Drug Research Unit, Department of Mental Health and Alcohol Research
National Public Health Institute, Helsinki

Adverse effects of anabolic androgenic steroids on the cardiovascular, metabolic and reproductive systems of anabolic substance abusers

Tuomo Karila

Institute of Biomedicine
Pharmacology
University of Helsinki

Department of Orthopaedics and Traumatology
Helsinki University Central Hospital
University of Helsinki

ACADEMIC DISSERTATION

To be publicly discussed with the permission of the Faculty of Medicine of the University of Helsinki, in Lecture Hall 1 at Biomedicum, Haartmaninkatu 8,
on June 13th, at 12 noon.

Helsinki 2003

Supervisors: Docent Timo Seppälä, MD
Drug Research Unit
Department of Mental Health and Alcohol Research
National Public Health Institute, Helsinki

Professor (emer.) Heikki Vapaatalo, MD
Institute of Biomedicine, Pharmacology
University of Helsinki

Reviewers: Docent Markku Kupari, MD
Department of Internal Medicine
Helsinki University Central Hospital
Docent Simo Taimela, MD
Department of Physiology
Institute of Biomedicine
University of Turku

Opponent: Docent Markku Alén, MD
Faculty of Sports and Health Sciences
University of Jyväskylä

Publications of the National Public Health Institute A 12/ 2003

ISBN 951-740-388-2 (printed version)
ISSN 0359-3584

ISBN 951-740-369-0 (pdf-version; http://ethesis.helsinki.fi)
ISSN 1458-6290

Reprotalo Lauttasaari Oy 2003

CONTENTS

1. LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following articles referred to in the text by Roman numerals I-V:

I. **Karila T**, Karjalainen J, Mäntysaari M, Viitasalo M, Seppälä T.
Anabolic androgenic steroids produce dose–dependent increase in left ventricular mass in power athletes, and this effect is potentiated by concomitant use of growth hormone. *Int J Sports Med 2003; 24: 1-7.*

II. Stolt A, **Karila T**, Viitasalo M, Mäntysaari M, Kujala U, Karjalainen J.
QT interval and QT dispersion in endurance athletes and in power athletes using large doses of anabolic steroids. *Am J Cardiol 1999; 84: 364-366.*

III. **Karila T**, Laaksonen R, Jokelainen K, Himberg J-J, Seppälä T.
The effects of anabolic androgenic steroids on serum ubiquinone and dolichol levels among steroid abusers. *Metabolism 1996; 45: 844-847.*

IV. Pärssinen M, **Karila T**, Kovanen V, Seppälä T.
The effect of supraphysiological doses of anabolic androgenic steroids on collagen metabolism. *Int J Sport Med 2000; 21: 406-411.*

V. **Karila T**, Hovatta O, Seppälä T.
Concomitant abuse of anabolic androgenic steroids and human chorionic gonadotrophin impairs spermatogenesis in power athletes.
Submitted (Int J Sports Med).

These publications are reprinted with the permission of Excerpta Medica Inc. (II), Thieme New York (I, IV) and W.B. Saunders Company (III). Some unpublished data have also been included.

2. MAIN ABBREVIATIONS

AAS	anabolic androgenic steroids
ALAT	alanine aminotransferase
ANOVA	analysis of variance
ASAT	aspartate aminotransferase
BMI	body mass index
E/A ratio	early-to-atrial peak velocity ratio
ECG	electrocardiography
FSH	follicle-stimulating hormone
GC/MS	gas chromatography/mass spectrometry
GH	growth hormone
HCG	human chorionic gonadotrophin
HDL	high-density lipoprotein
HP	hydroxylysyl pyridinoline mature crosslinks of collagen
HPLC	high-performance liquid chromatography
i.m.	intramuscular
ICTP	carboxyterminal telopeptide of type I collagen
IGF-bp3	insulin-like growth factor binding protein 3
IGF-I	insulin-like growth factor
LDL	low-density lipoprotein
LH	luteinizing hormone
LP	lysylpyridinoline mature crosslinks of collagen
LV	left ventricle
p.o.	peroral
PICP	carboxyterminal propeptide of type I procollagen
PIIINP	aminoterminal propeptide of type III procollagen
RelWT	relative wall thickness
s.c.	subcutaneous
SD	standard deviation
SHBG	sex hormone-binding globulin

3. ABSTRACT

A large number of young adults abuse anabolic androgenic steroids (AAS) to enhance physical fitness and appearance. Although AAS have been banned in organized sports for nearly thirty years, their use remains one of the main health-related problems in sports today because of their availability and low price. According to recent statistics of the International Olympic Committee, over half of positive doping cases are due to AAS abuse. Confiscation of doping substances by Finnish customs authorities increased during the 1990s concomitantly with the lower black market prices and easier availability of AAS.

The present study elucidates the adverse effects of AAS abuse. Its focus is on the effects of massive doses of AAS when abused with or without other anabolic substances such as growth hormone (GH), human chorionic gonadotrophin (HCG) or antiestrogens under authentic conditions.

Twenty-six healthy male power athletes were followed up during their self-regimen of substance abuse and during a six-month withdrawal period. None of the volunteers were competitive sports athletes subject to doping regulations, and they abused the drugs, which they had obtained from the black market, independently of this study.

The results indicate that AAS abuse is dose-dependently associated with myocardial hypertrophy and that concomitant use of GH is associated with concentric remodelling of the left ventricle (LV). Despite the similar heart size of elite endurance athletes and AAS-abusing power athletes, marked differences were present in the electrocardiographic repolarization indices. Thus, QT dispersion was greater in AAS abusers in spite of short QT intervals, in contrast to endurance athletes, who had long intervals but less QT dispersion. The heart of endurance athletes did not morphologically vary substantially from the heart of the subjects abusing AAS, but abuse of AAS resulted in hypertrophy with pathological features.

AAS abuse increases QT dispersion, as measured from a 12-lead electrocardiogram. The autonomic tone appears to influence QT variables more than the left ventricular mass does. Pathological hypertrophy caused by AAS abuse has been suggested to alter repolarization of the myocardium.

Our result support earlier findings of an AAS-induced decrease in serum high-density lipoprotein concentration. AAS also have an influence on the by-products of the mevalonate pathway. Significant increases occurred in serum ubiquinone concentration and the ubiquinone to high-density lipoprotein ratio was increased. However, serum dolichol concentration tended to decrease concomitantly with high-density lipoprotein concentration during AAS abuse. Supraphysiological doses of AAS enhance collagen synthesis, especially in soft connective tissues.

Concomitant abuse of supraphysiological doses of AAS with HCG results in altered semen density. Regardless of AAS-induced hypogonadotrophic hypogonadism, HCG maintained spermatogenesis but reduced semen quality. Both morphology and motility of semen tended to be impaired. The average semen concentration reached normal levels six months after the cessation of substance abuse, although serum testosterone levels still tended to be low, especially among those subjects with a longer history of anabolic substance abuse.

To sum up, despite the low number of subjects and relatively short follow-up, numerous adverse effects were observed, including ventricular tachycardia, transient infertility, atherogenic changes in lipoprotein profile and pathological remodelling of the myocardium. The abuse of physical fitness-enhancing substances of all forms should be considered a health risk for young males, which may result in both sudden and long-term adverse effects.

Despite subjects being followed for approximately one year and receiving abundant information concerning their health status, none of them discontinued substance abuse after the study, which reflects the difficulties in reducing substance abuse with the means of counseling and educational programmes.

4. INTRODUCTION

The abuse of anabolic androgenic steroids (AAS) is under constant debate world-wide. A large number of young adolescents abuse AAS to improve their physical fitness and appearance (NIDA 2000). While athletes involved in recreational and minor league sports outnumber top-level competitive athletes in the abuse of AAS (American Medical Association 1990), AAS are nevertheless one of the main health-related problems in organized sports due to their availability and low price. According to the International Olympic Committee, AAS abuse is found in over 50% of positive doping cases (World Antidoping Agency 2002). Moreover, doping tests carried out by Finnish anti-doping authorities between 1996 and 2001 showed that 30% of positive results were due to AAS abuse (Finnish Antidoping Committee 2002). Confiscation of doping substances by Finnish customs authorities increased during the1990s (Statistics of Finnish Customs 2002) (**Figure 1**), concomitantly with lower black market prices and easier access to AAS (**Figures 2 and 3**). Adolescents who experiment with AAS are also more prone to abuse recreational drugs (Nilsson *et al*. 2001b).

All major tissues, including the brain, have androgen receptors. AAS possess large systemic and psychological effects (Haupt 1993, Shahidi 2000). However, when AAS are used at supraphysiological doses, the mechanism of action is still under debate. Due to widespread abuse, many side-effcts of AAS abuse may turn out to be significant risk factors when considering public health (Haupt *et al.* 1984, Yesalis *et al.* 1989). Many AAS-induced adverse effects are considered to be reversible.

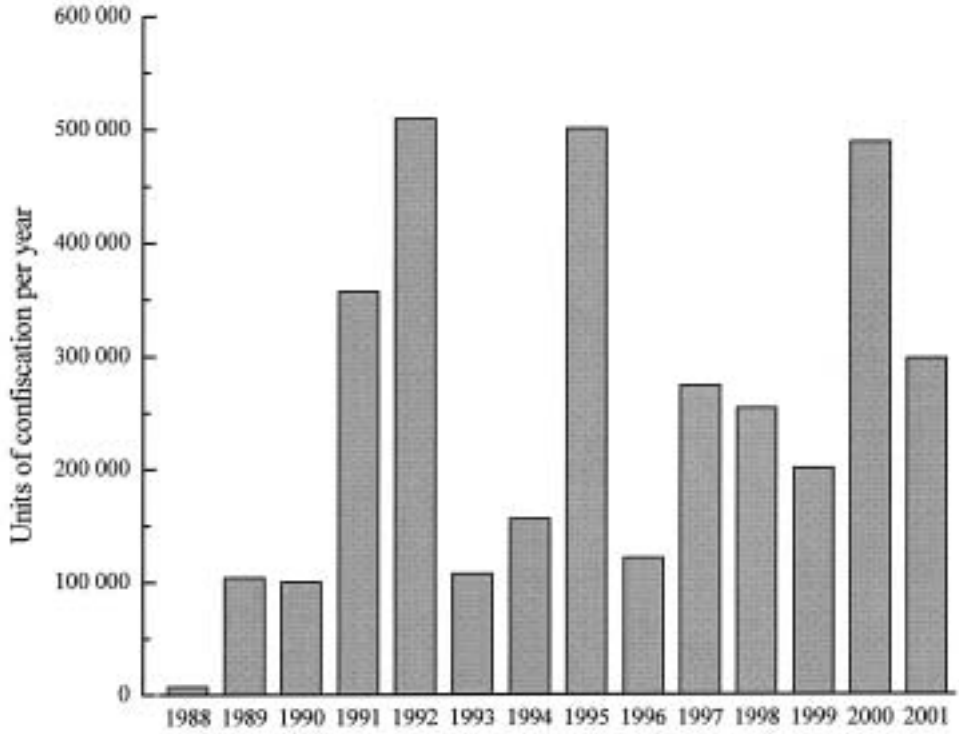


Figure 1. Confiscations of doping substances in Finland between 1988 and 2001 (Statistics of Finnish Customs 2002).

Of particular concern is the increased risk of cardiovascular adverse effects associated with AAS abuse, especially among persons predisposed to such events or diseases. Evidence exists that an increasing number of premature cardiac events are caused by AAS abuse (Lucas 1993, Taimela & Seppälä 1994). According to an epidemiological study among power lifters, the cause for premature cardiac death may lie in substance abuse (Pärssinen *et al*. 2000), though life expectancy among elite athletes on average is increased (Sarna *et al*. 1993).

AAS have profound effects on male endocrinological and reproductive systems. In previous studies, AAS-induced lowered male infertility has been found to be reversible. Reported sustained hypogonadotrophic hypogonadism caused by steroid abuse has also resolved after withdrawal of AAS (Alén *et al*. 1985b, Yesalis 1993). Prolonged abuse may, however, produce transient testicular impairment, observed as lowered steroidogenesis with normal gonadotrophin stimulus (Alén *et al*. 1985b, Ruokonen *et al*. 1985).

Substance abuse is strongly influenced by attitudes and trends in society, and recreational abuse is hard to control without proper legislation. Substance abuse is strongly associated with peer groups and the subgroups involved in fitness culture and may be better understood as a life-style. The abusers are very aware of the risks of their choice and yet are eager to put themselves at risk without deeper consideration. Fitness culture-related substance abuse should be viewed as a public health problem. For the previously mentioned reasons, there is a clear need to study the long-term adverse effects connected with this kind of life-style. With relevant counseling and adequate doping control, we stand a better chance of reducing AAS abuse.

Due to the secretive nature of anabolic substance abuse and for ethical reasons, conducting a study that would fulfill all of the criteria of a good clinical trial is difficult. To gain more profound knowledge of the health risks of this life-style we must therefore accept certain study limitations. The present study elucidates medical aspects of anabolic substance abuse. It was conducted to investigate cardiovascular and reproductive risks under authentic circumstances in young adult males who voluntarily predispose themselves to anabolic substances.

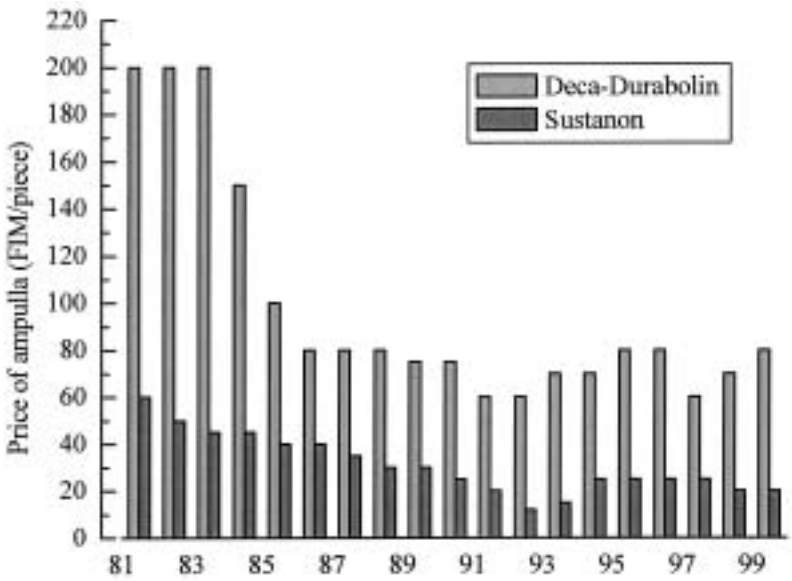


Figure 2. Price of the most common injectable anabolic androgenic steroid preparations abused in Finland between 1981 and 1999 (data based on questionnaire administered to study subjects).

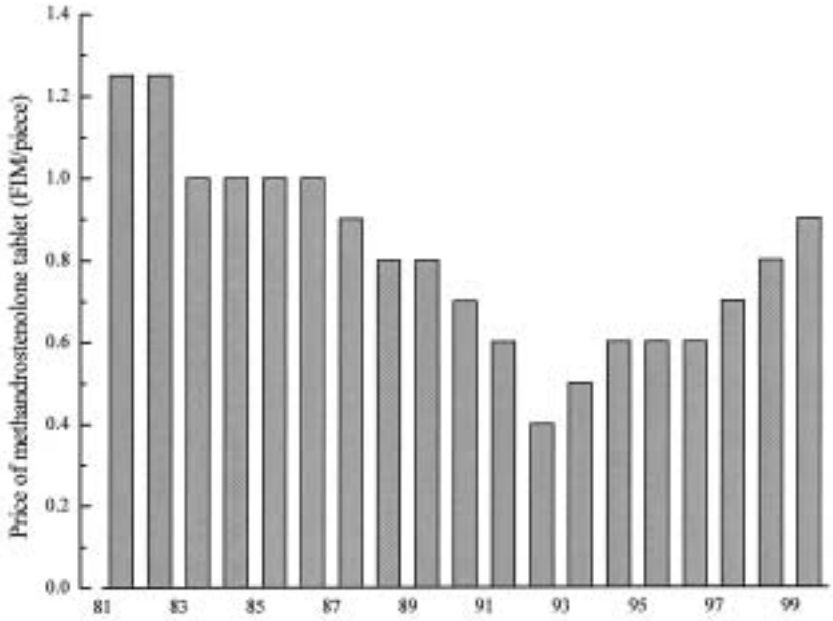


Figure 3. Price of the most common oral anabolic androgenic steroid preparation (methandrostenolone) abused in Finland between 1981 and 1999 (data based on questionnaire administered to study subjects).

5. REVIEW OF THE LITERATURE

5.1. Pharmacology of anabolic androgenic steroids

5.1.1. Testosterone

Testosterone is a steroid hormone produced by various tissues in the human body although it is mainly the product of endocrine glands, i.e. testes, ovaries and adrenal glands. Testosterone synthesis is under hypothalamic-pituitary-gonadal-axis control. Testicular steroid production is controlled by gonadotrophin, a luteinizing hormone (LH). In men, the majority of testosterone is of gonadal origin, and healthy adult males produce between 2.5 and 11 mg of testosterone daily. Male circulating testosterone levels are 10-fold higher than female levels. In women, the ovaries and adrenals contribute equally to testosterone production, each supplying about 25% of the total circulating level. The remaining 50% is derived from peripheral conversion of androstenedione in the liver, skin, brain and adipose tissue (Rosenfield 1972, Longcope 1986, Gagliardi 1991).

In men about 44% of the secreted testosterone is bound to the sex hormone binding globulin (SHBG), and around 2% occurs in free form. The remaining 54% is loosely bound to albumin, from which it can dissociate within the capillary beds (Pardridge 1986). Free and bound testosterone exist in equilibrium. Through aromatization and reduction, the molecule is converted to estrogen and more androgenic 5α-dihydrotestosterone, respectively (Huhtaniemi *et al*. 1992) (**Figure 4**).

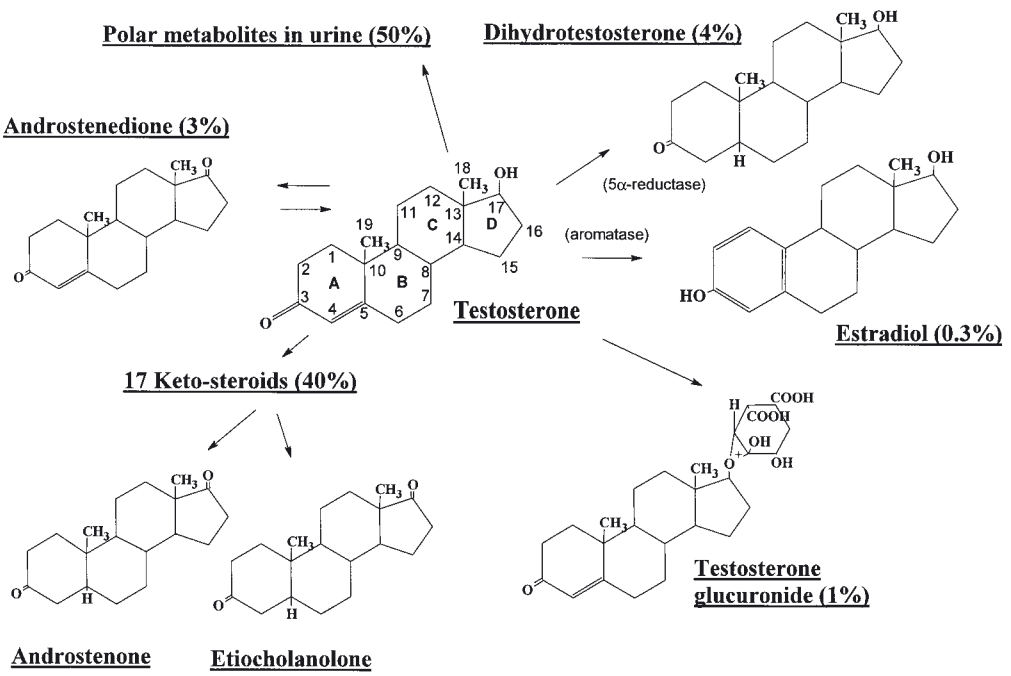


Figure 4. Metabolism of testosterone. Percentage converted and excreted is expressed in parentheses, and names of converting enzymes are given. Cyclic cholesterol rings are named by letters (A-D), and carbon atoms are numbered (1-19) (modified from Winters 1990).

The testosterone molecule is a four-ring structure of cyclic cholesterol rings. Both reproductive and non-reproductive tissues possess possible targets for testosterone. Testosterone has two different kinds of biological effects: 1) The androgen effect is responsible for the development of the male reproductive organs and secondary sexual characteristics, and 2) the anabolic effect can be seen in increased nitrogen fixation and protein synthesis (Huhtaniemi *et al*. 1992).

French physiologist Charles Edouard Brown-Sequard (1889) first recognized the anabolic effect of a testicular extract of dogs and guinea pigs when given subcutaneously. Since the isolation of testosterone from testicular extract in 1935, its virilizing properties have been recognized. To increase the therapeutic value of the molecule, scientists have been keen on developing molecules with emphasized anabolic and prolonged biological activity. Further, to avoid first-pass metabolism to increase systemic availability, the parent molecule needed modification, which led to the invention of numerous anabolic steroids (NIDA 2000, Shahidi 2001).

