with symptoms as decreased libido, erectile dysfunction, and reduced muscle mass and bone density and depression and distinct from 'andropause' or 'male menopause' (104). Few studies on what constitutes a 'normal' testosterone level at any particular age have been published. Moreover, there are large differences in serum concentration of testosterone among patients after androgen replacement therapy (105) and among healthy controls after testosterone administration. The variation may partly be ascribed to genetic differences. (106,107). A decline after food intake compared to samples taken in the fasting state has been noticed. Therefore, it is recommended to collect samples in the morning after an overnight fasting (108). Smoking and stress are other factors which could affect the testosterone concentrations (104). In conclusion, total testosterone concentrations vary largely among AAS abusers, depending on the time period since last injection but also depending on age, genetic and environmental causes.

## 1.8 ANDROGENS AND AAS

The androgens constitute of a family of hormones. The prototype and most well-known androgen is testosterone. Testosterone has several synthetic derivatives, created since its characterization as the mammalian male sex hormone in the mid-

1930s (109, 43). Androgens bind to the intracellular androgen receptors which are present in most organs but the biological effects are tissue specific (110,111).

## 1.9 ENDOGENOUS ANDROGENS

Cholesterol is the original substrate for formation of glucocorticoids, mineralocorticoids and sex steroids (Figure 2). Precursors of androgens are formed in the adrenals and biotransformed in the endocrine target organs, i.e the gonads and the prostate gland. Dihydrotestosterone is formed from testosterone in the prostate and is a more potent androgen than testosterone itself. Some of the testosterone precursors have weak androgenic effects such as dehydroepiandrosterone and androstenedione, (112).

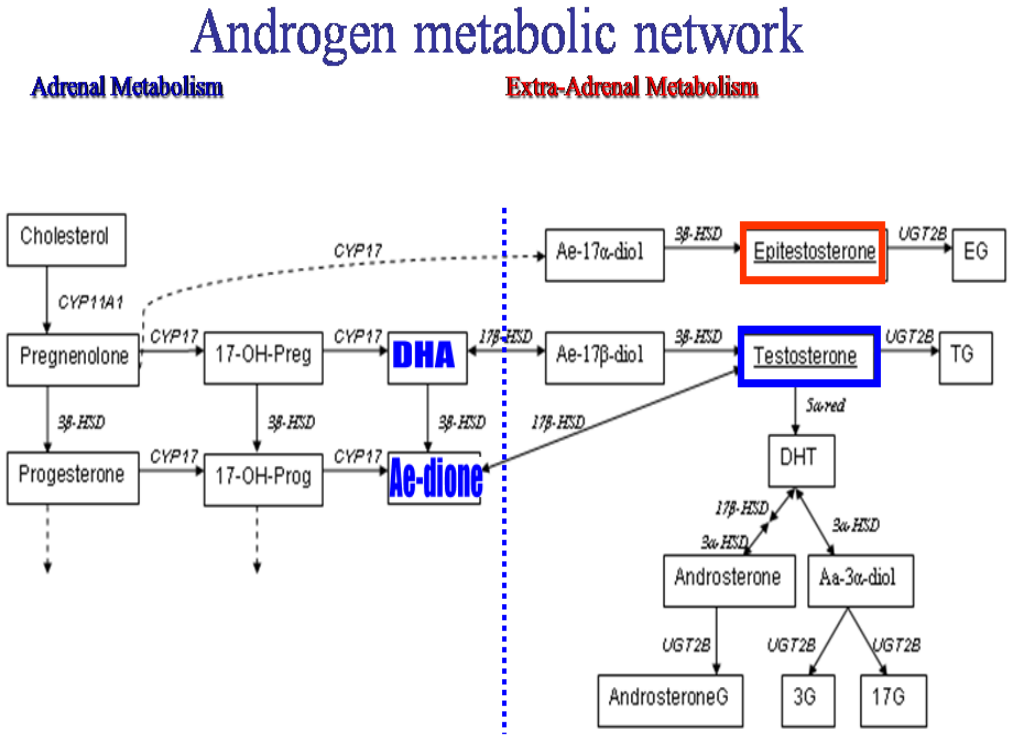



**Fig 2**. Metabolic network of androgens. Pathways to the left of the vertical striped line take place mainly in the adrenals, pathways to the right mainly in the gonads and the prostate. Preg = pregnenolone, Prog = progesterone, DHA = dehydroepiandrosterone, Ae-dione = androstenedione, Ae-17a-diol = Androstene-17a-diol, Ae-17b-diol = Androstene-17b-diol, Aa-3a-diol = Androstane3a-diol, DHT = dihydrotestosterone, G = glucuronide. Adapted from Rane and Ekström L, 2012

## 1.10 DOPING TEST VERIFYING AAS

AAS may be analyzed and verified in urine samples. Since testosterone is an endogenous compound abuse is analyzed by a ratio between testosterone and epitestosterone (T/E ratio) calculated after cleavage of glucuronide conjugates in urine (112). Epitestosterone levels are typically highest in young males; by adulthood, most healthy Caucasian males have a T/E ratio of about 1.1 (115). Administration of testosterone affects also the excretion of epitestosterone in the body due to the suppression of LH. However, there is a large interindividual difference (114). A T/E ratio of 4.0 has been used as the cut-off level in samples to be suspected for doping in sports, (www.wada-ama.org). For detection and verification of testosterone abuse in the society, a higher cut-off ratio is often applied to minimize the number of false positives. Interestingly, a number of subjects turned out to test falsely negative even with a ratio of 4.0 (113). A higher T/E ratio should therefore lead to even more falsely negative samples. Exogenous AAS are detected direct or by their metabolites (Table 2).

**Table 2) Commonly reported abused AAS**

| Generic name | Trade Name | Analysis |
|---|---|---|
| Testosterone enanthate | Various[1] | T/E ratio |
| Testosterone propionate | Various[2] | T/E ratio |
| Testosterone cypionate | Various[3] | T/E ratio |
| Nandrolone decanoate | Deca-Durabolin | metabolites |
| Stanozolol | Winstrol | metabolites |
| Methandrostenolone | Russian/Dianabol | metabolites |
| Boldenone undecylenate | Equipose | metabolites |
| Oxymetholone | Anadrol | metabolites |
| Oxandrolone | Anavar | metabolites |

For example: [1)] Testo, Test E, Testoviron, Testoject, Andro-100, [2)] Testovis, Testorapid, Testo-prop, Testex, [3)] Test-cyp, Deptrone, Andro-cyp, D-test

Gårevik 2013 Reported to Anti-Doping Hotline, at the division of Clinical Pharmacology at Karolinska University Hospital, Sweden 2012