5.1.2. Anabolic steroids

To strengthen the anabolic properties of testosterone, more than 100 synthetic steroid derivatives have been described for human purposes. The anabolic effect promotes protein synthesis, muscle growth and erythropoiesis. In clinical practice, substances with anabolic effect are needed to overcome various catabolic states (Shahidi 2001). However, none of these compounds are devoid of androgenicity. Androgenic and anabolic properties of anabolic steroids cannot be totally separated. Therefore, it is more appropriate to use the term anabolic androgenic steroids (AAS) (Yesalis 1993, Shahidi 2001) (**Figure 5**).

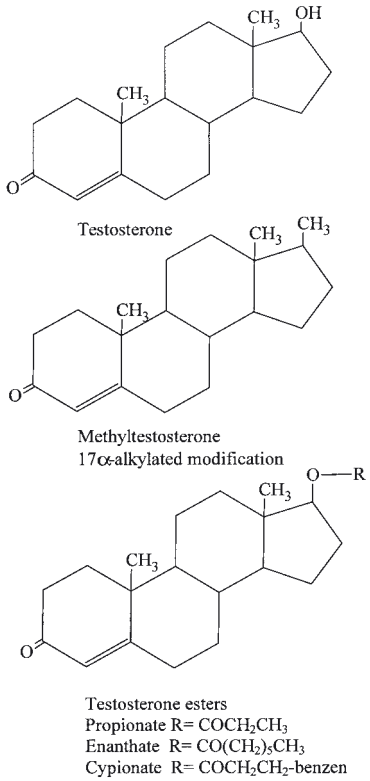


Figure 5. Testosterone molecule and those derivatives that can avoid first-pass metabolism.

Testosterone and its derivatives are well absorbed from the gastrointestinal tract but are rapidly metabolized during hepatic first-pass metabolism without reaching systemic circulation. Testosterone is inactivated primarily by the cytochrome P450 hepatic isoenzyme (Fotherby & James 1972). To increase systemic availability, AAS are modified as injectable 17β-esters or orally administered 17α-alkylated steroids (**Figures 6 and 7**). Orally administered testesterone undecanoate also avoids hepatic metabolism because it is absorbed from the alimentary canal through the lymphatic system (NIDA 2000).

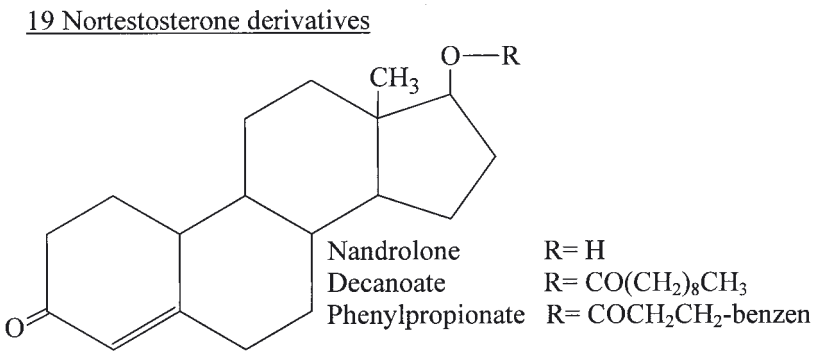


Figure 6. Molecule of an injectable anabolic androgenic steroid, 17β-ester, of 19 nortestosterone derivatives (Nandrolone).

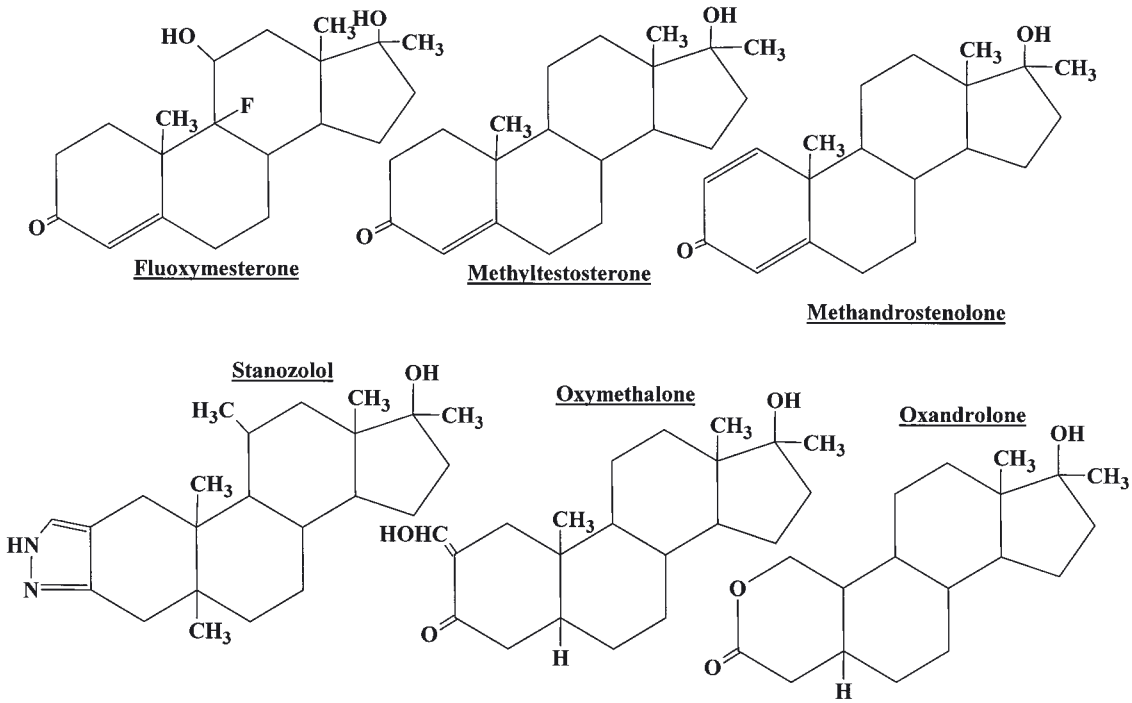


Figure 7. Most commonly abused oral (17α-alkylated) anabolic androgenic steroids.

After absorption from the gastrointestinal tract during hepatic first-pass metabolism, testosterone and AAS undergo biotransformation and are partly excreted via bile to the faeces. Testosterone in systemic circulation is also prone to metabolism in the liver, and once excreted, the steroid can be reabsorbed from the gastrointestinal tract. In peripheral tissues, testosterone is susceptible to glucuronization to androsterone and etiocholanolone, two major metabolites of testosterone, which are excreted to urine (Fotherby & James 1972, Gagliardi 1991) (**Figure 4**).

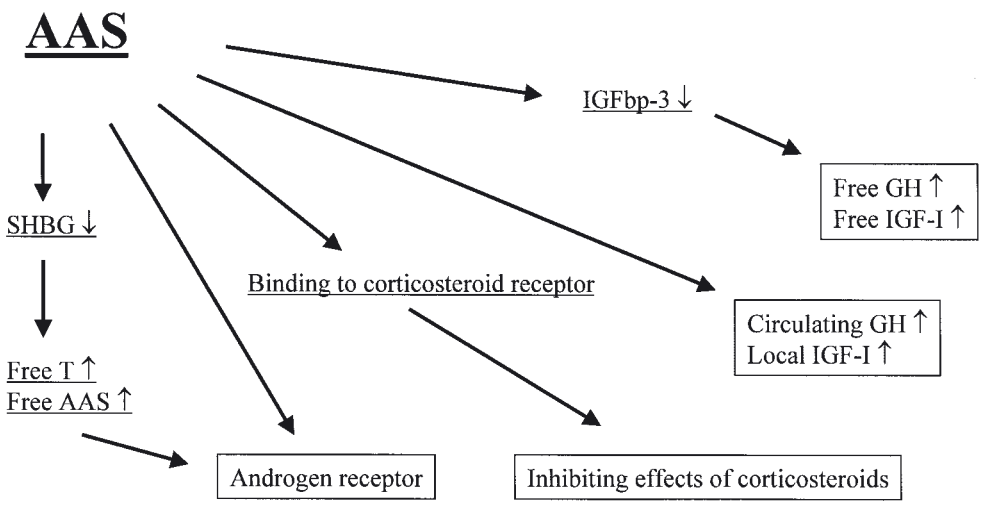
In vivo, different AAS are also potential targets for aromatization and reduction. Since the AAS molecule is susceptible to such enzymatic conversion, it possesses various biological properties (Yesalis 1993, NIDA 2000).

5.1.3. Mechanism of action

The effects of AAS on genes and consecutive gene expression are poorly understood. Recently, human myostatin has been cloned and is considered to be a negative regulator of muscle growth. Basaria *et al*. (2001) speculated that AAS might act by influencing myostatin concentration. Further, all tissues are susceptible to androgen action. No tissues are devoid of androgen receptors, and all androgen receptors distributed throughout the body possess the same binding affinity for a particular steroid. Receptor-binding studies have not demonstrated marked differences between AAS in receptor-binding affinity. Young adolescents are more susceptible to androgen action of AAS because they possess a higher number of cytosol androgen receptors. Even with the biologically active unbound fraction of testosterone in circulation, androgen receptor sites are already saturated in striated muscles (Sheridan 1983, Huhtaniemi *et al*. 1992, NIDA 2000, Shahidi 2000)

Supraphysiological doses of AAS induce gain in muscle size and strength, even without concomitant exercise (Alén *at al*. 1984, Bhasin *et al*. 1996, Giorgi *et* al. 1999). At a supraphysiological dosage, AAS interacts with various receptors, including progesterone, estrogen, and mineralo- and glucocorticoid receptors (NIDA 1990, Jänne *et al*. 1993). Supraphysiological doses of AAS have been speculated to mediate their anabolic action through interaction with glucocorticoid receptors by preventing glucocorticoid's catabolic effect (Hickson *et al*. 1990, Rogol & Yesalis 1992, Haupt 1993). Testosterone has in fact been shown to have a high affinity for glucocorticoid receptors and *in vivo* it acts as an antagonist to endogenous circulating glucocorticoids (Danhaive & Rousseau 1986, 1988).

AAS lower the levels of certain hormone-binding proteins in circulation. Thyroxin, cortisol, sex hormone, growth hormone and D-vitamin-binding globulin concentrations in circulation are decreased after AAS administration (Barbosa *et al*. 1971, Small *et al*. 1984, Ruokonen *et al*. 1985, Alén *et al*. 1987, Karila *et al*. 1998). Alterations in carrier protein concentration levels may increase biologically active steroid concentrations. One could also hypothesize that AAS-mediated anabolism could be partly due to increased concentrations of circulating biologically active human growth hormone (GH) and insulin like growth factor-I (IGF-I), particularly when supraphysiological doses are used (Hobbs *et al*. 1993, Karila *et al*. 1998). Alén *et al*. (1987) found 5 to 60 times higher serum GH concentrations in subjects on AAS, even without concomitant use of exogenous GH. Local stimulation of IGF-I may be required in the process of anabolic action (Fryburg 1994). Androgens are known to be needed in local production of IGF-I within the skeletal muscle (Mauras *et al*. 1998). These could partly explain the mechanism of action of supraphysiological doses of AAS. On the other hand, these findings might also provide an explanation for the unpredictable adverse effects of AAS (Bahrke *et al*. 1996, Pärssinen & Seppälä 2002) (**Figure 8**).



AAS = anabolic androgenic steroid
T = testosterone
GH = growth hormone
IGF-1 = insulin-like growth factor-1

SHBG = sex hormone binding globulin
IGFbp-3 = IGF binding protein-3

Figure 8. Suggested mechanisms of anabolic action of supraphysiological doses of anabolic androgenic steroids (Small *et al.* 1984, Alén *et al.* 1987, Hickson *et al.* 1990, Fryburg 1994, Karila *et al.* 1998)

5.2. Clinical indications

Anabolic androgenic steroids have established their usefulness in treating various types of anaemia, osteoporosis, androgen replacement therapy, muscle-wasting conditions, cachexia caused by various cancers, and HIV infection. Long-standing hypogonadism in adult males is associated with reduced bone remodelling and decreased bone formation. In treating muscle-wasting disorders with AAS, none of AAS preparations has proved to be superior to another (Francis *et al.* 1986, Strawford *et al.* 1999, Shahidi 2001, Pärssinen & Seppälä 2002). Recently, AAS have been studied for male andropause replacement therapy, but more studies are required before AAS can be used broadly for improving the quality of life of ageing males (Swerdloff *et al.* 1992) (**Table 1**).

5.3. Abuse of anabolic androgenic steroids

AAS abuse is widespread among all social levels of Western countries. While formely restricted to competing athletes, it now also impacts on recreational non-competitive adolescent athletes (Duchaine 1989, Terney & McLain 1990, Korkia & Stimson 1997). In fact, athletes involved in recreational and minor league sports outnumber top-level competitive athletes in AAS abuse (American Medical Association 1990). Moreover, there is evidence that high doses of AAS are also abused by non-athletic subgroups to gain euphoria (Handelsman & Grupta 1997, Kindlundh *et al.* 1999).

A vast amount of information is available on how to abuse AAS. Information is provided by "underground steroid manuals" and the internet, and peer groups play a particularly important role as an

Table 1. Clinical indications and clinical trials of anabolic androgenic steroids
(Bahrke *et al.* 1990, Tomoda 1999, Kochakian & Yesalis 2000, Basaria *et al.* 2001, Shahidi 2001)

Androgen replacement therapy
    Hypogonadism
Male contraception
Delayed puberty
Impotence
Osteoporosis
Muscle-wasting disorders
    Chronic obstructive pulmonary disease with weight loss.
    Human immunodeficiency virus-related
Anaemia
    Acquired and constitutional aplastic anaemia
    Systemic lupus erythematosus related aplastic anaemia
    Fanconi anaemia
    Sickle cell anaemia
    Anaemia of end-stage renal disease
    Hairy cell leukaemia
    Cyclic neutropenia
    Myelofibrosis
Bone marrow damage
Catabolia related to
    Severe burn injuries
    Major trauma
    Major surgical operation
    Chronic renal failure
    Cancer
Malnutrition states
    Alcohol hepatitis
Growth promotion of children with constitutional growth retardation
Turner's syndrome with growth retardation
Hereditary angioedema
Damaged myocardium
    Idiopathic dilated cardiomyopathy
Psychiatric disorders
    Depression
    Melancholia
    Involutional psychoses
    Schizophrenia
Male climateric symptoms
Postpartum breast pain and engorgement
Endometriosis

information source for adolescents (Duchaine 1989). Most AAS are acquired from non-medical sources such as the black market and gymnasiums (O'Sullivan *et al.* 2000, Green *et al.* 2001).

5.3.1. Epidemiology

In the early 1950s, AAS use spread among power sports, and soon thereafter its beneficial effects on all sports demanding peak physical performance were noticed. It is impossible to estimate the extent of AAS use in sports during the 1950s and 1960s. Most studies are based on case reports, and epidemiological

studies conducted retrospectively cannot fully be trusted due to the negative reputation of AAS abuse in sports. The International Olympic Committee included anabolic steroids on a list of prohibited substances in 1975 and testosterone in 1982. Despite the use of anabolic steroids being banned in the mid-1970s, their abuse continued extensively in numerous sports (NIDA 1990, Yesalis 1993).

Due to the secretive nature of doping, estimating the extent of doping abuse in modern organized sports is difficult. The percentage of positive doping test results in the summer Olympic games during 1976-1988 varied between 0 and 2.9% (NIDA 1990). Of doping tests endorsed by Finnish antidoping authorities in 1996-2001, 1.1% were positive. Throughout the world, the majority of positive doping test results are due to AAS abuse (World Antidoping Agency 2002). The prevalence of AAS abuse in organized sports based on these results is probably underestimated (Duchaine 1989).

All surveys of prevalence of AAS use are directed at adolescents and young adults, who are considered the most likely abusers. The non-medical use of anabolic androgenic steroids had increased by 50% among male adolescents between 1991 and 1999 in the United States (NIDA 2000). There is also contradictory evidence that abuse of AAS among adolescents in the US had decreased from 1988 to 1996 (Yesalis *et al*. 1997). Most surveys report that 3-12% of adolescent males living in the Western world admit to the former or present use of AAS. Among adolescent females, the prevalence is 1-2% (Yesalis & Bahrke 2000) (**Table 2**). According to a study conducted in Sweden, the size of municipalities does not have an effect on prevalence of AAS misuse (Nilsson *et al*. 2001a). Such findings may not, however, be applicable to Finland. According to the author's knowledge, abuse of AAS is mainly associated with urban life-style.

Table 2. Prevalence of AAS abuse among male adolescents

USA	3-7%	(Buckley *et al*. 1988, Windsor & Dunitru 1989)
Norway	0.8%	(Wichstrom & Pedersen 2001)
Great Britain	9.1%	(Korkia & Stimson 1997)
Sweden	2.8-3.6%	(Nilsson *et al*. 2001)
Finland	1.5%	(Seppälä *et al*. unpublished)

5.3.2. Fitness subculture

During the 1970s non-competitive fitness athletes adopted AAS use to improve outlook and physical fitness. Their motive for substance abuse thus derived from self-image improvement. The reason underlying substance abuse may be fulfilling social requirements (NIDA 1990, Kindlundh *et al*. 1999, Hartgens *et al*. 2001). Stimulants, diuretics and anabolic agents, such as adrenergic β_2-agonists, growth hormone and IGF-1, are taken for the same purpose. These recreational fitness athletes are aware of the adverse effects associated with such practices. Antiestrogens and human chorionic gonadotrophin (HCG) are used to avoid these effects (Macintyre 1987, Yesalis 1993, Pärssinen & Seppälä 2002). The term multipharmacy can be applied. An essential part of a fitness life-style is to follow strict diet and exercise regimen (**Table 3**).

Table 3. Additional self-administered pharmaceuticals among AAS abusers
(Yesalis 1993, Mäntysaari *et al.* 2002)

Human chorionic gonadotropin (HCG)
Antiestrogens
Human growth hormone
Growth factors (IGF-1)
Dopamine receptor agonists (bromocriptin)
Adrenergic β_2-agonists
Stimulants (ephedrine, amphetamine, coffeine)
Thyroxin
Finasteride
Diuretics
Insulin
Oral antidiabetic drugs
Aminogluthetimine
Antiacne medications
Non-steroidal anti-inflammatory drugs

5.3.3. Patterns of anabolic androgenic steroid abuse

Substance abuse follows a pattern that aims to optimize performance and minimize adverse effects and the development of tolerance, while somehow mimicking the natural hormonal status. Detailed information on such practices is easily accessible from a large selection of "underground" manuals that are available through the mail or by internet. These manuals provide volumes of information on substance abuse, the only purpose of which is to improve physical fitness and appearance. Without proper pharmacological and physiological understanding, abusers expose themselves to great danger.

According to the "underground manuals", AAS abuse is periodical, referred to as cycles lasting 6-12 weeks (Duchaine 1989). A typical abuser keeps wash-out period between cycles ranging from a couple of weeks to several months. During the wash-out period the body is assumed to recover from the cycle without developing a tolerance to AAS (Pärssinen & Seppälä 2002). The instructions often ignore the fact that many injectable preparations are long-acting. Furthermore, the author has noticed that a recent tendency in Finland is continuous abuse of AAS without wash-out periods. The duration of the cycle depends on the availability of substances and the level of experience of the abuser. More experienced abusers tend to elevate the AAS doses and prolong duration of cycles until they abuse AAS on a more or less continuous basis. Athletes combine various AAS preparations in a cycle, a process referred to as stacking. The reason for this practice lies in the belief that, due to depression of endogenous steroidogenesis, the body requires different steroids to mimic the normal hormonal balance. Different steroids are also used during different phases of the cycle to avoid development of tolerance (Duchaine 1989). AAS dosing is carried out in a pyramidical fashion. At the beginning of the cycle, the dose is elevated to maximal, decreasing the amount towards the end of the cycle. This is done in the belief of achieving the full benefit of AAS without developing tolerance, and relieving withdrawal symptoms connected with discontinuing the drug.

The abusers usually exceed the AAS doses used in clinical practice by 10 to 100 times. Availability and price of AAS on the black market strongly impact on the dosage and preparation used. The black market functions according to the rules of demand and supply. AAS abusers often abuse other drugs alongside AAS to further improve physical fitness and to counteract the adverse effects of AAS with self-treatment (Yesalis 1993) (**Table 3**).

5.3.4. Public health considerations

Life expectancy of former Finnish elite competitive athletes is longer than that of sedentary controls (Sarna *et al*. 1993). Power training itself does not increase mortality (Sarna *et al*. 1993), but in an epidemiological study conducted by Pärssinen *et al*. (2000), substance abuse was found to increase risk for premature death. The causes of death among the powerlifters were suicide, acute myocardial infarction, hepatic coma and non-Hodgkin's lymphoma; at least some of these may be related to AAS abuse.

Regardless of the abundant and readily available information on health risks associated with AAS abuse, the number of abusers is high according to surveys and customs confiscations (Finnish Customs, www.tulli.fi). During 2002 the number of confiscations of medical preparations increased by more than one third, to 520 cases, and about half of these were doping substances (232). This was also the first time when in 3 cases raw material was found (Finnish Customs, www.tulli.fi). Further, the relative amounts of growth hormone and growth factors have increased (Custom Authorities, personal communication).