### 1.10.1 FALSELY NEGATIVE VERSUS FALSELY POSITIVE AAS ANALYTICAL RESULTS

Jacobsson et al found a large interethnic variation in testosterone glucuronide excretion and a strong association with a deletion polymorphism in the uridine diphospho-glucuronosyl transferase (UGT 2B17). This polymorphism challenges the accuracy of the T/E ratio test. The gene can be either inserted or deleted, the three genotype groups are; *ins/ins* (two alleles), *ins/del* (one allele) or *del/del* (both alleles deleted). After administration of a single im dose of 500 mg testosterone enanthate to healthy male volunteers a large variation in T/E ratio was noticed. The degree and rate of increase in the testosterone glucuronide excretion rate were highly dependent on the UGT2B17 genotype with a 20-fold higher average maximum increase in the *ins/ins* group compared to the *del/del* group. Of the *del/del* subjects, 40% never reached a T/E ratio of 4.0 within 15 d after dose (114). Even after long term abuse of high doses of testosterone the T/E ratio can be below 4 (paper II). Genotype-specific T/E ratio cutoffs have been suggested to solve this problem (115). Moreover some males have T/E values greater than the accepted ratio value in sport (4.0), even without testosterone abuse. The main reason for such false-positive results is a low epitestosterone glucuronide concentration rather than a high level of testosterone glucuronide, which is partly due to a polymorphism in the CYP17 gene (116).

## 1.11 NANDROLONE

Nandrolone is a commonly abused AAS and detected by its metabolite 19-norandrosterone (19-NA) together with 19-noretiocholanolone. The threshold level is 2 ng/mL in both males and females. This cut-off ratio was implemented to consider possible endogenous production of nandrolone metabolites in humans. It is possible to detect nandrolone during pregnancy and there are reports that a generous amount of boar tissue containing endogenous 17β-nandrolone may cause excretion of 19-NA and 19-noretiocholanolone in the urine whitin hours after consumption (117,118). After single injections of 50, 100 and 150 mg nandrolone the metabolite is traceable in urine at concentrations higher than the threshold value of 2 ng mL for 6 months after injection of a single dose of 150 mg nandrolone decanoate (119).

Some individuals in paper II the 19-NA was detected up to one year after their last injection of nandrolone decanoate. Traceability over time depends mainly on the pharmaceutical formulation and the route of administration. Due to fast phase I

metabolism, nandrolone itself is rapidly excreted and traceable in urine for a few days (2–6) after intake of a single oral dose (120,126).

## 1.12 SUGGESTED DIAGNOSTIC CRITERIA FOR AAS ABUSE/DEPENDENCE

In the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, (DSM-IV) anabolic androgenic steroid dependency is found in the "other substance-related disorder" section and can be coded, depending on which diagnostic criteria are used. International Statistical Classification of Diseases and Related Health Problems - Tenth Revision (ICD-10) codes steroids and hormones in a section on "abuse of non dependence producing substances." ICD-10 goes on to state that "although it is usually clear that the patient has a strong motivation to take the substance, there is no development of dependence or withdrawal symptoms as in the case of the psychoactive substances." In 1994, the DSM-IV Sourcebook evidence review stated that "despite increasing clinical descriptive data on anabolic steroid withdrawal, dependence, and abuse, there are insufficient substantial basic or clinical research data to support the inclusion of these syndromes in DSM-IV" (122). Kanayam et al discuss a number of areas concerning AAS dependency on purpose to clarify the diagnostic criteria for AAS dependence for DSM-V. They suggest that the existing *DSM* criteria could be adapted for diagnosing AAS dependence with only small interpretive changes (123). Scally and Tan highlights that the symptoms of hypogonadism are identical to many of those described for the adapted AAS dependency criteria. As such, AAS-induced hypogonadism is a possible confounding variable in the diagnosis of anabolic-androgenic steroid dependency. They conclude properties must be taken into consideration regarding an AAS dependency syndrome (121). To be noticed, detection of AAS abuse is not included in the suggested criteria.

# 2. AIMS

The overall aim of this thesis was to increase the knowledge about two of the most commonly abused AAS, nandrolone and testosterone, and to investigate the influence on the lipid profile and gonadotropins.

The specific aims of the respective papers were:

**I)** To investigate and describe the co-abuse of AAS and narcotics in a group suspected of infringement of the narcotic laws in Sweden

**II)** To study the influence and the time course and reversibility of the effects on blood lipids and gonadotropins in abusers of nandrolone and testosterone. To study the relation between urinary biomarkers for testosterone and nandrolone abuse in relation to UGT2B17 genotype and time profile

**III)** To investigate whether a single dose of testosterone enanthate affects the cholesterol profile and the expression of HMGCR in healthy volunteers.

**IV)** To study the effect and time profile of different doses of testosterone enanthate on the blood lipid profile and gonadotropins

# 3. MATERIALS AND METHODS

## 3.1 SUBJECTS

All studies were approved by the Regional Ethics Committee and all subjects gave informed consent to participate, except in paper 1.

The study groups in the different papers are summarized in table 2.

| Study | Subjects | Sex | Design | AAS Substances | Age ± SD | N |
|---|---|---|---|---|---|---|
| Interview abuse pattern, I | Abusers | Male | Descripitive | several | 30 ± 7.4 | 56 |
| Gonadotropins, blood lipids and excretion, II | Abusers | Male | Experimental | nandrolone and testosterone enanthate | 26.4 ± 7.2 | 56 |
| Total cholesterol and HMG CoA Reductase, III | Healthy volunteers | Male | Experimental | testosterone enanthate | 29.4 ± 2.3 | 39 |
| Gonadotropins, blood lipids and excretion, IV | Healthy volunteers | Male | Experimental | testosterone enanthate | 33.8 ± 4.7 | 25 |

## 3.2 SUBJECTS-PAPER I

In the first study data on 56 individuals suspected of infringement of the narcotic laws in Sweden with confiscated and/or confirmed use of AAS. 45 of the subjects were confirmed abusers who tested positive for AAS. The mean age (SD) for those with only possession was 25±4.2. Information about subjects' present and past drug use was obtained through mandatory interviews with individuals. Data were collected between May 2007 and May 2008.

## 3.3 SUBJECTS-PAPER II

Fifty-six men between 18 and 57 years old were recruited through Anti-Doping Hot-Line to the project between 1998 and 2002. Mean onset of AAS abuse was 21 ± 5.01. The duration of AAS abuse was 5.2 ± 4.1 years. An intention and a promise to give up abusing AAS was a prerequisite to be included. No economical compensation was given to participants. Individuals were clinically investigated and a series of endocrine parameters were monitored in blood and urine samples that were collected

at different time points. At each visit they met a research nurse who could answer questions and check their social and psychological condition. If necessary, individuals were referred to qualified medical assessment and treatment at the Departments of Psychiatry and Endocrinology at the hospital.

### 3.4 SUBJECTS-PAPER III

Thirty nine healthy male volunteers were given 500 mg testosterone enanthate as a single intramuscular dose of Testoviron®–Depot. All participants were males aged 18-50 years. All participants underwent a medical examination, including laboratory tests, before enrollment to exclude any disease. Further inclusion criteria included a negative screening for illegal drugs, anabolic androgenic steroids, HIV, and hepatitis B or C virus. For inclusion it was also required that the subject was not a member of any organization belonging to the Swedish Sports Confederation, or had a malignancy within the past 5 years or an allergy to the study substance or its constituents.

### 3.5 SUBJECTS-PAPER IV

All participants underwent a medical examination including laboratory tests before enrolment. They were negative on screening for illegal drugs, AAS, HIV, hepatitis B or C virus. None was taking any substance interfering with testosterone. The participants were given 500 mg, 250 mg and 125 mg testosterone enanthate as a intramuscular dose of Testoviron®—Depot with a washout period of 6-8 weeks between the doses. Two participants did not take part in the 125 mg dose study.

### 3.6 METHODS-PAPER I

The compulsory data collection was done by two police units in Stockholm: the. Juvenile Drug Unit of the Stockholm County Police responsible for work with abusers of narcotics less than 25 years of age, and the “Club Commission” of the Stockholm County Police working with crimes associated with night clubs and restaurants. Both units are staffed by members of the narcotics police. Data was documented through an anonymous questionnaire. Data consisted of information about age, sex, confiscated AAS substances (type and amount), reasons for AAS abuse, experience of AAS use and regular gym training. Information gathered also included age at onset of AAS or narcotic agents (opioids, cocaine, amphetamine, cannabis, and marijuana) as well as information about ingestion of other medicines

such as growth hormone, insulin, antiestrogens, hCG, clenbuterol, ephedra, sildenafil and benzodiazepines.

### 3.7 METHODS-PAPER II, III AND IV

#### 3.7.1 URINARYANALYSES

Determination of urinary 19-NA level and T/E ratio was made using a validated gas chromatography–mass spectrometry (GC–MS) method (124,125) at our WADA accredited Doping Laboratory within the Division of Clinical Pharmacology.

#### 3.7.2 UGT2B17 GENOTYPING

Preparation of genomic DNA from serum samples was performed using Qiagen Mini Blood kit. Genotyping of the UGT2B17 deletion polymorphism was assessed by real-time PCR using UGT2B17 specific primers and VIC labeled gene specific probes. As controls the expression of β-actin and albumin were analyzed. If signals were obtained for β -actin and albumin but not for UGT2B17 the sample was identified as *del/del*, whereas if signal were produced in both UGT2B17 and control reactions the sample was identified as an ins-carrier *(ins/del or ins/ins).* The quality and quantity of serum derived DNA did now allow us to discriminate between *ins/ins* and *ins/del*.

#### 3.7.3 SERUM ANALYSES

All serum (S-FSH, S-LH, s-testosterone) and plasma analyses (P-Cholesterol, P-LDL, P-LDLD, P-Apolipoprotein B, P-HDL, P-Apolipoprotein A1, P-Lipoprotein(a), P-Apolipoprotein B and P-triglycerides) alanine transaminase (ALAT) and aspartate transaminase (ASAT) were determined by routine methods at the Division of Clinical Chemistry (Karolinska University Hospital, Stockholm). Total testosterone concentrations in serum in paper III were previously measured by GCMS (paper III).

#### 3.7.4 HMGCR

The concentration of the HMGCR in whole blood samples was analysed by Western blotting. The mRNA level of HMGCR in testosterone treated HepG2 cells was determined by real-time PCR. RNA extraction and cDNA was performed according to the manufacturer´s protocol as described in paper III.

### 3.7.5 STATISTICS

Statistical software programs were used to calculate significance of differences and correlations. See the respective paper

# 4. MAIN FINDINGS AND COMMENTS

## 4.1 PAPER 1: DUAL USE OF ANABOLIC ANDROGENIC STEROIDS IN SWEDEN

Urine samples and abuse information were collected by two police units in Stockholm. A majority of the AAS users (73%) also used narcotic agents. Only a minority (21%) of these individuals began their drug abuse with AAS and 55% with narcotic agents. Data about the initial type of abuse was missing for 24% of subjects. Twenty seven percent abused only AAS. The most commonly co-used substances were cannabis (35%), cocaine (28%), diazepam (26%), amphetamine (15%), ephedrine (11%), sildenafil (8%), heroin (4%) and other medicines (28%) such as: growth hormone, insulin, antiestrogens, hCG, clenbuterol, ephedra, sildenafil and benzodiazepines. The most commonly confiscated AAS were testosterone, nandrolone, trenbolone, boldenone, stanozolol, oxymetholone, methandienone and methenolone.
The use of AAS is prohibited in Sweden why our denomination is abuse. In an internationally perspective use is more proper since Sweden is quite unique with this legislation compared to other countries world-wide. In the published articles we therefore denominated it: “AAS use”.

### 4.1.2 COMMENTS

This study was based on information from 45 male AAS abusers subject to analyses and mandatory interview. This group was recruited to the study by two police units staffed by members of the narcotic police, thus it can not disclose to what extent AAS abuse generally exists among users of narcotic agents or vice versa. The presumption that AAS abuse is primarily part of a “healthy lifestyle” pattern that includes body-building and use of nutritional supplements was not confirmed. Only one-fifth of the subjects in the study had abused AAS prior to narcotics. The study does not inform anything about the abuse pattern, it could be sporadic or regular abuse of AAS or narcotics in the investigated group of co-abuse. The small number of gym customers among the dual abusers indicates that esthetic or strength purpose was not the main aim. This is also consistent the high presence of cannabis, due to the fact it is the most common drug at onset of abuse of narcotics. Our study does not give any information about the reasons for AAS abuse in the non- gym customers group of subjects. Supraphysiologic doses of

AAS may increase fat-free mass and muscle size even without strength training (12). This may attract some potential abusers without motivation for regular gym training. Further reason could be they simply did not visit any gym but exercised physically not at a gym but in another environment. AAS are also known to increase aggressiveness and inhibit impulse control, and this may be desirable among those who intend to commit criminal acts (15). The most commonly abused AAS substance was testosterone and nandrolone. This is consistent with other studies in Swedish populations (10, and Table 2, page 12). The reasons for nandrolone and testosterone to be two of the most preferable AAS of choice were not explored in this study.