Evidence on the effectiveness of educational programme in fighting against recreational AAS abuse is controversial (Goldberg *et al*. 1990, 1991). After receiving medical advice concerning the adverse effects of AAS, only 19% of counselled subjects refused to abuse AAS in the future (O'Sullivan *et al*. 2000). However, recent studies demonstrate beneficial effects of educational interventions, which might reflect the present generation being more receptive to counselling (Goldberg *et al*. 1996, Nilsson *et al*. 2001b). During the last decade public awareness of the adverse effects of AAS has increased and attitudes have become more negative forwards AAS abuse, even among adolescent athletes involved in competitive sports (Seppälä, personal communication).

AAS abuse resulting in addiction is one reason for continuation of substance abuse. After discontinuing the abuse, unpleasant withdrawal symptoms often drive users back to steroids. After the AAS cycle, abusers have been reported to frequently encounter depressive feelings (Sheridan 1983, Yesalis 1993, Pope & Katz 1994, Pope *et al*. 2000).

Some individuals have a genetic predisposition to develop depressive symptoms if deprived of androgen action (Seidman *et al*. 2001). Evidence exists that AAS abusers are also more susceptible to use of recreational drugs. AAS abuse seems to lower the threshold to experiment with recreational drugs (Nilsson *et al*. 2001).

AAS are prohibited in organized sports. In order to reveal the abuse, doping tests are mandatory. Recreational abuse and abuse in non-competitive sports are out of control. The US Congress has criminalized AAS abuse and included them as class III controlled substances. Some Western countries have acted similarly including Sweden and Finland (Shahidi 2001).

5.4. Anabolic androgenic steroid-induced adverse effects

AAS have been extensively studied. Within clinical dosages, they are well tolerated. Many of the AAS-induced adverse effects are reversible. Most adverse effects are gender-dependent, females, for instance, experiencing virilizing effects (Shahidi 2001). Studies with controlled supraphysiological doses, a proper study design and a matching control group have not been published. Adverse effects associated with use of supraphysiological doses of AAS are mainly based on case reports and follow-up studies without dosage controls. In the literature, there are numerous case reports of myocardial infarction

(McNutt *et al*. 1988, Lyndberg *et al*. 1991, Ferenchick & Adelman 1992, Appleby *et al*. 1994, Huie 1994), coronary atherosclerosis (Mewis *et al*. 1996), sudden death (Lyndberg *et al*. 1991, Fineschi *et al*. 2001), congestive heart disease (Ferrera *et al*. 1992), serious arrhythmia (Appleby *et al*. 1994, Nieminen *et al*. 1996), atrial fibrillation (Sullivan *et al*. 1999), intraventricular thrombosis (Gaede & Montine 1992), pulmonary embolus (Gaede & Montine 1992) and arterial and venous thrombosis (Ferenchick 1991) associated with AAS abuse.

AAS abuse has also been associated with hepatic dysfunction and various neoplasias. Alén (1985) reported that use of AAS significantly increased serum concentrations of hepatic aminotransferases, although measurements remained within normal limits. He concluded that sustained high-dose use of AAS produces mild impairment in liver function. AAS do not, however, cause irreversible damage to liver function (Zimmerman & Lewis 1987). Previous reports stating that AAS administration causes hepatic dysfunction are mainly based on elevated serum aminotransferase concentrations (Dickerman *et al*. 1999). Cholestatic jaundice is related to use of 17α-alkylated AAS, not to structurally different steroids. Peliosis hepatis (dilated hepatic venous sinuses), by contrast, is not related to C17-alkylating, but manifesting with testosterone administration (Burger & Marcuse 1952).

In the literature, evidence can be found of AAS promoting tumor formation in mice by enhancing the effects of carcinogens (Lesna & Taylor 1986), without the AAS being mutagenic in the Ames test (Ingerowski *et al*. 1981).

AAS have proven to be aetiological factors for some cancers. According to Chen *et* al. (1997), AAS are included as a risk factor for hepatocellular carcinoma together with viral hepatitis, alcohol consumption and some genetic factors. Benign hepatic neoplasia, diffuse hyperplasia, nodular regenerative hyperplasia and focal nodular hyperplasia have also been attributed to the use of 17α-alkylated AAS (Ishak & Zimmerman 1987). Histologically, a rare androgen-specific form of a hepatic tumour can be distinguished in man that appears to act more like a benign hepatocellular adenoma (Anthony 1975, Craig *et al*. 1989). Interestingly, these androgen-related tumours have a tendency to regress after androgen medication has ceased (Cocks 1981, Drew 1984, McCoughan *et al*. 1985).

Hepatocellular carcinoma is connected to long-term treatment with AAS (Ishak & Zimmerman 1987). However, the malignant nature of AAS-induced hepatocellular carcinoma is questionable since regression occurs in the majority of cases after withdrawal of AAS administration (Shahidi 2001). In addition, there is contradictory evidence about the role of androgens in prostate cancer (Signorello *et al*. 1997, Heikkilä 1999). AAS have also been associated with development of soft tissue sarcomas (Zahm *et al*. 1997). While clear convincing evidence of the mutagenicity of AAS is still lacking, they do at least possess tumour growth-promoting activity.

Depending on the administration route, infections at the injection site of bacterial or fungal aetiology have been reported. There is also an increased risk of hepatis and AIDS as a result of shared needles and syringes (Rich *et al*. 1999). AAS abuse is shown to increase the prevalence of acne formation also (Kiraly *et al* 1988).

AAS abuse is associated with various psychiatric and behavioural effects. One-fourth of AAS abusers report major mood syndromes, such as mania, hypomania or major depression, while on AAS (Pope & Katz 1994). Despite many of the studies in this area suffering from methodological inadequacies, they clearly do demonstrate that increased aggression and irritability are associated with AAS abuse (Bahrke *et al.* 1990). Moreover, evidence exists that severity of psychiatric adverse effects is dose-related (Porcelli & Sandler 1998). However, contradictory reports suggest that at least some psychiatric symptoms are associated with life-style and exercise regimen (Bahrke & Yesalis 1994).

Various psychiatric symptoms are related to withdrawal of AAS, and these increase AAS dependence (Brower *et al*. 1991) (**Tables 4-6**).

Because the present study aimed to elucidate selected somatic adverse effects of AAS abuse, psychiatric and behavioural effects were ignored.

Table 4. Self-reported adverse effects probably associated with AAS abuse
(Korkia *et al*. 1997, O'Sullivan *et al*. 2000)

Male-reported	
Altered libido	(61%)
Mood changes	(48%)
Reduced testis volume	(46-56%)
Acne	(43%)
Gynaecomastia	(52%)
Elevated blood pressure	(36%)
Fluid retention	(56%)
Injuries to tendons	(26%)
Nosebleeds	(22%)
More frequent flu	(16%)
Sleeping problems	(37%)
Female-reported	
Menstrual irregularities	(62%)
Fluid retention	(62%)
Clitoral enlargement	(31%)
Reduced breast size	(23%)
Elevated blood pressure	(15%)
Sleeping problems	(31%)

Table 5. Psychological and behavioural adverse effects associated with AAS use
(Brower *et al*. 1991, Uzych 1992, Pope *et al*. 1994)

- Altered libido
- Depression
  - Minor
  - Major
  - Connected to withdrawal of AAS
  - Relief of depression
- Psychotic symptoms/episodes
- Manic episodes
- Hypomania
- Euphoria
- Mood imbalance
- Body image dissatisfaction
- Increased
  - Aggressive behaviour
  - Uncontrollable behaviour
  - Irritability
  - Restlessness
- Suicide
- Dependence (addiction)

Table 6. Metabolic adverse effects associated with AAS use
(Ekert *et al*. 1971, Frerenchich *et al*. 1992, Ajayi *et al*. 1995, Cohen *et al*. 1997)

- Glucose intolerance
- Increased insulin resistance
- Impaired platelet function
- Decreased tolerance to anticoagulants

5.4.1. Effects on cardiovascular system

The risk for cardiovascular disease has been found to be increased among AAS abusers (Wilson 1988). Melchert *et al*. (1995) speculated that AAS-induced cardiovascular changes are related to four mechanisms: atherogenic lipoprotein changes; trombogenic changes in the blood coagulation cascade

and platelet function; predisposition to vasospasm; and direct cardiotoxicity. Average 24-hour blood pressure levels were not elevated in bodybuilders during AAS administration, but chronic AAS abuse did result in an abnormal 24-hour blood pressure pattern (Palatini *et al*. 1996). Evidence about the effect of AAS abuse on the function of the left ventricle (LV) is contradictory (Pearson *et al*. 1986, Urhausen *et al*. 1989, Thompson *et al*. 1992).

5.4.1.1. Cardiac adaptation to exercise

Physical activity has a significant effect on heart size, shape and function that is noticeable even after a short exercise period. Six weeks of moderate endurance training results in both hypertrophy and dilatation of the LV. These beneficial exercise-induced changes vanish in three weeks after cessation of physical activity (Shapiro & Smith 1983, Wight & Salem 1995). LV mass is 45% greater in competitive athletes than sedentary controls (Maron 1986). Diastolic function in athletes' heart is generally normal (Finkelhor *et al*. 1986, Lewis *et al*. 1992, Yeater *et al*. 1996), in contrast to pathological LV hypertrophy, like in hypertension, where impaired LV filling is often detected (Post *et al*. 1994).

Common opinion has previously held that athletes participating in dynamic type endurance sports develop larger LV cavity dimensions without a significant increase in wall thickness (eccentric hypertrophy) (**Figure 9**), whereas athletes involved in static exertion and exposed to a pressure load are more likely to develop greater LV wall thickness without a significant increase in cavity dimensions (concentric hypertrophy). However, resistance training without AAS produces the same positive effect on cardiac dimensions, diastolic function and blood lipids as aerobic training (Yeater *et al*. 1996). Further, echocardiographic studies have shown greater LV wall thickness to be common in endurance athletes, while this is often undetectable in athletes engaged in intense power training (Maron 1986, Pelliccia *et al*. 1993). Yeater *et al*. (1996) also reported that LV internal diastolic diameter was similar in endurance athletes and power athletes with or without AAS use. Karjalainen *et al*. (1997) have speculated that despite similar training and exercise capacity considerable differences in LV mass and geometry are present among top-level endurance athletes and that this could be due to genetic predisposition. Recently, it was shown that angiotensinogen gene M235 polymorphism is associated with LV mass in endurance athletes (Karjalainen *et al*. 1999). Despite earlier beliefs about the effect of exercise on myocardial morphology, it has been thoroughly documented that LV morphology depends on numerous factors.

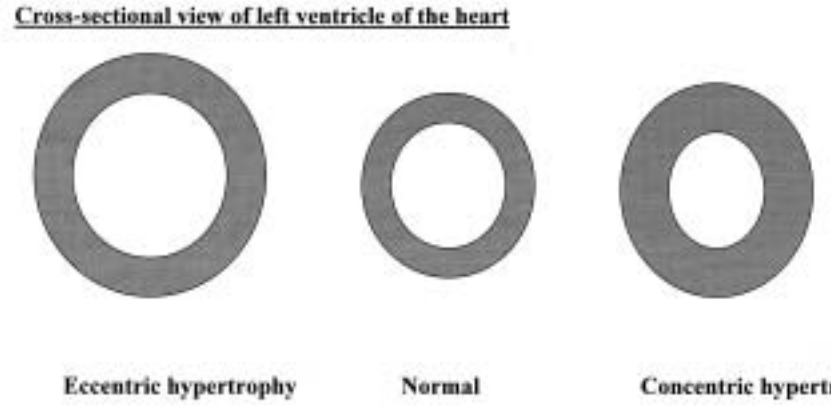


Figure 9. Left ventricle (LV) concentric hypertrophy of the heart. In concentric hypertrophy, LV wall thickening is disproportional to internal cavity size and is reflected as increased relative wall thickness [RelWT= (septum thickness + posterior wall thickness)/ left ventricle end diastolic diameter]. In eccentric hypertrophy, LV cavity size and LV wall thickness are increased proportionally, and RelWT is normal or decreased.

5.4.1.2. Anabolic androgenic steroids and cardiac hypertrophy

The heart of males in many species is larger than that of females, even after factoring in difference in body weight (Silver 1991). Stolt *et al.* (2000) reported that LV mass among elite female endurance athletes does not significanty exceed LV mass measured from sedentary male controls, 176 ± 29 g (mean ± SD) vs. 167 ± 37 g, respectively. Experimental studies support the assumption of a direct effect of androgens in the heart (Krieg *et al*. 1978). These findings are in line with another study that suggests that estrogens have a preventive role in the pathogenesis of LV hypertrophy (Lip *et al*. 2000). Marsh *et al*. (1998) demonstrated the presence of androgen receptors in human cardiac myocytes in both sexes and that androgens can directly mediate a significant hypertrophic response in cardiac myocytes. Experimental studies have shown that prolonged treatment with AAS leads to dose-dependent reversible myocardial hypertrophy together with irreversibly reduced compliance of the LV and decreased inotropic capacity of the myocardium (Rämö 1987, Karhunen 1988). Nevertheless, similar findings without altered contractility of the heart have also been demonstrated (Trifunovic *et al*. 1995).

Urhausen *et al*. (1999) found that among bodybuilders only those using AAS have clearly higher hypertrophic indces. Weight training combined with the use of AAS increases LV wall thickness, end-diastolic volume and mass, and isovolumetric relaxation time is also prolonged significantly (De Piccoli *et al*. 1991, Sachtleben *et al*. 1993, Dickerman *et al*. 1997a). Deligiannis *et al*. (1992) demonstrated in an echocardiographic study that use of AAS significantly increases LV end-diastolic volume (16%) and LV mass (17%) as well as total LV volume (17%) in athletes. These augmentations were in proportion to the increase in skeletal muscle mass.

LV hypertrophy is an independent risk factor for cardiovascular morbidity and mortality, and it has been linked to atrial fibrillation, ventricular arrhythmia and sudden cardiac death (Lip *et al*. 2000). Diastolic filling is impaired in pathological LV hypertrophic states where the heart is under a pressure load such as in aortic stenosis (Fifer *et al*. 1985). In arterial hypertension, the degree of pathological LV hypertrophy is directly related to the impairment of diastolic filling (Fouad *et al*. 1984). In the literature, discrepant results have been obtained for whether AAS abuse-related LV hypertrophy alters diastolic function (Urhausen *et al*. 1989, Yeater *et al*. 1996). However, Sader *et al*. (2001) did demonstrate that AAS abuse is not significantly associated with abnormalities of arterial structure or function.

5.4.1.2.1. Structure of the heart

Spirito *et al*. (1994), who studied 947 elite athletes, found significant gender-related differences in LV dimensions. Females elite athletes had a LV diastolic cavity dimension that was 2.0 mm smaller and a LV wall thickness 0.9 mm less than age-, body size- and sport- matched males. Among elite athletes, LV wall thickness exceeding 13 mm is uncommon (Pelliccia *et al*. 1991). Pellicicia *et al*. (1993) concluded that the presence of LV wall thickening exceeding 13 mm should suggest an alternative explanation to intensive power training. Among elite athletes, 2–13% possess a LV wall thicker than 13 mm, but none exceeds 15 mm (Pelliccia *et al*. 1991, Henriksen *et al*. 1996). AAS abuse potentiates concentric remodelling of LV hypertrophy (Urhausen *et al*. 1989, Dickerman *et al*. 1997a). This concentric increase in LV wall thickness is related to body weight and LV mass.

5.4.1.2.2. Histopathological changes

AAS-induced cardiac hypertrophy is associated with similar histopathological changes as those encountered in dilated cardiomyopathy (Ferrera *et al*. 1997). In autopsy samples and myocardial biopsies taken after AAS exposure, myocardial fibrosis and inflammation have been present (Kennedy & Lawrence 1993, Nieminen *et al*. 1996). Deleterious effects of AAS on myocardial cells depend on

the dose administered and the length of exposure (Melchert *et al*. 1992). Experimental studies with myocardial cell cultures reveal cell destruction associated with depressed contractile activity, increased lysosomal fragility and depressed mitochondrial activity (Melchert *et al*. 1992). Further, Tagarakis *et al*. (2000) demonstrated that muscular exercise combined with AAS impairs the cardiac microvascular adaptation to physical conditioning. These findings support a direct toxic effect of AAS on the myocardium (Melchert *et al*. 1995).

5.4.1.3. QT interval and dispersion

QT interval in the electrocardiogram (ECG) is described as the time from onset of ventricular activation to the end of electrical recovery. QT dispersion is the dispersion of QT intervals between the leads of a 12-lead ECG. It is an indirect measure of the heterogeneity of ventricular repolarization (Zabel *et al*. 1995). The lengthening of QT interval among endurance athletes is considered to be due to increased vagal tonus or adaptive cardiac hypertrophy (Browne *et al*. 1982). Lengthening of QT interval predicts death in patients with heart disease but not in healthy subjects (Karjalainen *et al*. 1997). QT dispersion exceeding 90 ms increases the risk of cardiac death, resulting in 2.8-fold higher mortality among heart failure patients (Anastasiou-Nana *et al*. 2000). Several studies also suggest that increased QT dispersion is associated with increased risk of arrhythmic events (Higham & Campbell 1994, Pye *et al*. 1994, Mänttäri *et al*. 1997). Furthermore, LV hypertrophy is associated with increased QT dispersion and increased mortality in hypertensive patients (Mayet *et al*. 1996, Perkkiömäki *et al*. 1996). "The athlete's heart" is hypertrophied and often has altered ECG, although no reports are available on QT dispersion among top-level athletes.

5.4.1.4. Alteration of lipoprotein profile

Sex hormones influence serum lipoprotein and apolipoprotein concentrations (Morrison *et al*. 1998). AAS alter lipoprotein profile towards atherogenicity (Alén & Rahkila 1984). Sustained use of AAS result in profound alterations of serum high (HDL) and low (LDL) -density cholesterol concentrations (Webb *et al*. 1984, Alén *et al*. 1985a). Glazer (1991) reviewed 15 articles and found that serum HDL levels were 40-70% lower due to use of AAS. HDL_2 fraction was lowered by 80%, and HDL_3 fraction by 35%. There was a concomitant increase of 40% in serum LDL concentration. AAS lowered apolipoprotein concentration, mainly via the apo A-I fraction (McKillop & Ballantyne 1987, Glazer 1991). However, Cohen *et al*. (1996) suggested that among male bodybuilders AAS have a beneficial effect on serum Lp(a) levels, although the HDL/ LDL–ratio is reduced. Lipoprotein alterations are assumed to be caused by induction of the HDL- catabolizing enzyme hepatic triglyceride lipase (Glazer 1991, Bausserman *et al*. 1997). These lipoprotein profile alterations during AAS administration reverse after discontinuation of treatment (Shahidi 2001). While the mechanism is still not resolved, two explanations exist for HDL reduction: one is based on AAS androgenic properties and the other is associated with 17α-alkylation.

5.4.1.4.1. Atherogenic changes

Numerous case reports on AAS atherogenicity have been published. Lipoprotein profile alterations due to AAS abuse are considered to be an aetiological factor for premature coronary heart disease (Haffner *et al*. 1983, Alén & Rahkila 1984, Webb *et al*. 1984, Glazer 1991, Mewis *et al*. 1996). Because low HDL levels have a negative correlation with coronary heart disease (Miller & Miller 1975, Mjos *et al*. 1977), AAS abuse is a risk factor of coronary heart disease (Glazer 1991). However, discordant reports claim that even though AAS result in marked depression in HDL serum concentration, the use of AAS is not associated with significant abnormalities of arterial structure or function (Sader *et al*. 2001). Dickermann *et al*. (1997b) also concluded that despite the significantly higher total/HDL cholesterol ratio, the low serum total cholesterol levels and low plasma triglyceride levels among AAS abusers raise questions

concerning the exact role of androgens in increasing risk of cardiovascular disease. The assumptions are based mainly on epidemiological studies on lipoprotein profile and case reports of sudden cardiac events in AAS abusers. Long-term follow-up studies on AAS abusers are needed to reveal causality.

5.4.1.5. Increased risk of cardiovascular events

Several case reports have suggested that AAS abuse is associated with sudden cardiac events such as ventricular arrhythmias, acute myocardial infarction and pulmonary embolism (McNutt *et al*. 1988, Ferrera *et al*. 1997). In epidemiological studies, LV hypertrophy has been cited as an independent risk factor for cardiovascular morbidity and mortality (Lip *et al*. 2000). Pärssinen *et al*. (2000) concluded in their epidemiological study that competitive powerlifters had an increased risk for premature death due to suspected substance abuse. Risk of premature death among top-level powerlifters (n=62) was 4.6 times higher than among the control population. Suicide and myocardial infarction were the main reasons for premature death (3 of each, out of 8 deaths). Former elite endurance athletes' life expectancy is increased mainly due to decreased cardiovascular mortality (Sarna *et al*. 1993).

Pathophysiological mechanisms of cardiovascular events among AAS abusers have been suggested to be the result of enhanced myocardial sensitivity to cathecholamine stimulation, coronary artery disease, myocardial fibrosis and inflammation as well as enhanced thrombogenesis (Shozawa *et al*. 1982, Ferenchick 1990, Ferenchick *et al*. 1992b, Kennedy & Lawrence 1993, Nieminen *et al*. 1996).

5.4.1.5.1. Arrhythmia and sudden death

It is widely accepted that AAS abuse increases risk of sudden death (Kennedy & Lawrence 1993). This idea is based on anecdotal evidence relying on case reports. No relevant large-scale follow-up studies have, however, been published which could verify causality. The incidence of sudden death among young healthy non-substance abusing athletes is ~1/200 000 (Maron *et al*. 1996), with hypertrophic cardiomyopathy being the most common cause (Maron *et al*. 1995). Different arrhythmias have been reported to be associated with abuse of AAS, including atrial fibrillation and ventricular tachycardia (Appleby *et al*. 1994, Nieminen *et al*. 1996, Sullivan *et al*. 1999). LV hypertrophy has also independently been associated with development of atrial fibrillation, ventricular arrhythmias and sudden cardiac death (Lip *et al*. 2000). Despite the lack of large-scale studies, the numerous case reports compellingly associate AAS abuse with increased risk of sudden death. In addition, because AAS abuse is not socially accepted anamnestic premortal AAS abuse may remain concealed.

5.4.1.5.2. Ischaemic heart disease

In medical literature, many case reports exist of premature acute ischaemic heart disease and myocardial infarction related to AAS abuse (Ferenchick *et al*. 1992, Appleby *et al*. 1994, Huie 1994). In most of the reports, aetiological factors other than previous AAS abuse have been ruled out. However, Fineshi *et al*. (2001) reported two cases where there were no findings in coronary arteries in autopsy. They speculated that a myocardial infarct without vascular lesions is rare and does not prove without doubt the direct cardiac toxicity of AAS. They also suggested that studies on AAS action on the neurogenic control of cardiac function in relation to regional myocardial contraction and vascular regulation are needed. Supraphysiological doses of AAS increase myocardial mass, with this growth likely having pathological features, and oxygen consumption is also increased (Deligiannis *et al*. 1992). One can easily conclude that AAS abuse increases the risk of ischaemic myocardial event.

5.4.2. Effects on metabolic system

5.4.2.1.Effects on non-sterol isoprenoid metabolism

Cholesterol and isoprenoid compounds have a critical and essential role in the growth of all eukaryotic cells (Siperstain 1984). Ubiquinone, also known as coenzyme Q_{10} (CoQ_{10}), is a non-sterol isoprenoid compound derived as a by-product from cholesterol synthesis (Olson & Rudney 1983). *In vivo*, ubiquinone acts as a lipid-soluble electron carrier in the electron transport chains of the mitochondria (Olson & Rudney 1983). It is strongly correlated with the serum LDL cholesterol fraction (Johansen *et al*. 1991). Karlsson *et al*. (1989, 1990) found that the serum CoQ_{10}/LDL ratio remained constant in all conditions examined. However, Aberg *et al*. (1994) demonstrated in rats that serum ubiquinone levels could be increased by 20% with probucol without altering serum cholesterol levels. Physical exercise also seems to have an influence on blood coenzyme Q_{10} concentration and interindividual variation exists (Karlsson 1987).

Ubiquinone and its reduced form ubiquinol have been assumed to possess antioxidant properties (Mohr *et al*. 1992). Stocker *et al*. (1991) have shown that ubiquinol has a powerful antioxidant effect on LDL, and therefore, one can conclude that it has an important role in the prevention of atherosclerosis.

Dolichols are α-saturated polyisoprenoid alcohols that are synthesized in microsomes and stored in lysosomes (Rip *et al*. 1985). Polyisoprenoid alcohols are present in all living cells (Rip *et al*. 1985). The liver has an important role in regulating blood dolichol supply (Marino *et al*. 1994). Phosphorylated dolichols function in the biosynthesis of N-linked glycoproteins as a main lipid carrier (Rip *et al*. 1985). Moreover, as free alcohol and fatty acid esters form, they modify fluidity, stability and permeability of biological membranes, and affect the process of fusion (Lai & Schutzbach 1984, Valtersson *et al*. 1985). A linear correlation is present between serum HDL and dolichol concentrations from which it is easy to conclude that the dolichols are transported with the HDL fraction (Yasugi & Oshima 1994). Agents that inhibit dolichol synthesis can possibly prevent an increase in plasma membrane IGF-I receptors, thus potentiating retarded cancer growth by down-regulation of the IGF-I effect. Dolichol and IGF-I appear to be essential for angiogenesis (McCarty 2001).

No previous reports of the effects of supraphysiological AAS doses on isoprenoid synthesis and blood concentrations exist.

5.4.2.2. Effects on collagen metabolism

AAS have increased collagen synthesis in an *in vitro* study when applied to human dermal fibroplasts (Falangia *et al*. 1998). AAS abuse has been suggested to deteriorate the form and function of connective tissues (Laseter & Russell 1991). Several case reports indicate that AAS abuse weaken the tendons, therefore considerably increases the risk of muscle and tendon ruptures (Bach *et al*. 1987, Kramhøft & Solgaard 1986). Among AAS-abusing athletes, tendon and muscle insertion traumas are suspected to be more frequent (Taimela & Seppälä 1994). Experimental studies have shown that high doses of AAS produce ultrastructural and biochemical alterations in tendons that may decrease the tensile strength of tendons (Michna 1986, Laseter & Russell 1991, Inhofe *et al*. 1995). Tendon degeneration and the development of dysplastic collagen fibrils have been demonstrated in rats during AAS administration, and these effects were most pronounced when exercise was combined with AAS administration (Wood *et al.* 1988). Moreover, the quantity of collagen molecules in tendons is decreased, while the number of collagen fibrils in the extracellular matrix increases. Further, depending on the duration of the AAS exposure, the relative number of dysplastic collagen fibrils in rat tendons increases (Michna 1986).

Type I collagen is the most abundant collagen type in the body, and its synthesis can be measured by serum carboxyterminal propeptide of type I procollagen (PICP), whereas carboxyterminal telopeptide of type I collagen (ICTP) reflects the degradation of type I collagen. Serum PICP has been shown to reflect the remodelling of bone. Serum ICTP implicates the resorption of bone (Risteli *et al*. 1988, Melkko *et al*. 1990). Metabolism of type III collagen, the major constituent of many dense and most loose connective tissues, except bone, tendon and cartilage, can be measured by serum aminoterminal propeptide of type III procollagen (PIIINP). PIIINP reflects the overall metabolism of all soft connective tissues in the body (Risteli *et al*. 1988).

Serum levels of ICTP and PIIINP are reported to increase during hormone replacement therapy with nandrolone decanoate (Hassager *et al*. 1994). This is thought to be due to increased breakdown of collagen types I and III in non-bone tissues. ICTP was suggested to derive relatively more from non-bone tissues than from bone because it did not correlate with histomorphometric measurements of bone turnover (Hassager *et al*. 1994). Oikarinen *et al*. (1992) found that during corticosteroid treatment the serum levels of PICP and PIIINP decreased, suggesting that corticosteroids suppress the synthesis of type I and III collagens. AAS at high concentrations may also bind to glucocorticoid receptors, and thus, AAS may have an impact on collagens by counteracting the effect of corticosteroid (Rogol & Yesalis 1992, Haupt 1993).

AAS may also influence collagen metabolism by direct stimulation of androgen receptors, which are known to be present in low densities in osteoblasts and bone marrow, thus also inderectly inhibiting osteoclast precursors and bone resorption. However, most of the androgen effects on bone turnover and bone mass occur via the estrogen receptor with prior aromatization of androgens into estrogens (Vanderschueren *et al*. 1995).

5.4.3. Effects on reproductive system

5.4.3.1. Endocrinological effects

AAS are derivatives of testosterone and, via negative feedback to the hypothalamus, they induce hypogonadotrophic hypogonadism associated with decreased serum testosterone concentrations (unless exogenous testosterone used), testicular atrophy, impaired steroidogenesis and spermatogenesis (Kilshaw *et al*. 1975, Schurmeyer *et al*. 1984, Jarow & Lipshultz 1990). There is a marked depression of serum testosterone and sex hormone-binding globulin (SHBG), especially when C17α-alkylated steroids are used, as well as of gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (NIDA 1990, Sader *et al*. 2001). LH and FSH control steroidogenesis and spermatogenesis, and their secretion is regulated by gonadal steroids and inhibin through negative feedback (de Kretser *et al*. 1998, 2000). LH and FSH secretion are equally suppressed after 4-6 months of testosterone administration at a physiological to moderately supraphysiological dosage (25-300 mg/week) (Matsumoto 1990). Normal spermatogenesis requires a 50-fold higher androgen concentration in the testis than in the peripheral serum (Adamopoulos *et al*. 1984, Turner *et al.* 1984). AAS also induce changes in other hormone levels and endocrinological systems, probably mediated by multiple receptor interactions, but these effects seem to be reversible (O'Connor *et al.* 1990, Brower 1993).

5.4.3.2. Effects on male fertility

Androgens have been used to treat male subfertility, but inadequate evidence exists to evaluate the usefulness of androgens for this purpose (Vandekerckhove *et al*. 2000). A few studies have also been published on AAS abuse-induced impaired fertility during a steroid cycle and following the cessation of

abuse. In these reports, lowered fertility has always been reversible (Yesalis 1993). AAS abuse induces oligozoospermia and sometimes azoospermia (Schurmeyer *et al*. 1984). Torres-Calleja *et al.* (2001) showed that abuse of AAS not only reduces the concentration of sperm, but in some subjects also impairs the percentage of morphologically normal semen. Lower semen density is supposed to occur because of AAS-induced hypogonadotrophic hypogonadism. No reports of the direct effects of AAS on testicular semen production are available.

In healthy male subjects, human chorionic gonadotrophin (HCG) used alone at a dosage of 5000 IU three times a week can maintain normal spermatogenesis (Matsumoto *et al*. 1983). The role of FSH in spermatogenesis is controversial, but at least it has a qualitative role in human spermatogenesis (Tapanainen *et al*. 1997). However, long-term HCG treatment has been shown to suppress spermatogenesis in an experimental model (Cusan *et al*. 1982), and HCG has a direct effect on spermatogenesis which leads to poorer sperm quality (Dunkel *et al*. 1997). Knowledge of male reproduction is mainly based on fertility control studies. Large variability exists in interindividual fertility, and thus the androgen dose required to suppress spermatogenesis also varies substantially between individuals (Schurmeyer *et al*. 1984, Knuth & Nieschlag 1987). Despite using a moderately large testosterone dosage (300mg/week), azoospermia is not reliably achieved in normal men (Matsumoto 1990). Further, this dosage failed to stimulate spermatogenesis (Matsumoto 1990).

5.5. Effects of growth hormone on cardiovascular system

Human growth hormone (GH) is a peptide hormone that is segregated from the posterior hypophysis. Different forms of GH are present in the circulation. This is a major peptide formed from a chain containing 191 amino acids. GH is mainly a regulator of vertical growth until closure of the epiphyseal plates, after which it also functions as an anabolic hormone, increasing the body's overall protein synthesis (Pelkonen 1992). Although the effectiveness of GH as an anabolic substance is undisputed, its benefit as a performance-enhancing substance in athletes is debatable (Macintyre 1987). Nevertheless, the abuse of GH to gain muscle mass and strength has increased due to its better availability since its synthetic manufacturing (Macintyre 1987). At present, a doping test to reveal GH abuse is lacking.

GH excess in prepubertal subjects leads to gigantism, and in postpubertal subjects excessive GH causes acromegaly. GH excess also has diabetogenic effects (Macintyre 1987). Before the advent of recombinant DNA technology, GH was derived from the pituitary glands of human cadavers (Biosynthetic growth hormone 1985). Several cases of Creutzfeldt-Jakob disease were attributed to the use of cadaver pituitary glands that were infected with this virus (Problems with growth hormone 1985). Due to its injectable route of administration, there is an increased risk of hepatis and AIDS as a result of shared needles and syringes.

GH directly affects the growth of the heart (Oparil 1985). The GH receptor gene is expressed in the myocardium of the rat heart (Mathews *et al*. 1989). In man, it controls cardiac wall stress and performance through its effect on myocardial growth (Fazio *et al*. 1997). In acromegaly, the majority of patients manifest LV hypertrophy even without hypertension (Lie 1980), and aberrant diastolic filling has also been reported (Smallridge *et al*. 1979). Acromegalic heart enlargement does not correlate with a person's weight or height (Lie 1980). Among acromegalic patients, the major causes of morbidity and mortality are cardiovascular complications such as premature coronary artery disease, hypertension, congestive heart failure and arrhythmia (Klein & Ojamaa 1992). Acromegalic patients have a higher prevalence and severity of ventricular arrhythmia, the severity of which correlates with LV mass (Kahaly *et al*. 1992). GH concentration does not affect the prevalence of arrhythmia, unlike the duration of exposure (Kahaly *et al*. 1992). Suppressing GH hypersecretion with the somatostatin analogue

octreotide improves the heart's systolic and diastolic functional indices at rest (Giustina *et al*. 1995). Moreover, LV mass is reduced with octreotide therapy (Tokgözoglu *et al*. 1994). However, prolonged hypersecretion of GH in acromegalic patients causes irreversible impairment in left ventricle filling (Rossi *et al*. 1992).

In GH-deficient adults, substitution therapy increases LV mass, mainly due to increased LV dimensions, but cardiac output is also increased (Caidahl *et al*. 1994). Contrary reports in which neither structure nor function was improved after GH replacement therapy are also available (Nass *et al*. 1995). The cardiac growth-promoting effect of GH has been studied under different conditions. Patients with idiopathic dilated cardiomyopathy have been reported to benefit from therapy with recombinant GH. GH therapy increases myocardial muscle mass and reduces dimensions of the LV (Lim *et al*. 1992, Fazio et al. 1996, Genth-Zotz *et al*. 1999). Patients with ischaemic cardiac failure, by contrast, do not benefit from GH therapy. No improvement was seen in LV function, either in mass or myocardial perfusion, after six months of therapy (Smit *et al*. 2001).

AAS and GH may have a synergetic effect on the myocardium. In experimental studies, concomitant use of androgens with GH increases the effects of both substances on the myocardium (Scow & Hagan 1965). Krieg *et al*. (1978) have also speculated that androgen receptor concentration may depend on GH concentration, which itself induces significant metabolic activities in the heart (Hjalmarson *et al*. 1975, Mowbray *et al*. 1975). Both AAS and GH have a direct impact on the myocardium. Typically in anabolic substance abuse, both of those hormones are abused concomitantly. No reports addressing possible effects of combined abuse have yet been published.

6. AIMS OF THE STUDY

This series of studies was conducted to evaluate various effects of abuse of anabolic substances on cardiovascular, metabolic and reproductive systems under authentic conditions.

The specific aims were:

1. To evaluate effects of anabolic androgenic steroid abuse with or without concomitant abuse of growth hormone on the size, morphology and function of the myocardium.

2. To reveal potential predictive signs in electrocardiograph depolarization indices of pathological growth induced by abuse of anabolic androgenic steroids.

3. To clarify anabolic androgenic steroid-induced alterations in cholesterol synthesis and effects on serum isoprenoid concentrations.

4. To elucidate effects of anabolic androgenic steroids on collagen synthesis, degradation and overall metabolism, to reveal a possible biochemical mechanism of anabolic androgenic steroids on collagen metabolism.

5. To study how abuse of anabolic substances affects male fertility, gonadotrophin plasma concentration and spermatogenesis.

7. SUBJECTS AND METHODS

7.1 Subjects recruitment

Between March 1992 and August 1994 a total of 26 healthy, non-obese, male power athletes aged 22-40 years responded to advertisement attached to the bulletin boards of various fitness clubs in the Helsinki metropolitan area (**Table 7**). They represented various sports (mainly bodybuilding) that use weight lifting as their main training modality. None of the volunteers represented a competitive sport subject to doping regulations. Participants abused drugs independently of this study, obtaining them from the black market. Subjects were consecutively recruited and all available subjects at the time were included in each substudy at the respective starting point.

Table 7. Characteristics of subjects (mean±SD)

	Study I			Study II			Study III	Study IV	Study V
	AAS	AAS+GH	Controls	Subjects	Endurance	Controls			
Number of subjects	15	4	15	15	30	15	13	17	18
Age (years)	30±4.6	32±7.4	26±2.9	30±5	25±3	26±3	24±4	30±6	28±4
Height (cm)	182.5±6.7	179±9.3	180±6.0	183±7	181±6	180±6	178±6	180±7	180±6
Weight (kg)	105±17	98±10	78±10	105±15	67±5	78±10	94±10	104±13	97±12
Body mass index (kg/m^2)	31.4±3.7	30.6±0.6	24.1±2.7	31.6±2.4	20.6±1.0	24.1±2.7	30.0±2.6	31.8±3.3	29.9±3.2
Lifetime abuse (years)	5.7±4.6	6.3±2.6		6.4±3.8			5.0±4.0	4.8±4.2	4.9±4.0

In Study II, 30 endurance athletes aged 22-31 years with running as their main training modality were included as a comparison group. All male runners of the national training group of the Finnish Orienteering Association as well as high-ranking long distance male runners (events from 3000 metres to marathon) from Southern Finland were invited to take part (participation rate 84%). The mean ± SD maximal oxygen uptake of the endurance athletes was 76 ± 5 (range 69-89) ml/kg/min and did not differ significantly between orienteering and long distance runners.

The control group (Studies I and II) consisted of 15 sedentary men, either Finnish army conscripts or physicians, with none exercising more than two hours per week. Their mean result in the Cooper test (distance run in 12 min) was 2730 metres.

All subjects and controls underwent a routine medical examination, and none had a history of any chronic diseases or took any continuous medication, except for the power athlete group.

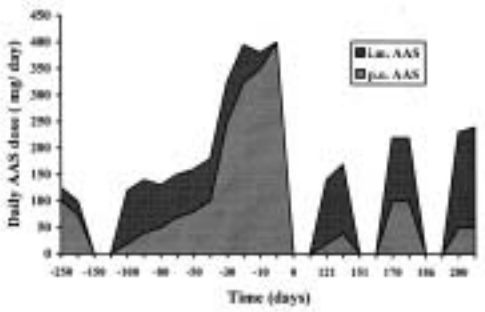
7.2. Ethics

The power athletes were counseled against substance abuse and given written information on adverse effects. All subjects and controls provided their written informed consent. The Ethics Committee of the National Public Health Institute, Finland, approved the study protocol. All participants signed a statement declaring that they were not under official doping control.

7.3. Study design and drugs

The power athletes were followed up during their self-regimen substance of abuse and the subsequent withdrawal period. Subjects were asked to maintain a six-month withdrawal, but some subjects did not follow these instructions. All subjects kept accurate daily records of the individual preparations and doses they used. Since each had an individual drug selection and dosage, the participants were asked

to confidentially surrender a sample of the preparation used (**Figures 10 and 11)**. The ingredients of various anabolic steroid preparations were determined by mass spectrometry (Donike *et al*. 1988).



Pharmaceutical preparation abuse between -125 and 0 days

- Primobolan depot® i.m.	(metelenolone)
- Winstrol 50® i.m.	(stanozolol)
- Sustanon 250® i.m.	(testosterone)
- Testosterone propionate 5%® i.m.	(testosterone)
- Metahandrostenolone® p.o.	(metahandrostenolone)
- Proviron 25 mg® p.o.	(mesterone)
- Anadrol 50 mg® p.o.	(oxymethalone)
- Winstrol 2 mg® p.o.	(stanozolol)
- Methyltestosterone® p.o.	(methyltestosterone)
- Anavar 2.5 mg® p.o.	(oxandrolone)
- Halotestin 5 mg® p.o.	(fluoxymesterone)
- Panteston 40 mg® p.o.	(testosterone)
- Nolvadex 10 mg® p.o.	(tamoxiphen)
- Spiropent 40 mg® p.o.	(clenbuterol)
- Parlodel 2.5 mg® p.o.	(bromocriptin)
- Profasi 5000 IU® i.m.	(human chorionic gonadotrophin)
- Somatogen 4 KY® s.c.	(growth hormone)
- Insulin s.c.	

Figure 10. Self-regimen of anabolic androgenic steroid abuse of subject number 4. i.m. = intramuscular injection; p.o. = peroral administration. Generic names of the pharmaceutical preparations are given in parentheses.

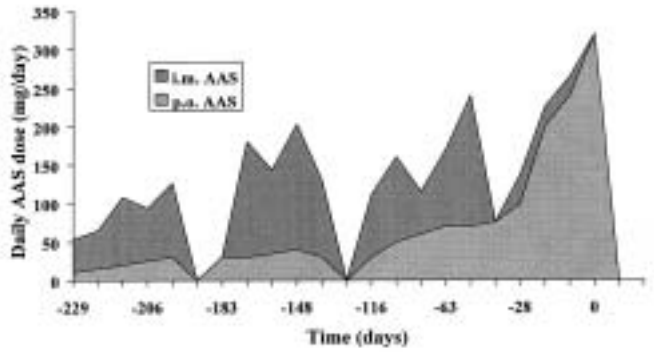


Figure 11. Self-regimen of anabolic androgenic steroid abuse of subject number 6. The subject abused a total of 3440.5 units (tablets and ampullae) during his 229-day cycle. Abused pharmaceutical preparations included testosterone i.m. and p.o., methandrostenolone, stanozolol i.m. and p.o., oxandrolone p.o., methyltestosterone p.o., clenbuterol p.o., growth hormone s.c., tamoxiphen p.o., human chorionic gonadotrophin i.m.
i.m. = intramuscular injection; p.o. peroral administration.