### 4.1.3 INTERPRETATIONS

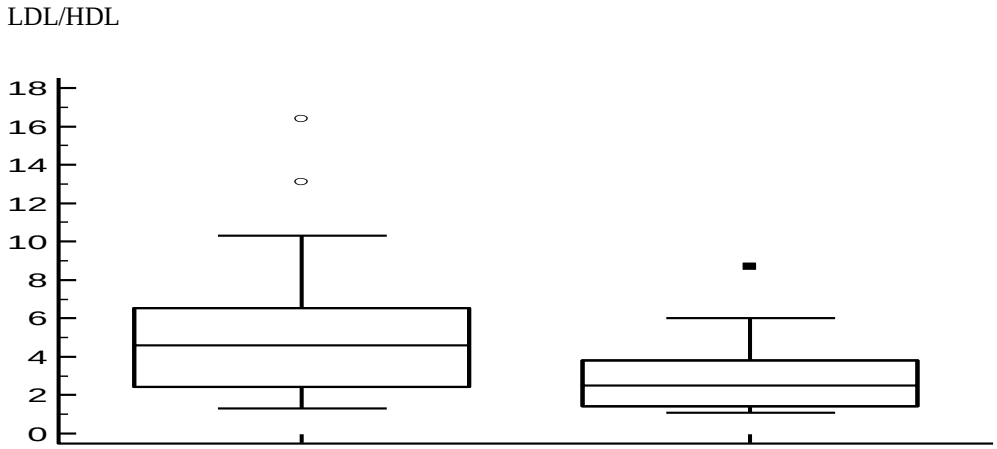
Our study has established that AAS abuse can be part of an advanced abuse pattern. The study did not lend support to the hypothesis that AAS abuse is a common "gateway" to narcotic use. Nor did it support the contention that AAS abuse is primarily part of a "healthy lifestyle" pattern that includes body-building, at least not in the investigated group of individuals. Further research in clean AAS abusers is needed to exclude the abuse of narcotic substances.

## 4.2 PAPER II: LONG TERM PERTURBATION OF ENDOCRINE PARAMETERS AND CHOLESTEROL METABOLISM AFTER DISCONTINUED ABUSE OF ANABOLIC ANDROGENIC STEROIDS

Fifty-six men between 18 and 57 years old, working out at gym facilities were recruited via Anti-Doping Hotline between 1998 and 2002. Intention to stop abuse of AAS was a prerequisite to be included. Some of them also admitted the use of other doping agents (hCG, efedrin, GH, tamoxifen) as well as otherdrugs (prescribed and/or OTC). Three individuals were positive for narcotics in urine analysis and were subsequently excluded from the study. Based on the interpretations in paper I, we found this group appropriate to investigate the alteration, time course and possible reversibility of blood lipids and gonadotropins in nandrolone and testosterone abusers. Of the 53 individuals, 23 individuals were confirmed as nandrolone and testosterone abusers by urine analysis and fulfilled the inclusion criteria. Of 21 individuals testing positive for 19-NA at the first visit, 6 months later 80% had 19-NA levels still above 2 ng/mL. Nine months later 58% (N=12) tested positive. After one year only 4 subjects remained in the study and 2

of them were still positive for 19-NA. All of the 21 nandrolone positive showed initial signs of compromised endocrine function as revealed by suppressed concentrations of LH and FSH. Fifty % and 70% of the individuals had FSH and LH concentrations below the detection limit of 0.1 IU/L and 0.7 IU/L, respectively. There was a significant correlation between 19-NA and LH and FSH concentration during 16 weeks. First visit ($r = -0.57$, $p = 0.02$ and $r = -0.75$, $p < 0.001$, respectively). 9–16 weeks after the last nandrolone intake, ($r = -0.76$, $p < 0.001$ and $r = -0.72$, $p < 0.001$, for LH and FSH respectively ($N = 15$).

Six months (visit 2) after last injection LDL was significantly lower than at the first visit ($p = 0.0061$). The concentration of HDL was significantly higher ($p < 0.001$) in 31 of the nandrolone and testosterone abusers. Mean concenctration of HDL at first visit 0.90 ± 0.05 mmol/L and at second visit, mean 1.13 ± 0.05 mmol/L. Mean concentration LDL at first visit: 3.24 ± 0.25 mmol/L and 2.63 ± 0.17 mmol/L at the second visit. The ratio of LDL/HDL concentration was significantly lower ($p < 0.004$) 6 months after discontinued abuse. Mean ratio at first visit: 5.1 ± 3.7 and after 6 months 2.9 ± 1.8, (Fig 3). The concentration of total cholesterol did not differ between the two visits.




LDL/HDL ratio in 31 individuals at their first visit to the clinic and 6 months later. All were positive for AAS at visit 1 and at visit 2 they had not relapsed into AAS-abuse. Significantly lower LDL/HDL ratios were observed at visit 2 (paired t-test).

The major enzyme responsible for testosterone glucuronidation is UGT2B17. Three of the 12 testosterone abusers were identified as homozygous for the

UGT2B17 gene deletion (*del/del*). When tested for testosterone abuse 1–5 weeks after their last testosterone injection, none of them were positive in the doping test using the T/E-ratio as biomarker. All individuals expressing UGT2B17 enzyme (*ins/del ins/ins)* displayed a T/E ratio above the cut off level 4 (positive in doping test). The T/E ratio was monitored in weeks 2, 4, 6 and 8 after the first visit and found to decrease with time. Two individuals relapsed into testosterone abuse, but only the one expressing UGT2B17 was suspected on the basis of the T/E as a biomarker. The *del/del* individual was never suspected.

### 4.2.1 COMMENTS

We believe the population in this study was as similar to one can get to the earlier common understanding of "AAS abusers less likely to abuse other illicit drugs based looking upon on themselves as healthy persons" (25, 26). It would be hard to collect this population today, since abuse of narcotics is common among AAS-abusers in Sweden today (paper 1, 128). The subjects in this study were only abusing AAS and individuals with narcotics abuse were excluded (only three). The urinary nandrolone metabolite 19-NA remained detectable for a long period of time, for some of the individuals up to one year after their last injection of nandrolone decanoate. One previous study showed that exogenous 19-NA could be detected for 6 months in some individuals after a single dose of 150 mg nandrolone decanoate (119). Exctreted 19- NA were greater after first urine analysis (day 0) compared to later performed urine analysis. We have no reason to believe this mirror a relapse in AAS abuse. The time since they took their last injection and the dose various which must be taken into consideration. The urinary excretion profile of 19-NA showed in our study as well as in healthy volunteers (119, 126, 127) disclose an inter-individual variation in the 19-NA excretion kinetics. It is not known why these metabolites reside in the body for such as long time since the parent substance nandrolone itself is only detectable in serum for 2–5 weeks (119). We show here for the first time that the long presence and slow elimination of nandrolone metabolites are associated with endocrine consequences. Possible long-term medical consequences of these effects need to be addressed in further studies. The relations between endocrine consequences and common reported side effects as hypogonadism and depression are also important further studies.