Urine specimens and serum samples were obtained from the subjects once every two weeks during substance abuse and the subsequent withdrawal period. A standard screening procedure for AAS was performed on each sample using GC/MS (Donike *et al*. 1988). No discrepancies were present between subjects' records and the results of chemical analyses.

For comparative purposes, we calculated various AAS doses based on milligrams of preparation used (although not all doses are equipotent). There is no way to differentiate AAS by administration route in terms of biological effectiveness. However, because the subjects lived in the Helsinki metropolitan area and were subject to the same drug availability, similar preparations were abused.

The subjects followed the same physical training regimen throughout the study.

7.3.1. Study I

Twenty substance-abusing power athletes (16 abusing AAS and 4 AAS and GH) and 15 controls were included. Echocardiography and Doppler echocardiography were performed during the last days of the AAS cycle. Tests were done between 8:00 and 12:00 after a light breakfast. The subjects did not take any medicine on that morning.

Mean AAS dose per day prior to cardiac evaluation was determined by dividing the total cumulative AAS dose (in mg) by the duration (days) of the cycle thus far. Both oral and injectable steroid preparations were included in the cumulative dose. Four of the subjects took 2-4 international units (IU) of GH in the evening on an irregular basis and by subcutaneous injection.

7.3.2. Study II

Fifteen substance-abusing power athletes (11 abusing AAS, and 4 AAS and GH), 30 substance-free endurance athletes and 15 controls were included. Twelve-lead ECGs were taken during the last days of the AAS cycle. ECGs were done between 8:00 and 12:00 after a light breakfast. The subjects did not take any medicine on that morning. Calculations of abused doses were determined as in Study I.

7.3.3. Study III

Thirteen substance-abusing power athletes were included. The average length of AAS administration during the study was three months, with the subsequent withdrawal period averaging six months.

Daily steroid dose (mg/day) was determined by calculating the average daily dose during the last ten days before a blood sample was drawn. The daily dose of an injectable steroid preparation was obtained by dividing the dose contained in each individual injection by the number of days between injections.

Every second week during the abuse cycle and withdrawal period, blood samples were drawn for assay of serum ubiquinone and dolichol, total and HDL cholesterol, triglycerides and liver aminotransferases (S-ALAT, S-ASAT).

7.3.4. Study IV

Seventeen AAS-abusing power athletes were included. Each subject gave four blood and urine samples, two of which were taken during the AAS administration period and two during the subsequent withdrawal period. The first sample was drawn at one month and the second sample at two months after the beginning of AAS abuse, and the third and the fourth samples at one and two months, respectively, after the cessation of AAS abuse.

Average daily doses of AAS were determined by dividing cumulative dose of AAS (p.o. and i.m. included) by duration (days) of the cycle. The weight used for the calculations in statistical analyses was the maximum weight measured during the course of the study. All subjects, except one, kept accurate records of the drugs and doses used during the AAS administration period. The exception was taken into account during statistical analyses.

7.3.5. Study V

Twenty-one substance-abusing power athletes were included. Three of those were excluded; one had a history of infertility treatment and the other two withdrew from the study for personal reasons, thus

resulting as 18 subjects participating.

Semen samples were obtained by masturbation after 2-7 days of sexual abstinence [95% confidence interval (CI) of the mean: 64-94 hours]. The samples were taken at the end of the AAS cycle (sample 1), at 1.5 months (95% CI: 39-52 days) after cessation of AAS abuse (sample 2) and at six months (95% CI: 134-185 days) after cessation (sample 3).

Both oral and injectable steroid preparations were included in the calculation of total cumulative AAS dose. The mean daily AAS dose was determined by dividing the total cumulative AAS dose by duration (days) of the cycle. The HCG dose was calculated as the total cumulative HCG dose (in IU) used during the cycle.

To study the effect of AAS dose, the subjects were divided into two groups. Those whose total cumulative dose was at or below the median (12 785 mg) were referred to as minor users (n=10), and those whose dose was above the median were referred to as major users (n=9). To determine the effect of HCG dose on semen variables, the subjects were also divided into another two groups: either below or above 12 000 IU [n=6 (mean dose 7875 IU) and n=13 (mean dose 32 000 IU), respectively]. The subjects were divided into groups based on self-reported information on substance abuse.

The subjects were contacted at 6 ± 0.65 years (mean ± SD) after cessation of the AAS cycle and asked about the number of children conceived and successful pregnancies during the period since cessation.

7.4. Echocardiography

Echocardiographic and Doppler measurements were made by the same observer, and obtained directly from the screen monitor with the aid of calipers and the instrument trackball. The investigator was blinded to drug selection and dosage. Due to marked differences in body shape between subjects, the investigator could not be blinded to study groups.

Echocardiographic and Doppler studies were performed with an Acuson 128 instrument and a 2.5 – 3.5 MHz transducer. Subjects were positioned at 45 degrees in left lateral position. To avoid including trabeculations in the wall thickness measurements, an integrated M-mode and two-dimensional study was done to determine interventricular septal and LV posterior wall thickness and LV end-diastolic cavity dimension. First, two-dimensionally targeted M-mode recordings were obtained in parasternal long-axis view (Sahn *et al.* 1978). Second, septal and posterior wall thicknesses were measured in parasternal long-axis view between mitral valve tips and papillary muscle, from expanded two-dimensional images. Smaller numbers from either M-mode or two-dimensional measurements were accepted to represent the actual thicknesses of the septum and posterior wall. The LV mass was calculated using the formula by Devereux (1987): Mass = 0.8*[1.04*(septal thickness + end-diastolic diameter + posterior wall thickness)^3- end-diastolic diameter]^3+ 0.6 g.

LV length was measured at end-diastole from the apical window at a view maximizing the ventricular length. Measurements were made from the mitral valve plane to the apical epicardium (L1) and to the apical endocardium (L2). The myocardial cross-sectional area of the LV was the difference between total LV area subtended by the epicardium (A1) and LV cavity area (A2) traced using a midventricular short-axis view at the level of papillary muscle tips. The concentricity or eccentricity of the LV myocardium was evaluated by calculating the relative wall thickness (RelWT) using the formula: RelWT=(septum thickness + posterior wall thickness)/LV end-diastolic diameter. The sphericity of the LV chamber was evaluated by calculating the ratio of LV end-diastolic diameter to ventricular length. In all two-dimensional measurements, the endocardial/cavity (black-white) interface was used for endocardial

border definition (Schiller *et al.* 1989). Measurements of LV diastolic filling velocities were obtained in an apical four-chamber view by positioning the pulsed Doppler volume sample about 1 cm below the mitral annulus. Early peak flow velocity (E) and peak atrial flow velocity (A) were measured and the ratio E/A calculated.

One power athlete in Study I underwent Doppler echocardiography three times. He was first examined during abuse of AAS and GH, second during the washout period and third during the next AAS cycle.

7.5. Standard 12-lead electrocardiogram and calculations of QT dispersion

The measurements were made from a standard 12-lead resting ECG. QT intervals were measured manually from the beginning of the Q wave to the end of the T wave by the same investigator, who remained blinded to the group and identity of subjects. The QT dispersion was calculated as the difference between the longest and the shortest QT interval. Since QT interval durations were different in each group, we also calculated the relative QT dispersion in two different ways:

1) QT dispersion divided by the longest QT interval,

2) Standard deviation of the QT interval divided by mean QT interval.

To compare the length of the QT intervals between groups, QT intervals were adjusted to the heart rate using the nomogram method (Karjalainen *et al*. 1994).

7.6. Clinical chemistry

After the blood samples were drawn, the serum was separated and aliquots stored at -20°C for lipids, carboxyterminal propeptide of type I procollagen (PICP), carboxyterminal telopeptide of type I collagen (ICTP), aminoterminal propeptide of type III procollagen (PIIINP) and hormones (testosterone, SHBG, LH, FSH) and at –70°C for ubiquinone/dolichol assays.

Total cholesterol levels, HDL fraction and serum triglycerides were measured using commercial kits from Boehringer-Mannheim Diagnostica (Mannheim, Germany). The HDL fraction was obtained by the Mg2+/dextran sulphate precipitation method (Finley *et al*. 1978), and LDL cholesterol was calculated using the Friedewald equation (1972).

The ubiquinone determinations were performed according to Laaksonen *et al.* (1995) by high-performance liquid chromatography (HPLC). The dolichols were analysed by an HPLC-method (Jokelainen *et al*. 1992). The serum levels of dolichols were expressed as the sum of the three homologues of 18, 19 and 20 isoprene units.

Analyses for PICP, ICTP and PIIINP were performed with a radioimmunoassay kit (Orion Diagnostica, Espoo, Finland)(Risteli *et al*. 1988, Melkko *et al*. 1990, Risteli *et al*. 1993).

Spot urine samples collected in the afternoon were used for the quantification of hydroxylysyl pyridinoline (HP) and lysylpyridinoline (LP) mature crosslinks of collagen. The HP/LP analysis was performed using equipment from Merck Hitachi according to Eyre *et al*. 1984, Black *et al.*1988, Palokangas *et al*. 1992). The results of HP and LP were given after comparison with standards, which were prepared from bovine cortical bone (Cheng *et al*. 1996) and calibrated with the aid of the authentic HP and LP standards kindly provided by Dr. Simon Robins (The Rowett Research Institute, Aberdeen, Scotland). The HP and LP results are expressed as µmol/mol creatinine. Creatinine (Cr) was quantified

in the unhydrolysed urine samples by the Jaffé procedure using a commercial reagent kit (Boehringer-Mannheim).

Total testosterone was determined in untreated serum by radioimmunoassay using a commercial kit (Coat-a-Count®) obtained from Diagnostic Products Corp., Los Angeles, CA, USA. Serum concentrations of LH and SHBG were determined by time-resolved fluoroimmunoassay (TRFIA) using commercial kits (Delfia®) obtained from Wallac Ltd., Turku, Finland. Serum FSH was determined by immunoluminometric assay using a commercial kit (ACS FSH®) obtained from Ciba-Coming Corp., Medfield, MA, USA.

7.7. Semen analyses

Semen analyses were carried out according to World Health Organization guidelines (World Health Organization Laboratory manual for the examination of human semen and semen-cervical mucus interactions, Cambridge WHO). Analyses of the concentrations and motility were, however, carried out using a Makler chamber at room temperature. The criteria for normozoospermia were a concentration of $\geq 20 \times 10^6$/ml, grade motility in 25% or grade A+B motility in 50% of spermatozoa, and normal morphology (in stained preparations) in at least 30% of spermatozoa.

7.8. Statistical methods

The results are expressed as mean ± standard deviation (SD), and 95% confidence intervals (CI) for the mean (Study I) or median (for semen variables, Study IV) are also given.

In Study I, non-parametric Kruskal-Wallis analysis of variance (ANOVA) was used to assess the statistical differences between the three groups, and when appropriate, multiple comparisons were made using the Mann-Whitney U-test with Bonferroni correction.

In Study II, one-way ANOVA was used to assess statistical differences between the three groups, and when appropriate, *post hoc* analyses were carried out using the LSD test.

In Study III, the results were recalculated from the original article. The mean for each subject during the AAS abuse and withdrawal period was calculated. After careful re-evaluation of the samples and the subjects, 8 subjects were included in the AAS group and 11 in the off AAS group. Wilcoxon´s paired test (n=8) was used for evaluation of the significance of differences between the groups.

In Study IV, the results were recalculated from the original article. The repeated measure of ANOVA was used to assess the statistical differences between the four samples, and when appropriate, *post hoc* analyses were carried out using Student's t-test to assess the statistical differences between the groups.

In Study V, the repeated measure of ANOVA was used to assess the statistical differences between the three samples and different groups, and when appropriate, *post hoc* analyses were carried out using Student's t-test to assess statistical differences between groups.

Pearson's correlation coefficients were calculated to assess the associations between echocardiographic measurements and various subject characteristics (Study I), AAS doses and serum PICP, ICTP and PIIINP and urine HP, LP and creatinine concentrations (Study IV), and semen variables and various subject characteristics (Study V). Spearman´s correlation coefficients were calculated to assess any relationship between the daily steroid dose (n=8) and serum ubiquinone, dolichol, cholesterol, HDL and LDL (n=11).

The determinants of LV mass (Study I) and anomalous spermatozoa (Study V) were studied using forward stepwise multiple regression analysis with α-to-enter = 0.150 and α-to-remove = 0.150.

In Study IV, the data on the subject who failed to supply accurate records of the drugs and doses used during the AAS administration period were not used when comparing the doses with other parameters. Probabilities of less than 0.05 were regarded as statistically significant. Statistical analyses were carried out using the software program Systat (1992) for Windows, and for Study II, the analyses were carried out using StatSoft's Statistica for Windows release 5.1.

8. MAIN RESULTS

8.1. Effects on left ventricular mass, geometry and filling

LV mass, height-indexed LV mass, RelWT, and septum and posterior wall thicknesses were all significantly greater in subjects using both AAS and GH (n=4) than those using only AAS (n=16) or among the sedentary controls (n=15) (**Table 3, Study I**). Moreover, all of these parameters were significantly higher among plain AAS abusers than among controls (**Table 3, Study I**). No significant differences were present between the subjects using only AAS and those using both AAS and GH in the period of lifetime AAS abuse or the duration of the previous cycle (**Table 2, Study I**).

Among the controls, the correlation between LV mass and body weight was high (r=0.72, p<0.01). In contrast, correlations among anabolic substance abusers (n=20) between LV mass and the person's height (r=0.24), weight (r=0.32), resting heart rate (r=0.09), systolic (r=-0.14) and diastolic blood pressure (r=0.22), period of lifetime AAS abuse (r=0.27) or length of AAS use prior to cardiac evaluation (r=0.13) were low and non-significant (**Table 4, Study I**). However, the correlation coefficient between LV mass and mean AAS dose was high (r=0.54, p<0.015) (**Figure 12**).

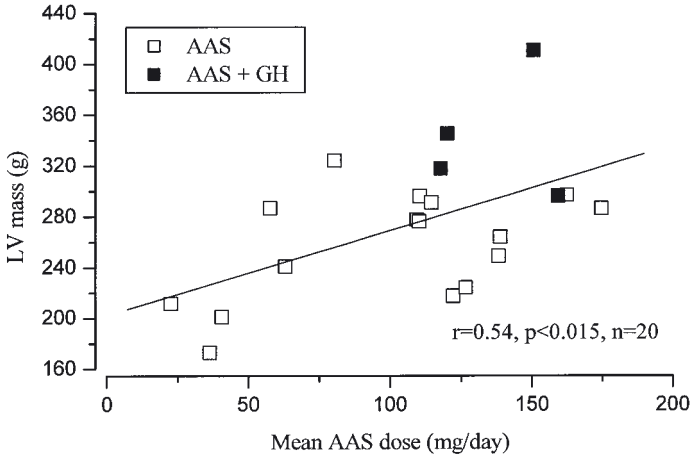


Figure 12. Correlation of mean anabolic androgenic steroid (AAS) dose with left ventricular (LV) mass. Subjects using AAS alone are indicated with open squares and those using both AAS and growth hormone (GH) with filled squares. Pearson's correlation coefficient and p-value for the whole population are shown.

When the simultaneous effects of background factors on LV mass in substance-abusing power athletes (n=20) were investigated, subject's physical characteristics, AAS abuse characteristics (period of lifetime AAS abuse, length of AAS use prior to cardiac evaluation, mean AAS dose), blood pressure and resting heart rate were included as independent variables in a forward stepwise regression analysis. The three factors constituting the final model were mean AAS dose, age and systolic blood pressure, accounting for 29%, 14% and 17%, respectively, of the variance in LV mass.

One of the subjects was studied three times: when he used both AAS and GH; then 237 days later, when he was not abusing any substances; and when he resumed abusing AAS alone. On these occasions, his LV mass was 3.8‰, 2.3‰ and 3.1‰ of his body weight, respectively (**Figure 2, Study I**).

To study LV diastolic function, the early peak flow velocity (E) and peak atrial flow velocity (A) were measured and the E/A ratio calculated. This ratio did not significantly differ between the groups. Among the substance abusers (n=20) the E/A ratio correlated negatively with the person's age (r=-0.70, p<0.01), height-indexed LV mass (r=-0.49, p<0.05) and RelWT (r=-0.60, p<0.01). A negative correlation was found between the E/A ratio and diastolic blood pressure (r=-0.49, p<0.05). RelWT correlated positively with resting diastolic blood pressure (r=0.58, p<0.05) (**Table 4, Study I**).

Both endurance (n=30) and power athletes abusing AAS (n=15, Study II) had significantly greater wall thickness and cavity dimensions than the sedentary controls (**Table III, Study II)**. The shape of the LV chamber was comparable in all groups, as revealed by the equal ratio of LV diameter to length. On average, LV mass was 56 % greater in the endurance and 72% greater in the power athletes than in the controls (**Table III, Study II)**. When expressed in relation to body surface area, the endurance athletes had 9% greater LV mass than the power athletes. Among all subjects (19 power athletes, 30 endurance athletes, 15 sedentary controls), RelWT is significantly correlated with LV mass (r=0.56, p<0.001) (**Figure 13**).

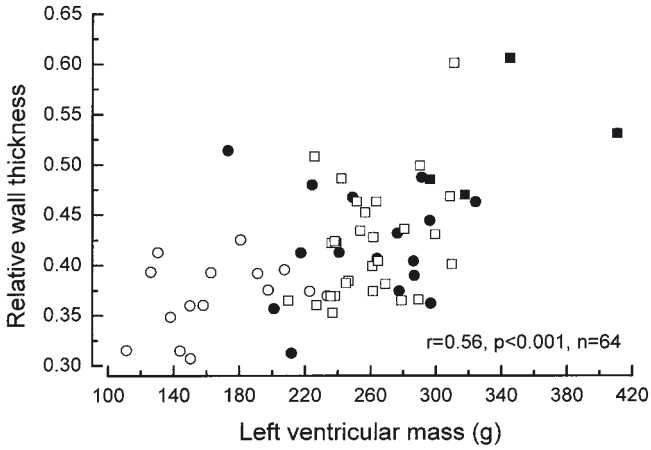


Figure 13. Correlation of left ventricular (LV) mass with LV relative wall thickness. Subjects abusing both anabolic androgenic steroids (AAS) and growth hormone are indicated as filled squares (n=4). Subjects abusing AAS alone are indicated as filled circles (n=15). Elite endurance athletes are indicated as open squares (n=30). Sedentary controls (n=15) are indicated as open circles. Pearson's correlation coefficient and Bonferroni corrected p-value are shown.

8.2. Effects on QT dispersion

The endurance athletes (n=30) had the lowest heart rates, and the power athletes (n=15) and controls (n=15) had similar heart rates on the average (**Table II, Study II**). Resting blood pressures were comparable between all three groups. Endurance athletes had the longest QT intervals in every lead, the differences being highly significant even when QT intervals were adjusted for heart rate (**Table IV, Study II**). The power athletes had shorter QT intervals than the controls, but the difference did not reach statistical significance (**Table IV, Study II**). This finding was also consistent over all 12 leads. When adjusted for heart rate, the power athletes still had the shortest QT intervals, but when compared with controls, the differences remained significant in only four leads.

The power athletes exhibited the greatest amount of QT dispersion and the endurance athletes the least. When the dispersion was expressed as a percentage of the longest QT interval duration or as the standard

deviation of the QT interval divided by the mean QT interval, the differences between the groups were clearly accentuated (**Table IV, Study II**).

Overall, no significant correlation was present between LV mass and QT dispersion. Low, although statistically significant correlations were found between QT dispersion and E/A ratio (r= -0.30), and LV end-diastolic diameter (r= 0.26) but not with LV mass. E/A ratio was significantly smaller in power athletes than in endurance athletes (mean ± SD; 1.59 ± 0.40 vs. 2.39 ± 0.61, p<0.001).

8.3. Effects on cholesterol metabolism

HDL cholesterol concentrations were 52% lower (p<0.05) during AAS abuse than during the withdrawal period (**Table 8**).

Table 8. Serum lipoprotein, ubiquinone and dolichol concentrations during steroid administration and withdrawal period (mean±SD)

Parameter	On Steroids (n=8)	Off Steroids (n=8)	
Cholesterol (mmol/l)			
Total	3.49 ± 0.56	3.66 ± 0.29	
LDL	2.48 ± 0.54	2.07 ± 0.47	
HDL	0.57 ± 0.36	0.87 ± 0.33	*
Triglycerides (mmol/l)	0.98 ± 0.34	1.59 ± 0.68	*
Ubiquinone (mg/l)	1.72 ± 0.60	1.17 ± 0.55	*
Total Dolichol (mg/l)	159.96 ± 69.76	192.85 ± 42.09	
Total dose (mg/d)	83.79 ± 62.44		

Significance of difference determined by Wilcoxon's paired test. *P≤0.05

AAS abuse significantly increased serum ubiquinone concentration by 47% (p<0.05). Moreover, a high positive correlation was found between serum ubiquinone concentration and daily steroid dose (r=0.76, p<0.05, n=8) (**Figure 14**). A high negative correlation was present between the ubiquinone and HDL cholesterol concentrations (r=-0.66, p<0.05, n=11) (**Figure 15**).

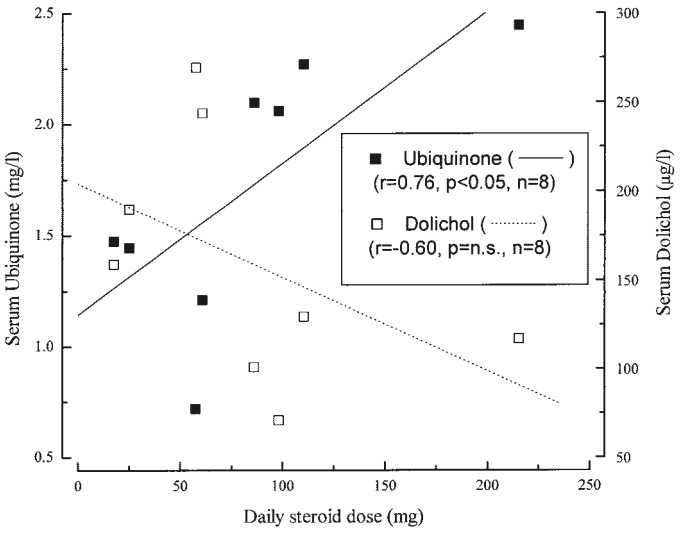


Figure 14. Correlations of daily AAS dose with serum ubiquinone and dolichol concentrations. Spearman's correlation coefficients, p-values and the number of observations are shown. n.s.= not significant.

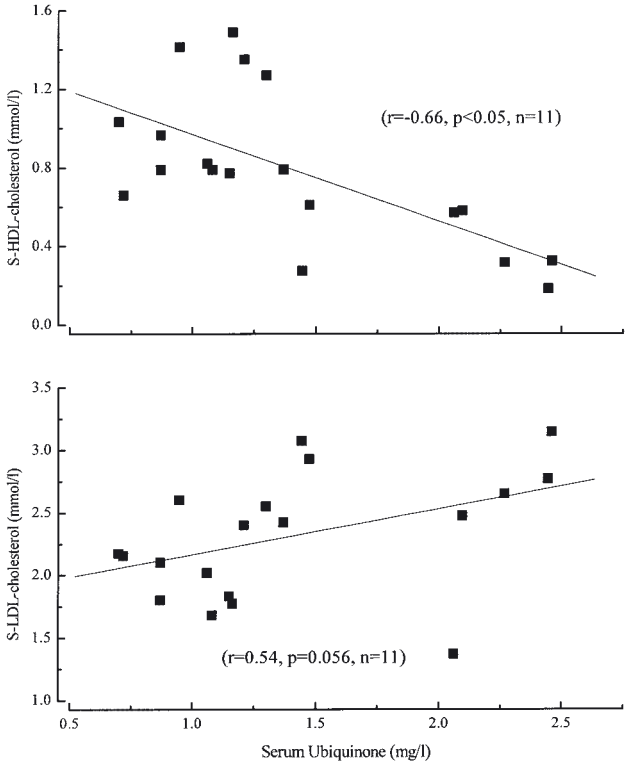


Figure 15. Correlations of serum ubiquinone with serum HDL and LDL cholesterol in subjects (n=13) both on anabolic androgenic steroids (AAS) and off AAS. Spearman's correlation coefficients, p-values and the number of subjects are shown.

Serum dolichol concentrations, by contrast, were 17% lower during AAS abuse than during withdrawal (**Table 8**). Daily steroid dose correlated negatively with serum dolichol concentration, but the correlation did not reach statistical significance (r=-0.60) (**Figure 14**). HPLC profiles of the dolichol fractions remained conctant throughout the AAS abuse and withdrawal periods.

8.4. Effects on collagen metabolism

In Study IV, the measured serum PICP, ICTP and PIIINP concentrations were within reference ranges for healthy adult males (**Table 9**).

Serum PIIINP concentrations during the abuse of AAS and one month after cessation were significantly higher than values obtained two months after cessation (**Table 9**). Serum ICTP measured after one month of AAS abuse was 3.10 ± 1.48 as compared with 3.90 ± 1.71 after one month of withdrawal. There was a borderline significance in differences in this type I collagen degradation (Repeated Measure of ANOVA, p= 0.057). No correlation was found between serum ICTP and urine LP.

The urine HP/LP ratio correlated positively with weight-indexed AAS dose (r=0.56, p<0.05, n=16) (**Figure 16**). Urine excretion of creatinine measured after two months' abuse was significantly higher than values obtained after one month's abuse or during the withdrawal period (**Table 4, Study IV**).

Table 9. Serum ICTP, PICP and PIIINP and urine HP and LP (mean±SD)

Parameter	Reference range (Mean±2SD)	Mean reference values	1 month on steroid	2 months on steroid	1 month off steroid	2 months off steroid	Repeated Measures of ANOVA p-value
S-ICTP (µg/l) (n=17)	1.8-5.0*	3.46±1.11	3.10±1.48	3.15±1.54	3.90±1.71	3.54±1.48	0.057
S-PICP (µg/l) (n=17)	38-202**	124±49	185.72±55.18	194.67±64.61	178.94±64.70	165.73±54.22	n.s.
S-PIIINP (µg/l) (n=17)	1.7-4.2***	3.04±0.68	3.15±1.87†	3.50±2.04‡	3.26±1.85‡	2.01±0.38	0.016
U-LP/creat (µmol/mol) (n=11)	-	-	2.36±1.68	2.82±1.47	2.86±2.18	2.72±1.84	n.s.
U-HP/creat (µmol/mol) (n=17)	-	-	14.92±6.40	15.44±7.42	16.15±8.11	17.11±5.64	n.s.
U-HP/LP (n=11)	-	-	4.96±2.87	6.00±1.95	4.56±2.45	4.56±2.50	n.s.

S = serum; U = urine; ICTP = carboxyterminal telopeptide of type I collagen; PICP = carboxyterminal propeptide of type I procollagen; PIIINP = aminoterminal propeptide of type III procollagen; LP = lysylpyridinoline crosslink; HP = hydroxylysylpyridinoline crosslink; creat = creatinine.
* = Risteli *et al.* 1993. ** = Melkko *et al.* 1993. *** = Risteli *et al.* 1988.
Comparison between the 4 samples performed by Repeated Measures of Analysis of Variance (ANOVA), and when appropriate, multiple comparisons were made using paired t-test. n.s. = not significant; † = p<0.05 vs. 2 months off steroid; ‡ = p<0.01 vs. 2 months off steroid.

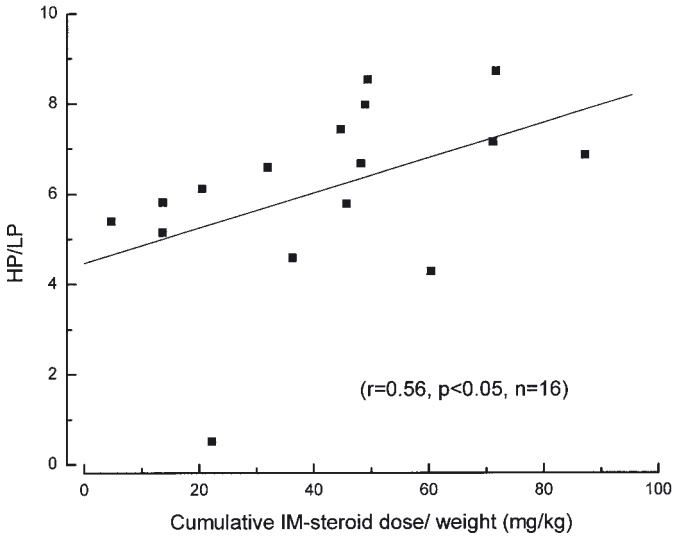


Figure 16. Correlation of HP/LP ratio with cumulative injectable intramuscular anabolic androgenic steroid dose relative to body weight (mg/kg) after 2 months' abuse. Spearman's correlation coefficient, p-value and the number of observations are shown.

8.5. Effects on spermatogenesis

In Study V, subjects' age had no significant influence on sperm concentration in any sample among our study population (Sample 1: r=-0.46, p=0.10; Sample 2: r=-0.30, p=0.29; Sample 3: r=-0.37, p=0.19). Nor was any association found between any semen variable and the length of lifetime AAS abuse (**Table 10**).

At the end of the AAS cycle (n=18), sperm count was 33 ± 49 x10^6/ml (mean ± SD) (median 11 x10^6/ml), and one subject was diagnosed with azoospermia. After 1½ months of cessation of AAS cycles (n=16), sperm concentration was 30 ± 42 x10^6/ml (median 7 x10^6/ml). Six months after the cessation of AAS abuse (n=16), the mean number of spermatozoa had increased to 77 ± 70 x10^6/ml (median 73 x10^6/ml) (95% CI 40-115 x10^6/ml). One of the subjects was diagnosed as azoospermic throughout withdrawal period; his sperm count even exceeded normal limits during the AAS cycle. The differences between the

Table 10. Correlations between semen variables and the length of lifetime anabolic androgenic steroid abuse in samples 1-3. Pearsons's correlations coefficients and p-values were determined.

	Sample 1 (n=18)	Sample 2 (n=16)	Sample 3 (n=16)
Semen volume	r=0.13 p=0.60	r=-0.11 p=0.68	r=0.11 p=0.68
Concentration of spermatozoa	r=-0.21 p=0.39	r=-0.17 p=0.54	r=0.11 p=0.70
Motility (WHO categories a + b)	r=-0.17 p=0.49	r=0.41 p=0.12	r=0.18 p=0.51
Normal morphology	r=0.001 p=1.00	r=-0.04 p=0.89	r=0.19 p=0.48

samples drawn six months after cessation of AAS abuse and both during and 1.5 months after the abuse were significant ($p \leq 0.05$, Repeated Measures of ANOVA) (**Table 3, Study V**).

In semen samples taken at the end of the AAS cycle, the correlation between sperm concentration and mean daily AAS dose nearly reached the level of statistical significance (r=-0.44, p=0.066) (**Figure 2, Study V**). A significant positive correlation was observed between HCG dose used during the cycle and the percentage of morphologically abnormal spermatozoa (r=0.60, $p<0.01$) (**Figure 2, Study V**).

When simultaneous background factors of morphologically abnormal spermatozoa were investigated, the HCG dose used was the only factor bearing significance in the model, accounting for 36.5% of the variance. In subjects using a higher dose of HCG, spermatozoa were significantly morphologically abnormal [mean: 80% vs. 28% (higher vs. lower)], although the higher HCG dose maintained spermatogenesis better during the cycle, the sperm concentration being 44 ± 54 $x10^6$/ml (mean ± SD) (median 21 $x10^6$/ml) vs. 10 ± 17 $x10^6$/ml (median 0.6 $x10^6$/ml) ($p<0.05$). No differences were present between the HCG groups in AAS dose.

Before starting the study, five of the 18 subjects reported having one or more children. Six years after completing the study, 10 of the subjects had got children and one couple had terminated a pregnancy after the study.

The main findings are summarized Table 11.

Table 11. Main study results

	On AAS	Off AAS	AAS + GH	Endurance training	Control
Left ventricular mass (LV mass)	↑	↓	↑↑	↑	↔
Left ventricle end diastolic diameter (LVEDD)	↑	↓	↑↑	↑	↔
Left ventricle posterior wall thickness (PWT)	↑	↓	↑↑	↑↔	↔
Relative wall thickening of left ventricule (RelWT)	↑	↓	↑↑	↔	↔
Aortic diameter (AO)	↔	↔	↔	↔	↔
Ratio of early and after diastolic flow (E/A)	↔(↑)	↓	↑	↔	↔
QT-dispersion	↑		↑	↓	↔
QT-interval	↓		↓↓	↑	↔
Serum ubiquinone	↑	↓			
Serum dolichol	↓	↑			
Serum high-density cholesterol	↓↓	↔			
Serum low-density cholesterol	↑	↔			
Serum triglyceride	↔	↔			
Serum carboxyterminal propeptide of type I procollagen (PICP)	↔(↑)	↓			
Serum carboxyterminal telopeptide of type I collagen (ICTP)	↔(↓)	↑			
Serum aminoterminal propeptide of type III procollagen (PIIINP)	↑	↓			
Urine hydroxylysylpyridinoline (HP)	↑	↔			
Urine lysylpyridinoline (LP)	↔	↔			
Urine HP/LP ratio	↑↑	↔			
Serum creatinine concentration	↔	↑			
Semen concentration	↓↓(↓)*	↔(↓)			
Semen volume	↔(↓)	↔			
Semen morphology	↓	↔			
Semen motility	↓(↔)	↔			
Serum luteining hormone (S-LH)	↓↓	↔			
Serum follicle-stimulating hormone (S-FSH)	↓	↔			
Serum testosterone	↓**	↔(↓)			

* = HCG abused concomitantly with AAS

** = Unless exogenous testosterone is abused

( ) = tendency, ↔ = unchanged, ↑↑ = marked changes

9. DISCUSSION

Several adverse effects of massive doses of AAS were detected in this series of studies, which were performed under authentic conditions of anabolic substance abuse. Novel findings were that abuse of AAS, especially with GH, resulted in marked pathological hypertrophy of the myocardium, which was observed as a significant concentric remodeling of LV and increased QT dispersion. Also, serum ubiquinone concentration was significantly increased during AAS abuse

In line with previous studies, LDL/HDL cholesterol ratio was significantly increased and, also, all of our subjects demonstrated hypogonadotrophic hypogonadism while on AAS. Although some subjects were able to maintain spermatogenesis during AAS abuse with regular injections of HCG, semen quality was impaired. After six months of AAS abstinence, mean semen concentrations reached the normal level, but mean serum testosterone concentrations remained low.

AAS abuse is suspected to result in fibrous tissue formation in the myocardium (Nieminen *et al*. 1996). Our results suggest that AAS abuse at supraphysiological doses decreases the degradation of type I collagen and significantly increases the overall metabolism of type III collagen in all soft tissues. Deleterious effects with massive doses of AAS were seen on various organs and metabolic systems strengthening the idea of dose-dependent adverse effects.

Knowledge of adverse effects of AAS at supraphysiological doses in the past has been mainly based on animal experiments and case reports. Some previous reviews have been speculating that most harmful adverse effects of AAS abuse are those that affect cardiovascular and reproductive systems (Lucas 1993, Taimela & Seppälä 1994, Pärssinen & Seppälä 2002). The findings of the present study indicate several, potentially life-threatening adverse effects of anabolic substance abuse, strengthening the evidence from the previous findings. Exercise-associated health benefits may not be gained when the training regimen is augmented with anabolic substances abuse. Weight training itself does not decrease life expectancy, but the substance abuse often associated with it can increase the risk of premature death (Sarna *et al*. 1993, Pärssinen *et al.* 2000).

9.1. Study limitations

Several limitations were encountered due to nature of this study, with the subjects' primary aim being to improve physical fitness and increase muscle mass. To reveal the health problems connected with this particular subculture abusing doping substances under authentic conditions, the limitations had to be accepted. For ethical reasons, controlling the use of steroids or the dosage or pharmaceutical agents chosen was not possible since the drug abuse occurred independently of this study. To compare the use of moderate and massive doses of AAS, we divided the subjects into groups based on self-reported substance abuse information.

Most previous studies used a similar follow-up method, and they also suffer from a lack of a matching control group. Arranging a matching control group with a reliable history of power training without any abuse of anabolic substances seems practically impossible due to the secretive nature of substance abuse. Moreover, training histories and training volumes of these groups would not be comparable. The number of subjects in the present study (26) is high compared with previous studies of a similar design. In addition, the duration of follow-up, 2-9 months of drug abuse and 5-9 months of withdrawal, was longer than in most earlier studies. Overall, our subject sample is reasonably representative of this highly selective group of subjects.

We were unable to control for the effects of training on the myocardium and on other parameters,

although the subjects maintained a similar heavy load resistance-training regimen throughout the study. All subjects had been practicing with weights for at least two years prior to the study. Diet was also not controlled, but subjects reported the diet having remained unchanged. In studies where recruitment is based on voluntariness, without controlling the substances abused, selection bias is inevitable. However, subjects with a medical history of diseases associated with the systems studied were excluded, and all underwent medical examination, after which only clinically healthy subjects were included.

9.2. Effects on cardiovascular system

LV mass, geometry and filling were studied among abusers of anabolic substances with echocardiography. Myocardial hypertrophy was evident and was dose-dependently associated with AAS abuse, and concomitant use of GH led to an even greater increase in LV mass. LV mass was unrelated to body dimensions among the subjects, although in the controls, a significant positive correlation was found. This supports the idea that AAS and GH have a direct effect on the myocardium. Several earlier studies also suggest that GH or concomitant IGF-I has a direct effect on the myocardium (Lie 1980, Mathews *et al*. 1989). Moreover, the GH receptor gene is expressed in the rat myocardium (Mathews *et al*. 1989).

A significant proportion of patients with GH hypersecretion, i.e. acromegaly, demonstrate LV hypertrophy, even without hypertension. Acromegalic heart enlargement does not correlate with body weight or height (Lie 1980). In GH-deficient adults, GH substitution increases LV mass mainly by increasing LV dimensions (Caidahl *et al.* 1994). On the other hand, suppressing GH hypersecretion in acromegaly with the somatostatin analogue octreotide causes a significant decrease in LV mass without altering body dimensions (Lim *et al.* 1992).

Strenuous physical exercise is known to increase LV mass (Maron 1986). According to Pellicicia *et al*. (1991, 1993), LV wall thickness exceeding 13 mm is uncommon in highly trained competitive athletes, and thus, its occurrence would suggest an explanation other than intensive athletic training. This study supports the conclusion because 4 out of 20 subjects (3 of whom concomitantly used GH) had an LV wall thickness greater than 13 mm. One can therefore reason that part of the myocardial hypertrophy observed in the subjects is due to self-administration of anabolic substances.

Our finding is in line with previous reports that AAS alter the LV morphology, making it more concentric (Dickerman *et al*. 1997a,c), and that concomitant abuse of GH leads to an even greater RelWT of LV. Isometric weight training is assumed to increase concentric LV hypertrophy, whereas endurance training is assumed to be associated with eccentric LV hypertrophy (Shapiro 1984, Maron 1986). Contrary reports are also available in which AAS-abusing power athletes demonstrate both concentric and eccentric enlargement of LV mass (Deligiannis & Mandroukas 1992, Sachtleben *et al*. 1993), in accordance with our findings. In fact, our echocardiographic findings in power athletes using massive doses of AAS but not GH resembled findings in top-level endurance athletes. We demonstrated that concentricity of LV is associated with LV mass.

Despite similarities in echocardiographic findings, substance-free endurance athletes and AAS-abusing power athletes differ markedly with respect to their electrocardiographic repolarization indices. Physiological LV hypertrophy in endurance athletes not using drugs did not increase QT dispersion, which has been shown to be associated with a pathological myocardium (Mayet *et al*. 1996, Perkiömäki *et al*. 1996). While QT intervals were prolonged in endurance athletes, they had significantly less QT dispersion. AAS users had the shortest QT intervals of all study groups but a greater QT dispersion.

The physiological lengthening of the QT interval, which has earlier been shown to also exist in female endurance athletes, may have two potential mechanisms (Stolt *et al*. 1997). First, physiologically adapted

LV hypertrophy could prolong the process of repolarization. Second, endurance athletes have enhanced vagal tone, which may lengthen the QT interval (Browne *et al*. 1984). Pathological hypertrophy of LV induced by AAS abuse does not demonstrate vagal bradycardia (author´s unpublished observation).

LV hypertrophy among power athletes was not physiological since it was partly induced by anabolic substances. This pathological hypertrophy was reflected as an increased dispersion of QT intervals, similar to that found in hypertensive LV hypertrophy (Mayet *et al*. 1996, Perkiömäki *et al*. 1996). It may also be reflected as a higher risk of malignant arrhythmia in AAS users. Two of the subjects participating in this study did show increased fat and fibrous tissue in endomyocardial biopsies, with one of these subjects experiencing exercise-induced ventricular tachycardia during the treadmill test (Nieminen *et al*. 1996). Thus, the LV mass as such is not a determinant of the QT variables, rather it is the histological quality of the myocardium that is decisive.

Cardiac systolic function has been reported to remain normal during AAS administration (Deligiannis *et al*. 1992), but there are contradictory reports concerning AAS effects on diastolic function (Pearson *et al*. 1986, Urhausen *et al*. 1989, Thompson *et al*. 1992, Dickerman *et al*. 1998). While we did not find any significant differences in the E/A ratios between study groups, the four subjects using both AAS and GH tended to have lower E/A ratios, demonstrating impaired diastolic LV function. Impaired diastolic filling has also been reported in the active phase of acromegaly (Smallridge *et al*. 1979, Rossi *et al*. 1992). The subject who underwent echocardiography three times over a period of 20 months showed alterations in the E/A ratio between cycles; he had the lowest E/A ratios while using anabolic substances and the highest during the withdrawal period.