In our study group the AAS abusers had an HDL concentration below 1.0 mmol/L at visit 1, whereas six months after AAS cessation they have reached a HDL concentration of 1.1 mmol/L. According to Framingham data individuals below 1.0 mmol/L had a fourfold increase in risk of coronary heart disease compared to those with concentrations of 1.03–1.27 mmol/L, and in agreement with previous conclusion, low HDL concentrations may account for an increased risk of coronary heart disease in AAS abusers (82). At the time for the investigation the reference range for men in this age group was 0.6-1.8 mmol/L therefore the majority did not leave a new sample for blood lipids analysis until six months after the first analysis. The reference range for LDL was 1.6-4.6 mmol/L, and the majority was within reference range at the first visit. However, the ratio LDL/HDL was above the upper recommended limit for a major part of the individuals at the first visit (reference range <5). The ratio is a better indicator for adverse blood lipids profile on the individual level. The blood lipid profile was significantly improved 6 months after cessation of the AAS-abuse.

None of the homozygous of UGT2B17 gene deletion (*del/del*) was positive for testosterone abuse. Two individuals relapsed into testosterone abuse, but only the one expressing UGT2B17 was suspected on the basis of the T/E as a biomarker. An increased sensitivity of the analysis of testosterone in urine is needed since even the use is forbidden according to Swedish law. Population cut-off value approach may only engage athletes that are repeatedly tested, and not in the analyses of illegal abusers in the society. Our results clearly show that genetic information would increase the sensitivity of the test. It will not only minimize the false negatives and improve the analytical part in AAS abuse medical investigation it would also be fair to the concerned individual and to the society.

As is obvious with this type of investigation there are inevitable limitations. In addition to their mixed intake of AAS, some of them also admitted the use of other doping agents (hCG, ephedrin, GH, tamoxifen) as well as other drugs not considered as narcotics. The varying time interval since they took their last injection is a further confounder that must be taken into consideration.

### 4.2.2 INTERPRETATIONS

The main findings in our study established a sustained suppression of LH and FSH lasting for several months. The urinary biomarker 19-NAwas correlated to the concentrations of LH and FSH. Some of the testosterone abusers did not test positive due to a genetic deletion polymorphism of the UGT2B17. Significantly increased concentrations of HDL and decreased concentrations of LDL and decreased LDL/HDL ratio were observed for 6-months after cessation of AAS abuse. In order to identify the pure effects of AAS another study design is required. Moreover the time and the dose of administrated AAS should ideally be known.

## 4.3 PAPER III: SINGLE DOSE TESTOSTERONE INCREASES TOTAL CHOLESTEROL LEVELS AND INDUCES THE EXPRESSION OF HMG CoA REDUCTASE

Thirtynine healthy volunteers were given 500 mg testosterone enanthate as a single intramuscular dose of Testoviron® Depot equivalent to 360 mg testosterone. Blood and serum was collected prior to (day 0), 2 and 15 days after testosterone administration. All samples were collected between 07 and 11 am and were directly frozen at -20°C. based on our results in paper II, we believe this study design is appropriate to investigate the time course of the effects on blood lipids and their possible reversibility. We also investigated whether a single dose of testosterone enanthate affects the expression of HMGCR, the rate limiting enzyme in the cholesterol synthesis chain,.

Total cholesterol level increased on day two to 4.87 ± 0.25 mmol/L from 4.23 ± 0.27 mmol/L on day 0 ($p = 0.007$). The total cholesterol level was back to baseline (4.23 ± 0.14 mmol/L) on day 15. There was no significant difference in HDL, LDLD or VDL between days 0 and 2.

There was a significant increase in HMGCR level in whole blood on day 2 ($p = 0.03$). The gene expression of HMGCR was 1.8 fold higher 2 hours after treatment ($p < 0.001$) but lower (0.8) fold after 24 hours ($p = 0.047$). A correlation analysis demonstrates that the increase in cholesterol concentrations correlated significantly to the total testosterone concentrations on day 2 ($r^2 = 0.14$, $p = 0.02$)

### 4.3.1 COMMENTS

To our knowledge, this is the first time an increase in total cholesterol level has been observed after only one single dose of testosterone. Moreover, we show for the first time that a supra-physiological dose of testosterone induces the gene expression of HMGCR *in vivo,* in blood, in healthy volunteers. In HepG2 cells exposed to testosterone we found a time dependent normalization and down regulation presumably because of a negative feedback on the cholesterol synthesis on a transcriptional level. It is conceivable that AAS may impact on the cholesterol homeostasis, partly via an increase of the HMGCR expression. Using whole blood as surrogate model for HMGCR expression may, however not reflect the expression profile in the liver.

We measured total cholesterol two days after testosterone administration, there were no diet restrictions. They were all in postprandial phase; the change in total cholesterol can hence be influenced by factors other than the testosterone injection.

### 4.3.2 INTERPRETATIONS

The immediate response to AAS is a cause for concern and warrants follow-up of the cardiovascular risk factors that may appear later in life in AAS abusers.

To measure lipid profiles in blood before and after administration of one dose of testosterone is the best possible design to interpret perturbations in the lipid profile.

## 4.4 PAPER IV: EFFECTS ON GONADOTROPINS AND BLOOD LIPIDS OF DIFFERENT DOSES OF TESTOSTERONE IN HEALTHY MEN

In this study we investigated the effect of different doses of testosterone enanthate on the lipid profile and on gonatropins in healthy volunteers. The lipoprotein profile and endocrine profile was analysed prior to, four and fourteen days after administration of 500, 250 or 125 mg testosterone enanthate. All participants had s-LH (reference range 1.2-9.6 IU/L) and s-FSH (reference range 1.0-10.0 IU/L) concentrations in the normal range for age prior to first dose (day 0) of testosterone enanthate 500 mg (mean value 3.46 ± 1.08 IU/L and 3.27 ± 1.63 IU/L respectively).

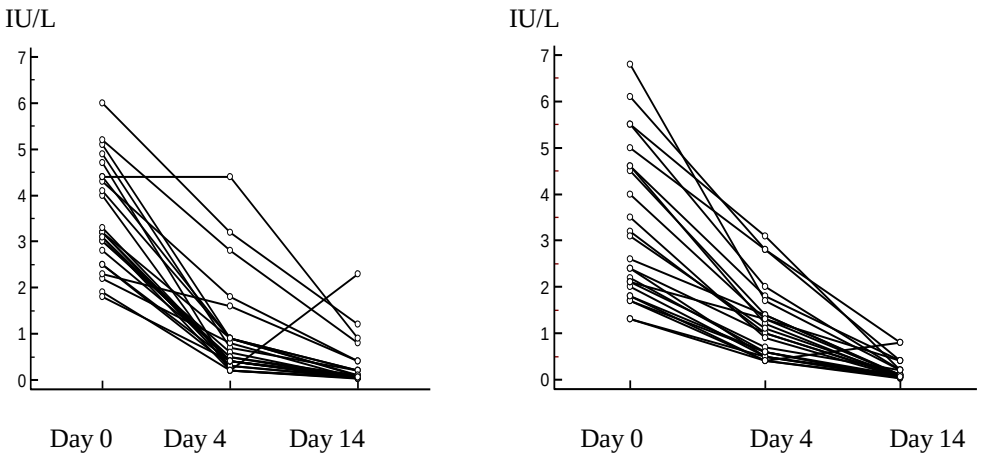
Before the last dose (125 mg) all s-LH concentrations were within the reference range (mean value 3.25 ± 0.34 IU/L).