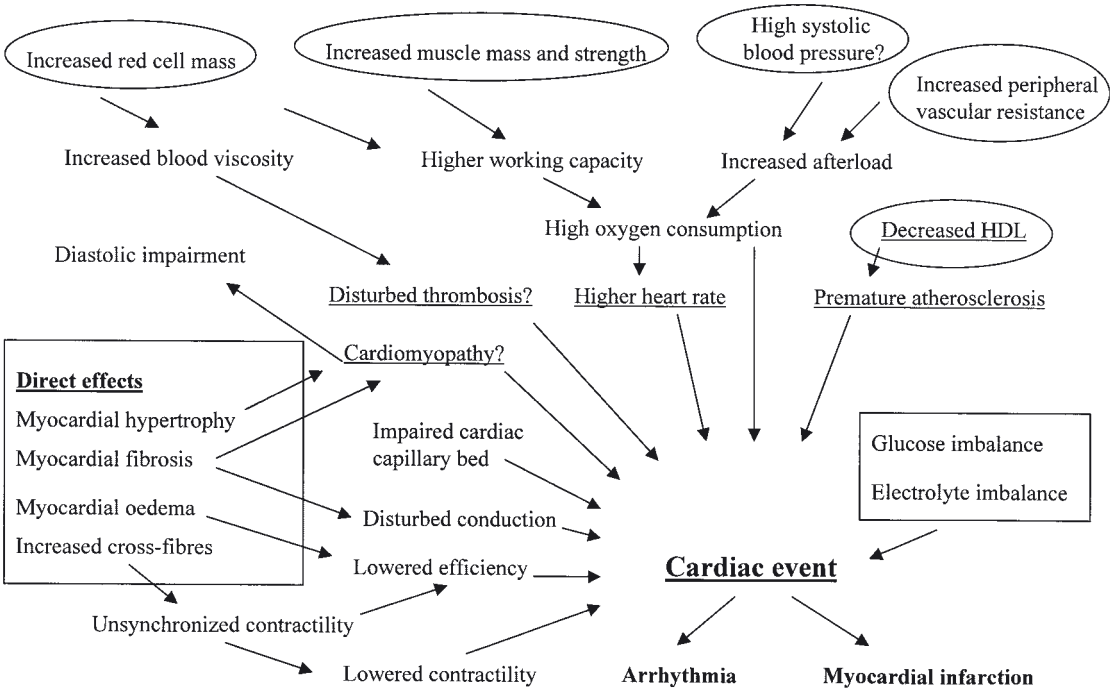
Our findings suggest that AAS and GH have very similar anabolic effects on the myocardium, and these two anabolic substances seem to potentiate the hypertrophic effect when used concomitantly. In earlier experimental studies, a similar effect of concomitant administration of androgens and GH has been demonstrated (Scow & Hagan 1965). This effect may be mediated through AAS decreasing the circulating levels of IGF-bp3, leading to increased concentrations of free GH and IGF-1 (Karila *et al*. 1998). However, Alén *et al*. (1985) found that serum GH concentrations were increased during AAS abuse without exogenous GH administration. It has also been speculated that androgen receptor concentration may depend on GH concentration (Krieg *et al*. 1978). However, these two anabolic agents clearly possess a profound deleterious direct effect on the human myocardium when concomitantly abused and may predispose the abuser to a premature cardiac event (**Figure 17**).

9.3. Effects on lipid metabolism

In addition to myocardial changes, AAS also affect metabolic systems connected with the cardiovascular system. Serum HDL levels are very low in AAS users, predisposing to premature atherosclerosis (Alén & Rahkila 1984, Webb *et al*. 1984, Glazer 1991). Several earlier reports have also indicated increased risk of arterial thrombosis during AAS use (Ferenchick *et al*. 1992, Gaede *et al.* 1992), but the specific pathomechanism remains to be elucidated (Ferenchick 1991, Nakao *et al*. 1991, Thorisdottir *et al*. 1992, Polderman *et al*. 1993). Our findings confirm that abuse of AAS results in profound alterations in serum HDL cholesterol concentrations. According to the study groups clinical experience, orally administered 17α-alkylated steroids appear to produce a greater reduction on HDL concentration than 17β-esters.

No previous studies have elucidated the effect of supraphysiological doses of AAS on the non sterol isoprenoid compounds ubiquinone and dolichol, which are by-products of the synthetic pathway of cholesterol (Olson & Rudney 1983). These isoprenoids may have an effect on the regulation of cell growth, but their exact physiological role is unclear (Siperstain 1984).

Figure 17. Proposed pathomechanisms of premature cardiac event due to abuse of anabolic androgenic steroids



Exogenous ubiquinone is administered because of its presumed preventive role in atherosclerosis and to enhance aerobic capacity in athletes. Ubiquinone acts as a lipid-soluble electron carrier in the electron transport chains of mitochondria (Olson *et al.* 1983). Its reduced form, ubiquinol, has a powerful antioxidant effect on LDL, and therefore, it is hypothesized to protect against atherosclerosis (Stocker *et al.* 1991). In this study, a significant rise in serum ubiquinone level was observed during AAS abuse, and the rise was even greater than that measured during oral administration of ubiquinone preparations (Laaksonen, personal communication). The atherogenic changes due to AAS abuse can be assumed to be partly countered by increased ubiquinone concentration. Ubiquinone is mainly carried in the circulation by LDL (Laaksonen *et al.* 1995). In earlier studies, the ubiquinone/LDL ratio has been invariable under all investigated conditions (Karlsson *et al.* 1989, 1990). In the present study, a correlation was also found between serum ubiquinone and LDL concentrations ($r=0.54$, $p=0.054$).

Dolichols are α-saturated polyisoprenoid alcohols that are synthesized in microsomes and stored in lysosomes (Rip *et al.* 1985), and the liver appears to regulate its circulatory dolichol supply (Marino *et al.* 1994). In phosphorylated form, dolichols function in the biosynthesis of glycoproteins, also modifying biological membrane fluidity, stability, permeability and fusion (Lai & Schutzbach 1984, Rip *et al.* 1985, Valtersson *et al.* 1985). In a manner dissimilar to that observed for ubiquinone, serum dolichol concentration decreased during AAS abuse. Dolichols are transported in the circulation mainly by HDL (Yasuki & Oshima 1994). In the present study, the serum dolichol/HDL ratio remained virtually unchanged regardless of AAS use. This could be due to common regulatory mechanism for HDL.

Isoprenoids have been shown to be involved in controlling cell growth (Siperstain 1984).

Further studies have revealed that isoprenylation of growth-regulating proteins produces a potential signalling factor for cellular proliferation (Santos & Nebreda 1989, Mendola & Baker 1990). Altered isoprenoid production due to AAS use may lead to interference with the cellular proliferation mechanism. Changes in isoprenoid metabolism may explain some anabolic effects of AASs or may be a launching mechanism for uncontrollable growth. The abuse of AAS has been associated with various tumor formations (Lesna & Taylor 1986). Increased isoprenoid metabolite concentration may have a role in AAS-induced promotion of tumor growth.

9.4. Effects on collagen metabolism

AAS have a quantitative and qualitative effect on connective tissue, which is made up of various collagen fibrils (Michna 1986, Laseter & Russell 1991, Inhofe *et al*. 1995). Fibrous tissue in the myocardium has been shown to be increased with AAS use (Nieminen *et al*. 1996). Further, AAS abuse has been demonstrated to weaken tendons and ligaments, exposing muscles and tendons to ruptures (Kramhøft & Solgaard 1986, Bach *et al*. 1987). Among AAS-abusing athletes, tendon and muscle insertion traumas are more frequent (Taimela & Seppälä 1994). Moreover, duration of exposure to AAS also seems to have an effect on quality of collagen fibrils (Michna 1986). However, tendons and muscle insertions are put under greater demands with increased muscle strength and consequently, decreased collagen quality may predispose these structures to ruptures. This study protocol did not examine the effect of increased load on tendon.

High doses of AAS were found to enhance collagen synthesis, especially in soft connective tissues. During the AAS cycle soft connective tissue collagen metabolism was increased. This effect tended to be dose-dependent. Degradation of type I collagen, by contrast tended to diminish; collagen type I is most abundant in bone tissue (Melkko *et al*. 1990). Urinary lysylpyridinoline (LP) mature crosslinks of collagen values remained unchanged. An increase in urinary LP values is generally considered to be a marker of collagen metabolism in bone tissue. In our study, collagen originating from bone was unaffected by AAS abuse, at least within the time interval evaluated. Concordant with this, the elevated urinary HP/LP mature crosslinks of collagen ratios during AAS abuse compared with values obtained during the withdrawal period suggest that the collagen increase originated from the degradation of soft connective tissue. Muscle mass was exceptionally high in our subjects, and because connective tissue in muscle can be a considerable source of HP (Fujimoto 1980, Palokangas *et al*. 1992, James *et al*. 1993), it was not unexpected that increased collagen metabolism result in an AAS-induced anabolic effect on muscle tissue over the time frame.

The mechanism of action of supraphysiological AAS doses on tissue metabolism remains unclear. Most of the androgen effects on bone turnover and bone mass have been suggested to occur via the estrogen receptor, with prior aromatization of androgens into estrogens (Vanderschueren *et al*. 1995). This may be the case in the present study. Alén *at al*. (1985) had measured increased estrogen concentrations in males taking AAS. AAS at high concentrations may also bind to glucocorticoreceptors, and thus, the effects of AAS on collagen may occur by reversing the effects of corticosteroids (Rogol *et al*. 1992, Haupt 1993).

Serum PICP has been used a marker for osteoporosis in clinical practice. Although AAS administration is indicated in treating osteoporosis, we were not able to demonstrate changes in serum PICP regardless of the AAS dosage. This may be due to the relatively short duration of the study. We did, however, confirm that AAS have at least a qualitative effect on collagen metabolism in soft connective tissue. These short-term changes in collagen metabolism may be due to increased anabolic effects in muscle or may be secondary effects of increased working capacity.

9.5. Effects on reproductive system

In agreement with previous studies, we found that abuse of supraphysiological doses of AAS results in transient severe oligozoospermia in males due to AAS-induced reversible hypogonadotrophic hypogonadism (Alén & Suominen 1984, Schurmeyer *et al*. 1984, Jarow & Lipshultz 1990). But contrary to these reports, our results also suggest that concomitant use of AAS and HCG cause deteriorated semen quality. This is in accordance with the recent finding of Torres-Calleja *et al*. (2001) that abuse of AAS alone not only reduces the sperm concentration but also impairs the percentage of morphologically normal semen.

All subjects were aware of the side-effects associated with substance abuse. In Finland, steroid abusers are accustomed to using one HCG injection (2500-5000 IU) a month during the cycle. Larger doses are used at the end of an AAS cycle; 10 000-20 000 IU of HCG divided into two to four separate injections, are typically used during the last two weeks of the cycle (author's unpublished data). In addition, some of our subjects used antiestrogens such as tamoxifen (10-20 mg/day) or clomifen (50-100 mg/day) to treat and prevent gynegomastia. Power athletes also use antiestrogens in the belief that they maintain gonadal function during the steroid cycle and promote faster hormonal recovery from AAS abuse. Our findings indicate that antiestrogens used during the AAS cycle do not increase serum gonadotropin concentrations

The mean duration of the AAS cycle was 4½ months and the mean daily dose of AAS was 96 mg, and only one subject became azoospermic soon after cessation of abuse. In a multicentre male contraception study conducted by WHO (1990), up to 65% of subjects became azoospermic during the six-month suppression period with a mean daily dose of 29 mg of testosterone enanthate. In our study, the mean daily dose of AAS seemed to have more effect on sperm concentration than did cycle duration. Alén & Suominen (1984) demonstrated azoospermia (6 out of 7) after three months of AAS abuse without HCG use, at a mean daily dose of 50 mg. Most of their subjects reached azoospermia at even lower AAS dosing and within a shorter period of time. One can therefore conclude that combined use of HCG and AAS can maintain endogenous spermatogenesis during AAS abuse.

In male contraception studies, the recovery of sperm concentration to baseline levels has been found to take about six months (WHO 1990). Most of our subjects also achieved normal sperm density after the six-month withdrawal period. Uppon cessation of substance abuse, one subject became azoospermic and did not recover during the six-month withdrawal. However, during the five-year follow-up the subject and his wife had conceived two healthy daughters.

Although spermatogenesis was recovered by most of the subjects during the six-month withdrawal period, serum testosterone concentrations did not reach normal levels among the heaviest AAS abusers. Alén *et al*. (1985b) reported that during a withdrawal period of 3 to 4 months subjects´ serum testosterone failed to reach normal levels even though gonadotrophin concentrations were within normal limits, indicating prolonged impairment of testicular function. This is consistent with the finding that long-term AAS abuse may produce transient testicular impairment, which can be seen in lowered serum testosterone and testosterone precursor levels with normal gonadotrophin stimulus (Ruokonen *et al*. 1985). We were able to demonstrate similar findings in subjects with a long history of AAS abuse, especially in those using massive dosages. Despite impaired steroidogenesis, these subjects had normal spermatogenesis after the withdrawal period. Further, the subjects did not suffer from any subjective symptoms that might have accounted for the testosterone deprivation.

In normal adult males with normal spermatogenesis, testosterone concentrations in the testes are 50-fold higher than in peripheral serum (Turner *et al.* 1984, Adamopoulos *et al*. 1997). One could hypothesize

that the required concentration could also be achieved by back flow of androgens from the circulation to the testes when massive doses of AAS are abused.

During transient hypogonadotrophic hypogonadism induced by abuse of AAS steroidogenesis seems to respond to HCG in a similar way as in prepubertal boys (Martikainen *et al*. 1986). Our study demonstrates that spermatogenesis can be maintained by using HCG regardless of AAS-induced suppression of gonadotrophin secretion. This finding is in line with the observation that normal spermatogenesis could be maintained with HCG after three months´ suppression of steroidogenesis with testosterone (Matsumoto *et al*. 1983). Normal sperm quality can be achieved with HCG alone in men who have hypogonadotrophic hypogonadism at postpubertal onset (Finkel *et al*. 1985). Our results suggest that HCG maintains spermatogenesis in AAS abusers with no FSH stimulus, but this regimen produces more abnormal and hypokinetic spermatozoa. This reduced semen quality may be due to lowered FSH, which has at the very least a quantitative role in human spermatogenesis (Tapanainen *et al*. 1997). However, HCG alone has also been shown to have a direct effect on spermatogenesis, resulting in poorer sperm quality (Dunkel *et al*. 1997). Other contrary reports that indicate when HCG was administered to patients with idiopathic oligo- or asthenozoospermia without hypothalamohypopituitary-hypogonadism 47% showed improvement in semen quality, mainly in motility and morphology (Homonnai et al. 1978). Sperm quality impairment in the present study cannot be explained by altered excretion of gonadotrophins, leaving concomitant abuse of AAS and HCG as a viable explanation.

9.6. Clinical implications

The use of performance-enhancing substances is associated with a higher risk for cardiac events and sudden death, which can be partly explained by pathological myocardial hypertrophy and subsequent increased QT dispersion.

AAS abuse induces a marked increment to the LDL/ HDL ratio, which is considered to be a marker of increased risk for atherogenic cardiovascular disease. However, atherogenic changes may in part be counteracted by the increased ubiquinone to dolichol ratio induced by AAS.

AAS use may expose abusers to soft tissue traumas due to altered collagen metabolism in those tissues. AAS abuse may induce direct effects on collagen metabolism, and also influence tendons and tendon insertions indirectly due to the increased working capacity of muscles.

AAS-impaired spermatogenesis is reversible, but may involve a lengthy recovery especially if long-acting AAS are used.

Although subjects were thoroughly counseled about the risks associated with substance abuse, all continued the abuse after the six-month withdrawal period. Even a subject who sustained an episode of ventricular tachycardia, which was treated with electronic cardioversion, continued a training regimen augmented with various anabolic substances. Contradictory evidence exists about the effectiveness of counseling against the substance abuse (Goldberg *et al*. 1996, O'Sullivan *et al*. 2000, Nilsson *et al*. 2001b). It was obviously that the lack of convincing evidence of adverse effects associated with abuse that was the major reason for the ineffectiveness of counseling with this selected group pf AAS abusers. Hence, more studies are needed to convince abusers to avoid the long-term adverse effects of abuse of anabolic substances.

10. CONCLUSIONS

Cardiovascular, metabolic and male fertility aspects associated with anabolic substance abuse were investigated. The effects of abuse, particularly of massive doses of anabolic androgenic steroids with or without concomitant abuse of the most common subsidiaries, were also evaluated. Taken together, the studies confirm that the abuse of anabolic substances produces profound, partly irreversible changes in various organs and systems, and that these changes tend to be dose-related.

The main conclusions are as follows:

1. Abuse of anabolic androgenic steroids induces dose-related left ventricular hypertrophy, and the concomitant abuse of growth hormone is associated with concentric remodeling of the left ventricle. The morphological changes resemble those of endurance athletes but show pathological features. Other factors, such as endocrinological or genetic components, may have a stronger role in the morphological adaptation of the myocardium to exercise than type of training.

2. Abuse of anabolic androgenic steroids increases QT dispersion measured by a 12-lead electrocardiogram. The left ventricular mass as such is not a determinant of the QT variables, but the morphological and histological qualities of the myocardium impact on QT variables and alter repolarization of the myocardium.

3. In line with previous reports, the abuse of anabolic androgenic steroids was found to induce profound changes in serum lipid concentrations. Serum high-density lipoprotein concentration during abuse at the highest doses was under the detection limit of the laboratory method used. The influence on cholesterol metabolism seems to be related to by-products of the mevalonate pathway. Serum concentration of ubiquinone is significantly increased without influencing serum low-density lipoprotein concentration.

4. Supraphysiological doses of anabolic androgenic steroids enhance collagen synthesis, especially in soft connective tissues, probably due to increased anabolic action in muscle tissue.

5. Concomitant abuse of supraphysiological doses of anabolic androgenic steroids with human chorionic gonadotrophin results in profound alteration of semen density. Anabolic androgenic steroids induce hypogonadotrophic hypogonadism, and combined with human chorionic gonadotrophin can maintain spermatogenesis but reduce semen quality. The abuse of anabolic substances results in transiently lowered male fertility, but the reduced semen density and semen quality appears to be reversible.

The subjects were followed for approximately one year, and despite receiving abundant information concerning their health status, none discontinued doping substance abuse after the study. This highlights the need for effective educational programme to discontinue the abuse. Because the more commonly abused anabolic substances increase the risk for cardiac event and lower male fertility, the abuse of such substances should be considered a public health problem.

11. ACKNOWLEDGEMENTS:

This work was carried out between 1992 and 2003 at the Drug Research Unit, Department of Mental Health and Alcohol Research, National Public Health Institute, in Helsinki. I am indebted to Professor Jussi Huttunen, the Director General of the Institute, and Professor Jouko Lönnqvist, the Head of the Department of Mental Health and Alcohol Research, for providing excellent research facilities.

This series of studies was carried out in a close collaboration with and with the support of the following institutes: Central Military Hospital, Division of Cardiology and Research Institute of Military Medicine; United Laboratories Ltd., Doping Control Section, Helsinki; Institute of Biomedicine, Pharmacology, and Unit for Sports and Exercise Medicine, University of Helsinki; Departments of Orthopaedics and Traumatology, and Internal Medicine, Helsinki University Central Hospital; Department of Clinical Pharmacology and Research Unit of Alcohol Diseases, University of Helsinki; Department of Health Sciences, University of Jyväskylä; Karolinska Institute, Department of Obstetrics and Gynaecology, Huddinge University Hospital, Sweden; Semen Laboratory at the Infertility Clinic of the Family Federation of Finland; ORTON Orthopaedic Hospital, Invalid Foundation, Helsinki.

My supervisor docent Timo Seppälä, to whom I owe deepest gratitude, suggested the topic of this project to me. His continuous patience and enthusiastic encouragement made it possible for me to conquer through this extremely exciting era of my life. I have been privileged to be the recipient of his sound advice, inspiring ideas and witty humour during the numerous meetings in his smoky office. Thank you Timo; you have given me the time and space to proceed in this study in my own way.

Special thanks are due to all of the subjects who so willingly participated in this study. I am honoured to get to know all of you, and flattered by your trust.

I thank Professor Heikki Vapaatalo, my supervisor, from the Institute of Biomedicine, University of Helsinki, for his warm and encouraging way of pushing me to complete this project. You do possess a magnificent everlasting engine to motivate people to reach their goals.

My endless gratitude is due to my fellow investigators, Jouko Karjalainen, MD, and Matti Mäntysaari, MD of the Central Military Hospital, for providing me with financial support as well as for sharing your vast knowledge on the physiology and morphology of the human heart.

I am also indebted to my other collaborator; professor Outi Hovatta, Mia Pärssinen MD, Vuokko Kovanen PhD, Reijo Laaksonen MD, Antti Leinonen MSc, Anu Stolt MD and docent Pekka Rauhala, for co-operation, friendship and support.

My sincere appreciation is due to the entire staff at the Drug Research Unit, National Public Health Institute, for helping me in so many ways, and remembering me warmly way during my athletic career. Special thanks belong to Esa Meririnne MD, Mr. Kari Ariniemi, Aino Kankanpää PhD, Mrs. Ulla Lindholm and Mrs. Sisko Loponen for generously assisting me over the years.

I owe a large dept of gratitude to docent Markku Kupari and docent Simo Taimela, the official reviewers appointed by the Faculty of Medicine, for their guidance, constructive criticism and in valuable comments. Further, I am extremely thankful to Carol Ann Pelli HonBSc for linguistic editing of the manuscript.

My sincere gratitude is due to docent Risto Tuominen for his generous help and for revealing the secret world of of statistics to me.

My heartfelt gratitude goes to my family for their unconditional support. Thank you Mother, Tiina, Ronja, Father, Sirpa and Reuwen.

Finally, I owe my deepest gratitude to my wonderful wife Heidi for her never-failing love and patience over these years. Her own courage and encouragement has made it possible for me to complete this challenging effort.