S- LH and s-FSH were significantly decreased after the first (500 mg), second (250 mg) and third dose (125 mg) on days 4 and 14 ($p = <0.0001$), (all s-LH and s-FSH concentrations shown in Figure 4).

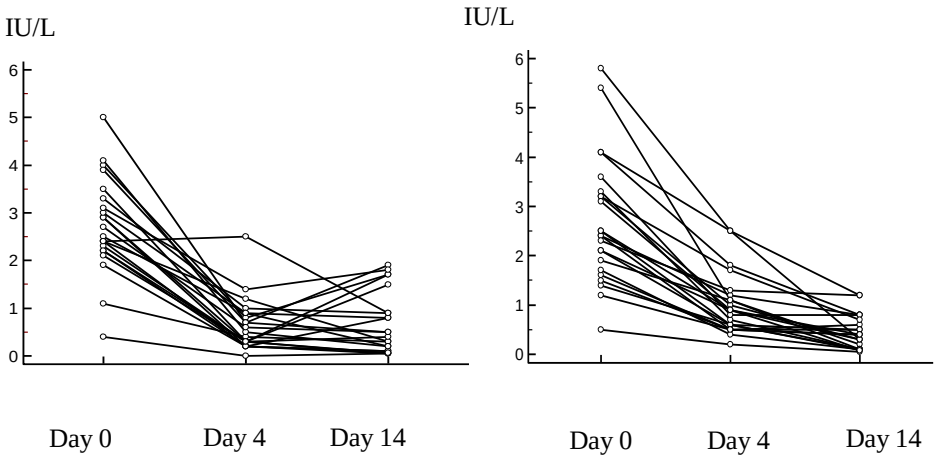
Prior to the second administration of testosterone enanthate (250 mg) and after a 6-8 weeks washout period there was a significant decrease (p= 0.01) in s-LH concentrations (mean value 2.85 ± 1.16 IU/L) compared to day 0 (3.46 ± 1.08 IU/L) before the first dose. Two individuals of these had even s-LH concentrations below reference range (0.4 and 1.1 IU/L) before the second dose (day 0), compared to day 0 before the first dose (3.2 and 2.8 IU/L respectively).

**Fig 4).** Dose dependent suppression of s-LH and s-FSH after different parenteral doses of testosterone enanthate. Day 0 = refers to values before administration.

**S- LH before and after 500 mg testosterone** **S-FSH before and after 500 mg testosterone**




**S- LH before and after 250 mg testosterone** **S-FSH before and after 250 mg testosterone**




**S- LH before and after 125 mg testosterone** **S-FSH before and after 125 mg testosterone**

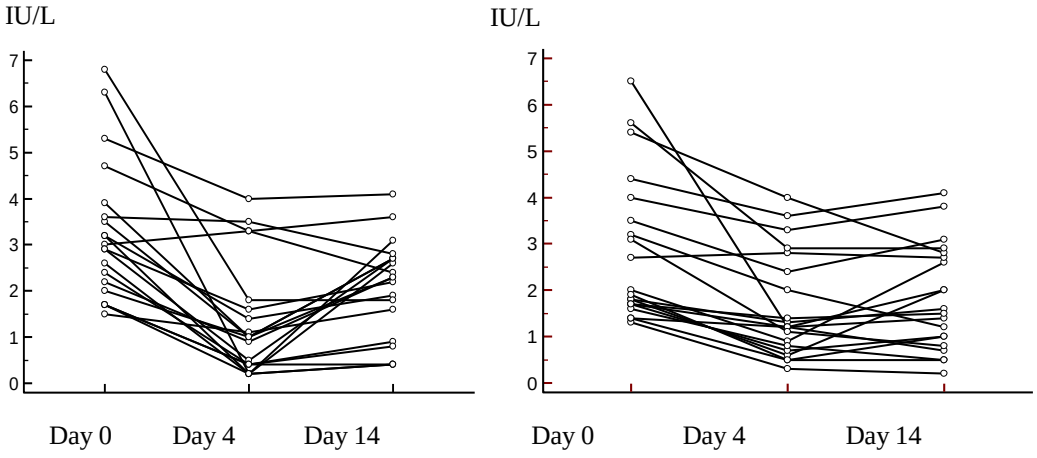
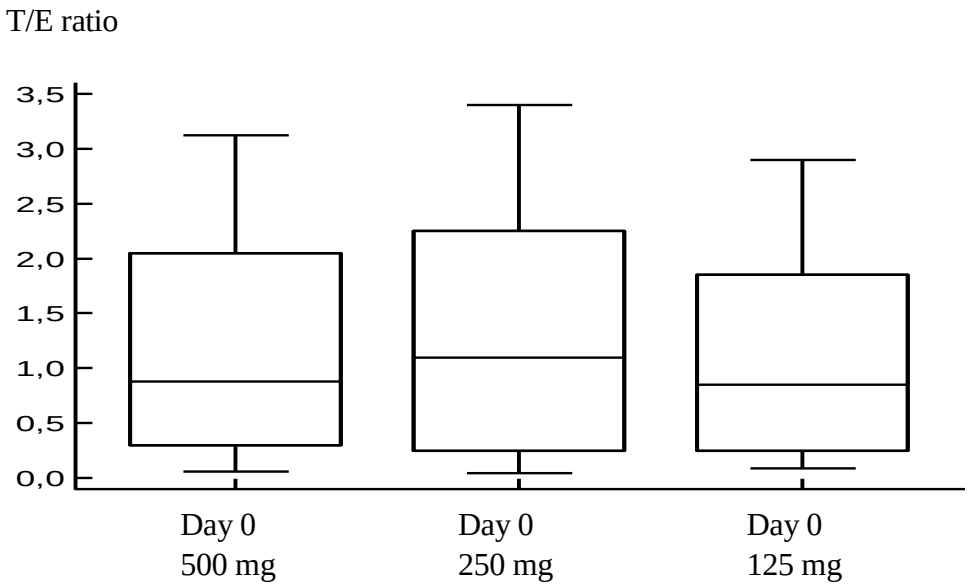


**Fig 5)** T/E ratio before parenteral testosterone administration, (day 0) with a 6-8 weeks washout period no significant changes in the T/E ratio




All urine T/E ratios were back to baseline values before each new intramuscular dose of testosterone enanthate. Results are given as mean ± SEM. Mean values prior to the 500, 250 and 125 mg doses were 1.19 ± 0.22, 1.32 ± 0.25, and 1.14 ± 0.21, respectively (Fig 5). Central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents the median. Horizontals line extends from the minimum to maximum.

All participants had s-testosterone concentrations in the normal range for age (10-30 nmol/L) before first dose of 500 mg (15.02 ± 0.76 nmol/L). The concentrations of s-testosterone reached a maximum on (+360 %) on day 4 and decayed to + 39 % on day 14 after the 500 mg dose. The peak increase on day 4 was + 112 % after the 250 mg dose ($p < 0.0001$). There was no increase day 14 after second dose and there was no significant increase after the third 125 mg dose on any day. However, there was a significant decrease ($p<0.05$) with 63% on day 14 after the 125 mg dose.

P-ApoA1 concentrations decreased 12 and 18 % four and 14 days after administration of 500 mg ($p <0.0001$). There was no change day 4 after second dose (250 mg) in p-ApoA1 concentrations but on day 14 there was a decrease with 12 % ($p < 0.05$). There was no significant change of the ApoA1 concentrations after the lowest dose. P-HDL cholesterol concentrations decreased with 8 and 10 % on days 4 and 14 after

500 mg testosterone ($p<0.001$). The corresponding values after 250 mg were 8 and 15 % ($p <0.001$). There was no significant change of 125 mg in p-HDL cholesterol concentrations. (All significant changes shown in table 3)

**Table 3)** Dose dependent increase in ApoB/ApoA1 and LDL/HDL ratio and dose dependent decrease in HDL, ApoA1 and Lp(a) concentrations 4 and 14 days after parenteral (day 0/4/14) testosterone doses. Day 0 = refers to values before administration.

| | 500 mg | | | 250 mg | | |
|---|---|---|---|---|---|---|
| **Day** | **0** | **4** | **14** | **0** | **4** | **14** |
| ApoB/ApoA1 | 0.49±0.03 | 0.47±0.03 | 0.61±0.03[a] | 0.52±0.03 | 0.53±0.03 | 0.55±0.03 |
| LDL/HDL | 2.41±0.14 | 2.61±0.18[b] | 2.86±0.19[b] | 2.48±0.19 | 2.86±0.19[b] | 2.62±0.17[a] |
| HDL nmol/L | 1.19±0.05 | 1.1±0.05[b] | 1.08±0.04[b] | 1.27±0.04 | 1.16±0.06[b] | 1.12±0.05[b] |
| ApoA1( g/L) | 1.72±0.07 | 1.57±0.06[a] | 1.41±0.05[a] | 1.67±0.05 | 1.58±0.04 | 1.56±0.04[c] |
| Lp(a) mg/L | 96 | 100 | 68[c] | 81 | 105 | 68 |
| Range Lp(a) | 50-589 | 50-542 | 50-472 | 50-603 | 50-623 | 50-567 |

Anova repeated measure adjustment for multiple comparisons: Bonferroni corrected. Results are given as mean ± SEM [a] p=<0.0001 [b]p=0.001 [c] p=<0.05 (data before and after administration of 125 mg testosterone are not shown)

### 4.4.1 COMMENTS

We have studied the metabolism and excretion of androgens in healthy volunteers. LH remained repressed even 6 weeks after dose of 500 mg and for two individuals even below lower limit of reference range. These results indicate that AAS have a more profound endocrine effect on the hypothalamic-pituitary-adrenal -axis than was previously known. All urinary T/E ratios were back to baseline values before each dose as expected. This study demonstrates that the minimal dose to increase serum testosterone concentrations and suppressed s-LH and s-FSH was found to be 250 mg of testosterone enanthate. The maximum significant difference in the lipid profile occurred 14 after 500 mg in ApoA1. A decrease of 12 % in dose of 250 mg also

occurred fourteen days after second dose. HDL followed the same pattern with a maximum decrease 14 days after dose. The ratios ApoB/ApoA1 maximum increased 24.5% and LDL/HDL 16.3 % 14 days after dose of 500 mg. Androgens regulation of p-Lp (a) is shown by the moderate decrease of 14 % after a single dose of 500 mg testosterone. There was no significant change in the lipid profile following the lowest dose of 125 mg. The minimal single dose for these effects on s-testosterone, ApoA1 and HDL was dose 250 mg testosterone enanthate. The results clearly show a dose dependent adverse effect on the gonadotropins and unfavorable changes in blood lipids.

### 4.4.2 INTERPRETATIONS

We presume a dose dependent increase in serum testosterone concentrations with suppression of s-LH and s-FSH in all men is 250 mg testosterone enanthate. This estimate should be interpreted with caution since the concentrations of LH and FSH were significantly decreased after 125 mg. The threshold dose for alterations in ApoA1 and HDL concentrations was also 250 mg and for LP (a) 500 mg. Another AAS, stanozolol was studied in respect of its effect on p-HDL and found to give a 71% decrease 7 days after 10 days treatment with this agent (76).

# 5. GENERAL DISCUSSION AND FUTURE PERSPECTIVES

The abuse of AAS (often in combination with other illegal drugs/narcotics) is a threat to the health of the abusers. The use of AAS may be part of a general pattern of using drugs for recreational purposes. All investigated individuals in papers I and II were controlled by urine analysis not only for AAS, but also for narcotics. Studies by other investigators on AAS in relation to gonadotropins and/or blood lipids did not control for possible co-abuse of AAS and narcotics (59, 73, 82, 90, 92). There is a lack of long term prospective studies on abuse of AAS and side effects. We have found long duration of suppressed gonadotropin concentrations after abuse of nandrolone which has previously not been shown. In paper II, LH and FSH were correlated to the 19-NA metabolites in urine. The prolonged decrease in LH 6 weeks after one dose of 500 mg testosterone in healthy volunteers indicates a long suppression also after testosterone exposure. These results indicate that AAS have a more profound endocrine effect on the hypothalamic-pituitary-adrenal -axis than was previously known.

There was an inter-individual variation in the 19-NA excretion kinetics. The long detection time of 19-NA and the slow excretion rate need to be further studied in a controlled setting. Moreover, in the group of AAS abusers several of the individuals had LH and FSH at, or below the lower normal limit. Most likely the repression is associated with hypogonadism which is a well known consequence as reflected by decreased libido, impotence and other signs. The long term consequences of these findings remain to be established.

In agreement with other studies we show that testosterone has a negative effect on concentrations of HDL and ApoA1, but a potentially “beneficial” effect on Lp (a) by a reduction of the concentration after 500 mg. Unfavorable long-term changes in blood lipid profile may increase the risk of coronary heart disease.
We could demonstrate a perturbation in the lipoprotein profile after only one single dose of testosterone. Four and fourteen days after dose we found a decrease in HDL and ApoA1. This is a rapid response in the blood lipid profile of AAS as well as a rapid normalization after discontinuation of the AAS abuse.

There was no decrease in HDL and ApoA1 after the 125 mg testosterone dose. Thus, the minimal dose with effects on the lipid profile is 250 mg. There was significant dose dependent decrease in LH and FSH that lasted for 14 days after the three different doses of testosterone. The clinical experimental setting is very different from the real life situation of the AAS abusers. AAS are generally taken at repeated courses known as "cycles", each lasting several weeks to several months to reach maximum anabolic effects.

It is believed that AAS exert some of their influence on the cholesterol profile by inducing the HDL-catabolising enzyme hepatic triglyceride lipase (HTGL) synthesis in the liver (76). We provide an alternative or additional explanation that AAS may impact on the cholesterol homeostasis via an increase of the HMGCR expression. The effect of 500 mg testosterone in Lp(a) is of special interest since the serum concentration of Lp(a) seems to be genetically determined and cannot be lowered by alterations in food as well as competitive inhibitors of the HMGCR (59).

We have confirmed that the UGT2B17 polymorphism has an impact on the T/E ratio in AAS abusers. Individual cut-off value approach may only be employed in athletes that are repeatedly tested, and not in the doping control of illegal abusers in the society (115). Differences in anabolic effects in due to genetic variation would be important to know but this has not yet been studied. Variations in androgen metabolism due to genetic differences may have clinical importance since the response to androgen therapy may be affected, for example in treatment of hypogonadism.

As the abuse in society took place during the late 1970s and 1980s (3-5) the first generation of AAS abusers may possibly suffer from atherosclerosis today as a consequence of changes in blood lipid profile. Future longitudinal studies should especially investigate the clinical significance of AAS induced impairment of blood lipids in subjects abusing AAS.

# 6. CONCLUSIONS

From our studies the following conclusions can be drawn;

I) Co-use of AAS and narcotics agents is frequent among people taken into custody for criminal activity. The study does not lend support to the hypothesis that AAS may serve as a gateway drug to narcotic use.

II) Nandrolone abuse leads to sustained suppression of LH and FSH for a period of one year whereas the cholesterol profile may be normalized within 6 months.

III) One single dose of testosterone causes a perturbation in the blood lipid profile. It is possible that AAS may partly impact on the cholesterol homeostasis via an increase of the HMGCR expression.

IV) There is a dose dependent increase in serum testosterone concentrations and a corresponding suppression of s-LH and s-FSH after a dose of 500 or 250 mg of testosterone. The threshold dose for blood lipids alterations is 250 mg.

# 7. SVENSK SAMMANFATTNING

Testosteron och nandrolon är två av de vanligaste missbrukade anabola androgena steroiderna (AAS) i samhället. Motiven bakom AAS-missbruk är oftast av estetisk karaktär, såsom en önskan om större och mer väldefinierade muskler. Under 1970- och 80- talet när missbruket fick fäste i samhället blev det kopplat till ambitiösa kost- och träningsprogram. Sedan dess har sammissbruket med narkotika ökat och motiven för missbruk av AAS förändrats. Från att enbart inbegripa maximal anabol effekt kan ett motiv idag vara att förstärka självkänslan eller bara vara en del i ett avancerat missbruksmönster.

Biverkningarna av detta missbruk inbegriper bland annat ogynnsamma effekter på blodlipiderna, hjärt-kärlsjukdomar, endokrina störningar framförallt en minskad utsöndring av gonadotropiner (luteiniserande hormone (LH) och follikel-stimulerande hormon (FSH). Det i sin tur kan leda till en minskad egen produktion av testosteron med medföljande symptom på hypogonadism. Även allvarliga psykiska biverkningar är vanligt rapporterade såsom affektiva symptom, förlorad impulskontroll, depression, aggressivitet och även självmord.
För att säkerställa graden av missbruk och eventuell effekt av behandling mäts metaboliter av AAS i urin. Metaboliten av nandrolon heter 19 norandrosterone (19-NA). För diagnos av missbruk av testosteron analyseras kvoten testosteron/epitesto-steron (T/E-kvoten).
Blodfetter studeras genom att mäta bland annat det totala kolesterolet i blodet, blodfetter med hög densitet (HDL) som fraktar bort kolesterol från kroppen, blodfetter med låg densitet (LDL) som fraktar kolesterol ut i kroppen. Men även apolipoprotein A1(transportör och grundläggande del av HDL) och apolipoproteinB (grundläggande del i LDL). De ogynnsamma effekterna vid missbruk av AAS på blodfetterna består främst av en sänkning av HDL och apolipoprotein A1 och vid längre tids missbruk ett ökat värde av LDL. Detta kan på lång sikt medföra en ökad risk för hjärt-kärlsjukdomar.

Syftet med denna avhandling var att öka kunskapen om nandrolon och testosteron och undersöka deras inverkan på blodfetter och gonadotropiner. Genom att studera detta får vi större förståelse för riskerna med missbruk av AAS och bättre förutsättning att

utvecklinga vård och behandling. Studierna inom ramen för avhandlingen har funnit att:

- Hos individer, anhållna av polis för narkotikabrott ett omfattande sammissbruk med AAS och narkotika förelåg. Ett flertal av dessa hade börjat sitt missbruk med narkotika.

- I ett tidigare insamlat material på AAS missbrukare utan narkotikamissbruk fann vi att 19-NA kunde analyseras i urin upp till 1 år efter sista dos. Den minskade utsöndringen av FSH och LH var korrelerade till 19-NA i urin. Blod fetterna normaliserades 6 månader efter avslutat missbruk.

- Uppreglering av HMGCR ses redan efter en enda dos av testosteron. Detta kan delvis förklara testosteronets effekter på blodlipider.

- Vi fann en sänkning av LH och FSH bland män vid dos av 500 mg och 250 mg testosteron. Tröskeldosen för blodfettsförändringar och för ökade koncentrationer av testosteron i serum låg på 250 mg.

Den minskade utsöndringen av gonadotropinerna efter missbruk av nandrolon var långvarig (upp till ett år). Detta tolkat tillsammans med resultat från en dos av testosteron på friska försökspersoner där LH var nedtryckt mer än 6 veckor efter en dos indikerar att AAS har en starkare inverkan på HPA-axeln (hypothalamus, pituitary/hypofys, adrenal/binjure) än vad som tidigare var känt. Vi fann också en ogynnsam effekt på blodfetterna genom en sänkning av HDL och APOA1 koncentrationer, men även gynnsamma genom en sänkning av Lp(a). Vi kunde visa dessa förändringar av en enda dos av testosteron, och bara några dagar efter dosen var given. En polymorfi i en gen UGT2B17 har stor inverkan för utfallet av testosteron analys även ibland missbrukare som tagit stora doser av testosteron. Genetisk information skulle minska risken för falska negativa svar.

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