Financial support from the Finnish Antidoping Committee, Helsinki, and the Ensio Hyvärinen Foundation is gratefully acknowledged.

12. REFERENCES

Aberg F, Zhang Y, Appelkvist E, et al. Effects of clofibrate, phthalates and probucol on ubiquinone levels. Chem Biol Interact 1994; 91: 1-14.

Adamopoulos D, Karamertzanis M, Nicopoulou S, et al. The combination of testosterone undecanoate with tamoxifen citrate enhances the effects of each agent given independently on seminal parameters in men with idiopathic oligozoospermia. Fertil Steril 1997; 67: 756-762.

Adamopoulos D, Lawrence DM, Vassilopoulos P, et al. Hormone levels in the reproductive system of normospermic men and patients with oligospermia and varicocele. J Clin Endocrinol Metab 1984; 59: 447-452.

Ajayi AA, Mathur R, Halushka PV. Testosterone increases human platelet thromboxane A2 reseptor density and aggregation responses. Circulation 1995; 91: 2742-2747.

Alén M. Androgenic steroids effects on liver and red cells. Brit J Sports Med 1985; 19: 15-20.

Alén M, Häkkinen K, Komi PV. Lihaksiston voimantuottokyvyn muutokset androgeenisia steroideja käyttäneillä voimailijoilla. [Changes in muscle power production capacity in power athletes self-administering androgenic anabolic steroids]. Duodecim 1984; 100: 1096-1104.

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

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

Alén M, Rahkila P, Reinilä M, et al. Androgenic-anabolic steroid effects on serum thyroid, pituitary and steroid hormones in athletes. Am J Sports Med 1987; 15: 357-361.

Alén M, Reinilä M, Vihko R. Response of serum hormones to androgen administration in power athletes. Med Sci Sports Exerc 1985; 17: 354-359. (1985b)

Alén M, Suominen J. Effect of androgenic and anabolic steroids on spermatogenesis in power athletes. Int J Sports Med 1984; 5(Supplement): 189-192.

American Medical Association. Medical and nonmedical uses of anabolic steroids. Council on scientific affairs. JAMA 1990; 264: 2923-2927.

Anastasiou-Nana MI, Nanas JN, Karagounis LA, et al. Relation of dispersion of QRS and QT in patients with advanced congestive heart failure to cardiac and sudden death mortality. Am J Cardiol 2000; 85: 1212-1217.

Anthony P. Hepatoma associated with androgenic steroids. Lancet 1975; 1: 685-686.

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

Bach BR, Warren RF, Wickiewicz TL. Triceps rupture: a case report and literature review. Am J Sports Med 1987; 15: 285-289.

Bahrke MS, Yesalis CE. Weight training. A potential confounding factor in examining the psychological and behavioural effects of anabolic-androgenic steroids. Sports Med 1994; 18: 309-318.

Bahrke MS, Yesalis CE, Wright JE. Psychological and behavioural effects of endogenous testosterone levels and anabolic-androgenic steroids among males. Sports Med 1990; 10: 303-337.

Bahrke MS, Yesalis CE, Wright JE. Psychological and behavioural effects of endogenous testosterone and anabolic-androgenic steroids. An update. Sports Med 1996; 22: 367-390.

Barbosa J, Seal US, Doe RP. Effects of anabolic steroids on hormone-binding proteins, serum cortisol and serum non-protein-bound cortisol. J Clin Endocrinol Metab 1971; 32: 232-240.

Basaria S, Wahlstrom JT, Dobs AS. Clinical review 138: Anabolic-androgenic steroid therapy in the treatment of chronic diseases. J Clin Endocrinol Metab 2001; 86: 5108-5117.

Bausserman LL, Saritelli AL, Herbert PN. Effects of short-term stanozlol administration on serum lipoproteins in hepatic lipase deficiency. Metabolism 1997; 46: 992-996.

Bhasin S, Storer T W, Berman N, et al. The effects of supraphysiological doses of testosterone on muscle size and strenght in normal men. N Engl J Med 1996; 335: 1-7.

Biosynthetic growth hormone. Med Lett Drugs Ther 1985; 27: 101-104.

Black D, Duncan A, Robins SP. Quantitative analysis of the pyridinium crosslinks of collagen in urine using ion-paired reverse-phase high-performance liquid chromatography. Anal Biochem 1988; 167: 197-203.

Brower KJ, Blow FC, Young JP, et al.Symptoms and correlates of anabolic-androgenic steroid dependence. Br J Addict 1991; 86: 759-768.

Brower KJ. Anabolic steroids. Psychiatr Clin North Am 1993; 16: 97-103.

Browne KF, Zipes DP, Heger JJ, et al. Influence of the autonomic nervous system on the QT interval in man. Am J Cardiol 1982; 50: 1099-1103.

Brown-Sequard E. The effects produced on man by subcutaneus injections of liquid obtained from the testicles of animals. Lancet 1889; 2: 105-107.

Buckley WE, Yesalis CE, Frield, et al. Estimated prevalence of anabolic steroid use among male high school seniors. JAMA 1988; 260: 3441-3445.

Burger RA, Marcuse PM. Peliosis hepatis. Report of a case. Am J Clin Pathol 1952; 22: 569-573.

Caidahl K, Edén S, Bengtsson BÅ. Cardiovascular and renal effects of growth hormone. Clin Endocrinol 1994; 40: 393-400.

Chen CJ, Yu MW, Liaw YF. Epidemiological characteristics and risk factors of hepatocellular carcinoma. Eur J Gastroenterol Hepatol 1997; 12: 294-308.

Cheng S, Kovanen V, Heikkinen E, et al. Serum and urine markers of type I collagen metabolism in elderly women with high and low bone mineral density. Eur J Clin Invest 1996; 26: 186-191.

Cocks J. Methyltestosterone-induced liver-cell tumors. Med J Australia 1981; 2: 617-619.

Cohen JC, Hickman R. Insulin resistance and diminished glucose tolerance in powerlifters ingesting anabolic steroids. J Clin Endocrinol Metab 1987; 64: 960-963.

Cohen LI, Hartford CG, Rogers GG. Lipoprotein (a) and cholesterol in body builders using anabolic androgenic steroids. Med Sci Sports Exerc 1996; 28: 176-179.

Craig J, Peters R, Edmondson H. Tumor of the liver and intrahepatic bile ducts. In Atlas of tumor pathology, second series. Armed Forces Institute of Pathology, Washington DC. 1989. 36-41.

Cusan L, Pelletier G, Bélanger A, et al. Inhibition of spermatogenesis and steroidogenesis during long-term treatment with hCG in the rat. J Androl 1982; 3: 124.

Danhaive PA, Rousseau GG. Binding of glucocorticoid antagonist to androgen and glucocorticoid hormone receptors in rat skeletal muscle. J Steroid Biochem 1986; 24: 481-487.

Danhaive PA, Rousseau GG. Evidence for sex-dependent anabolic response to androgenic steroids mediated by muscle glucocorticoid receptors in the rat. J Steroid Biochem 1988; 29: 575-581.

de Kretser DM, Loveland KL, Meinhardt A, et al. Spermatogenesis. Hum Reprod 1998; 13: 1-8.

de Kretser DM, Meinhardt A, Meehan T, et al. The roles of inhibin and related peptides in gonadal function. Mol Cell Endocrinol 2000; 30: 43-46.

Deligiannis AP, Mandroukas K. Noninvasive cardiac evaluation of weightlifters using anabolic steroids. Scand J Med Sci Sports 1992: 3: 37-40.

De Piccoli B, Giada F, Benettin A, et al. Anabolic steroid use in body builders: an echocardiographic study of left ventricular morphology and function. Int J Sports Med 1991; 12: 408-412.

Devereux RB. Detection of left ventricular hypertrophy by M-mode echocardiography. Hypertension 1987; 9(suppl II): 19-26.

Dickerman RD, McConathy WJ, Schaller F, et al. Echocardiography in fraternal twin bodybuilders with one abusing anabolic steroids. Cardiology 1997; 88: 50-51. (1997a)

Dickerman RD, McConathy WJ, Zachariah NY. Testosterone, sex hormone-binding globulin, lipoproteins, and vascular disease risk. J Cardiovasc Risk 1997; 4: 363-366. (1997b)

Dickerman RD, Pertusi RM, Zachariah NY, et al. Anabolic steroid-induced hepatotoxicity: Is it overstated? Clin J Sports Med 1999; 9: 34-39.

Dickerman RD, Schaller F, McConathy WJ. Left ventricular wall thickening does occur in elite power athletes with or without anabolic steroid use. Cardiology 1998; 90: 145-148.

Dickerman RD, Schaller F, Zachariah NY, et al. Left ventricular size and function in elite bodybuilders using anabolic steroids. Clin J Sport Med 1997; 7: 90-93. (1997c)

Donike M, Geyer H, Gotzmann A, et al. Dope Analysis. In Official Proceedings of International Athletic Foundation World Symposium on Doping in Sport – 1987. Bellotti P, Benzi G, Ljungqist A, eds. The International Athletic Foundation, London. 1988; 53-80.

Drew E. Androgen related primary hepatic carcinoma in a patient on long term methyltestosterone therapy. J Abdom Surg 1984; 26: 103-106.

Duchaine D. Underground Steroid Handbook II. 1989.

Dunkel L, Taskinen S, Hovatta O, et al. Germ cell apoptosis after treatment of cryptorchidism with human chorionic gonadotropin is associated with impaired reproductive function in the adult. J Clin Invest 1997; 100: 2341-2346.

Ekert H, Muntz RH, Colebatch JH. Decreased anticoaculant tolerance with oxymethelone. Lancet 1971; 2: 609-610.

Eyre DR, Koob TJ, Van Ness KP. Quantitation of hydroxypyridinium crosslinks in collagen by high-performance liquid cromatography. Anal Biochem 1984; 137: 380-388.

Falangia V, Greenberg AS, Zhou L, et al. Stimulation of collagen synthesis by the anabolic steroid stanozolol. J Invest Dermatol 1998; 111: 1193-1197.

Fazio S, Cittadini A, Sabatini D, et al. Growth hormone and heart performance. A novel mechanism of cardiac wall stress regulation in humans. Eur Heart J 1997; 18: 181-184.

Fazio S, Sabatini D, Capaldo B, et al. A preliminary study of growth hormone in the treatment of dilated cardiomyopathy. N Engl J Med 1996; 334: 809-814.

Ferenchick GS. Are androgenic steroids thrombogenic? (Letter). N Eng J Med 1990; 322: 476.

Ferenchick GS. Anabolic-androgenic steroids and thrombosis: is there a connection? Med hypothesis 1991; 35: 27-31.

Ferenchick GS, Adelman S. Myocardial infarction associated with anabolic steroid use in a previously healthy 37-year-old weight lifter. Am Heart J 1992; 124: 507-508.

Ferenchick G, Schwartz D, Ball M, et al. Androgenic-anabolic steroid abuse and platelet aggregation: A pilot study in weight lifters. Am J Med Sci 1992; 303: 78-82.

Ferrera PC, Putnam DL, Verdile VP. Anabolic steroid use as the possible precipitant of dilated cardiomyopathy. Cardiology 1997; 88: 218-220.

Fifer MA, Borow KM, Colan SD, et. al. Early diastolic left ventricular function in children and adults with aortic stenosis. J Am Coll Cardiol 1985; 4: 1147-1155.

Fineschi V, Baroldi G, Monciotti F, et al. Anabolic steroid abuse and cardiac sudden death - A pathologic study. Arch Pathol Lab Med 2001; 125: 253-255.

Finkel DM, Phillips JL, Snyder PJ. Stimulation of spermatogenesis by gonadotropins in men with hypogonadotropic hypogonadism. N Engl J Med 1985; 313: 651-655.

Finkelhor R, Hanak L, Bahler R. Left ventricular filling in endurance-trained subjects. J Am Coll Cardiol 1986; 8: 289-293.

Finley PR, Schifman RB, Williams RJ, et al. Cholesterol in high density lipoprotein: use of Mg^{2+}/ dextran sulphate in its enzymatic measurement. Clin Chem 1978; 24: 931-933.

Finnish Antidoping Committee. www.liite.com 2002.

Finnish Customs statistics. Suomen tullilaitos. www.tulli.fi 2002.

Fotherby K, James F. Metabolism of synthetic steroids. Adv Steroid Biochem Pharmacol 1972; 3: 67-165.

Fouad FM, Slominski JM, Tarazi RC. Left ventricular diastolic fuction in hypertension: relation to left ventricular mass and systolic function. J Am Coll Cardiol 1984; 3: 1500-1503.

Francis RM, Peacock M, Aaron JE, et al. Osteoporosis in hypogonadal men: Role of decreased plasma 1,25-dihydroxyvitamin D, calcium malabsorption, and low bone formation. Bone 1986; 7: 261-268.

Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.

Fryburg DA. Insulin-like growth factor I exerts growth hormone- and insulin-like actions on human protein metabolism. Am J Physiol 1994; 267: E331-E336.

Fujimoto D. Evidence for natural existence of pyridinoline crosslink in collagen. Biochem Biophys Res Comm 1980; 93: 948-953.

Gaede JT, Montine TJ. Massive pulmonary embolus and anabolic steroid abuse. JAMA 1992; 267: 2328-2329.

Gagliardi C. in Current Obstetric & Gynecologic Diagnosis & Treatment. Pernoll ML eds, Appleton & Lange, East Nordwalk. 1991; 1046.

Genth-Zotz S, Zotz R, Geil S, et al. Recombinant growth hormone therapy in patients with ischemic cardiomyopathy. Circulation 1999; 99: 18-21.

Giorgi A, Weatherby RP, Murphy PW. Muscular strength, body composition and health responses to the use of testosterone enanthate: a double blind study. J Sci Med Sport 1999; 2: 341-355.

Giustina A, Boni E, Romanelli G, et al. Cardiopulmonary performance during exercise in acromegaly, and the effects of acute suppression of growth hormone hypersecretion with octretide. Am J Cardiol 1995; 75: 1042-1047.

Glazer G. Atherogenic effect of anabolic steroids on serum lipid levels. A literature review. Arch Intern Med 1991; 151: 1925-1933.

Goldberg L, Bents R, Bosworth E, et al. Anabolic steroid education and adolescents: do scare tactics work? Pediatrics 1991; 87: 283-286.

Goldberg L, Bosworth EE, Bents RT, et al. Effect of an anabolic steroid education program on knowledge and attitudes of high school football players. J Adolesc Health Care 1990; 11: 210-214.

Goldberg L, Elliot D, Clarke GN, et al. Effects of a multidimensional anabolic steroid prevention intervention. The Adolescents Training and Learning to Avoid Steroids (ATLAS) Program. JAMA 1996; 276: 1555-1562.

Green GA, Uryasz FD, Petr TA, et al. NCAA study of substance use and abuse habits of college student-athletes. Clin J Sports Med 2001; 11: 51-56.

Haffner SM, Kushwaha RS, Foster DM. Studies on the metabolic mechanism of redused high-density lipoproteins during anabolic-steroid therapy. Metabolism 1983; 32: 413-420.

Handelsman DJ, Grupta L. Prevalence and risk factors for anabolic-androgenic steroid abuse in Australian high school students. Int J Androl. 1997; 20: 159-164.

Hartgens F, Van Marken Lichtenbelt WD, Ebbing S, et al. Body composition and anthropometry in bodybuilders: regional changes due to nandrolone decanoate administration. Int J Sports Med 2001; 22: 235-241.

Hassager C, Jensen LT, Pødenphant J, et al. The carboxy-terminal pyridinoline cross-linked telopeptide of type I collagen in serum as a marker of bone resorption: the effect of nandrolone decanoate and hormone replacement therapy. Calcif Tissue Int 1994; 54: 30-33.

Haupt HA. Anabolic steroids and growth hormone. Am J Sports Med 1993; 21: 468-474.

Haupt HA, Rovere GD. Anabolic steroids: A review of the literature. Am J Sports Med 1984; 12: 469-484.

Heikkilä R, Aho K, Heliövaara M, et al. Serum testosterone and sex hormone-binding globulin concentrations and the risk of prostate cancer. Cancer 1999; 86: 312-315.

Henriksen E, Landelius J, Wessle'n L, et al. Echocardiographic right and left ventricular measurements in male elite endurance athletes. Eur Heart J 1996; 17: 1121-1128.

Hickson RC, Czerwinski SM, Falduto MT, et al. Glucocorticoid antagonism by exercise and androgenic-anabolic steroids. Med Sci Sports Exerc 1990; 22: 331-340.

Higham PD, Campbell RWF. QT dispersion. Br Heart J 1994; 71: 508-510.

Hjalmarson AC, Rannels DE, Kao R, et al. Effects of hypophysectomy, growth hormone, and thyroxine o protein turnover in hear. J Biol Chem 1975; 250: 4556-4561.

Hobbs CJ, Plymate SR, Rosen CJ, et al. Testosterone administration increases insulin-like growth factor-I levels in normal men. J Clin Endoc Metab 1993; 77: 776-779.

Homonnai ZT, Peled M, Paz GF. Changes in semen quality and fertility in response to endocrine treatment of subfertile men. Gynecol Obstet Invest 1978; 9: 244-255.

Huhtaniemi I, Koskimies A, Pelkonen R. Kivekset. in Kliininen Endokrinologia. Lamberg B-A, Koivisto V, Pelkonen R eds. Duodecim, Jyväskylä. 1992; 458-478.

Huie MJ. An acute myocardial infarction occuring in an anabolic steroid user. Med Sci Sports Exer. 1994; 26: 408-413.

Ingerowski G, Scheutwinkel-Reich M, Stan H. Mutagenicity studies on veterinary anabolic drugs with the salmonella/ microsome test. Mutat Res 1981; 91: 93-98.

Inhofe PD, Grana WA, Egle D, et al. The effects of anabolic steroids on rat tendon. An ultrastructural, biomechanical, and biochemical analysis. Am J Sports Med 1995; 23: 227-232.

Ishak KG, Zimmerman HJ. Hepatotoxic effects of the anabolic/androgenic steroids. Semin Liver Dis 1987; 7: 230-236.

James IT, Walne AJ, Perrett D. The measurement of pyridinium crosslinks: a methodological overview. Ann Clin Biochem 1993; 33: 397-420.

Jarow JP, Lipshultz LI. Anabolic steroid-induced hypogonadotropic hypogonadism. Am J Sports Med 1990; 18: 429-431.

Jänne OA, Palvimo J, Kallio P, et al. Androgen receptor and mechanism of androgen action. Ann Med 1993; 25: 83-89.

Johansen K, Henning T, Karlsson J, et al. Coenzyme Q10, alpha-tocopherol and free cholesterol in HDL and LDL fractions. Annals Med 1991; 23: 649-656.

Jokelainen K, Salmela K, Humaloja K, et al. Blood dolichol in lysosomal diseases. Biochem Cell Biology 1992; 70: 481-485.

Kahaly G, Olshausen KV, Mohr-Kahaly S, et al. Arrhythmia profile in acromegaly. Eur Heart J 1992; 13: 51-56.

Karhunen MK, Rämö MP, Kettunen R. Anabolic steroids and the hemodynamic effects of endurance training and deconditioning in rats. Acta Physiol Scand 1988; 133: 297-306.

Karila TAM, Koistinen H, Seppälä M, et al. Growth hormone induced increase in serum IGFBP-3 level is reversed by anabolic steroid in substance abusing power athletes. Clin Endocrinol 1998; 49: 459-463.

Karjalainen J, Kujala UM, Stolt A, et al. Angiotensinogen gene M235T polymorphism predicts left ventricular hypertrophy in endurance athletes. J Am Coll Cardiol 1999; 34: 494-499.

Karjalainen J, Mäntysaari M, Viitasalo M, et al. Left ventricular mass, geometry, and filling in endurance athletes: association with exercise blood pressure. J Appl Physiol 1997; 82: 531-537. (1997a)

Karjalainen J, Reunanen A, Ristola P, et al. QT interval as a cardiac risk factor in a middle aged population. Heart 1997; 77: 543-548. (1997b)

Karjalainen J, Viitasalo M, Mänttäri M, et al. Relation between QT intervals and heart rates from 40 to 120 beats/min in rest electrocardiograms of men a simple method to adjust QT interval value. J Am Coll Cardiol 1994; 23: 1547-1553.

Karlsson J. Heart and muscle ubiquinone or CoQ10 as a protective agent against radical formation in man. Adv Myochem 1987; 1: 305-318.

Karlsson J, Diamant B, Folkers K. Skeletal muscle characteristics of significance for metabolism in health and disease. Adv Myochem 1989; 2: 283-291.

Karlsson J, Diamant B, Folkers K, et al. Plasma ubiquinone and cholesterol contents with and without ubiquinone treatment. In Highlights in ubiquinone research. Lenaz G, Barnabe O, Rabbi A, Battino M eds. Taylor and Francis, London. 1990; 296-302.

Kennedy MC, Lawrence C. Anabolic steroid abuse and cardiac death. Med J Aust 1993; 158: 346-348.

Kilshaw BH, Harkness RA, Hobson BM, et al. The effects of large doses of the anabolic steroids, methandrostenolone, on an athletes. Clin Endocrinol 1975; 4: 537-541.

Kindlundh AMS, Isacson DGL, Berglund L, et al. Factors associated with adolecent use of doping agents: anabolic-androgenic steroids. Addiction 1999; 94: 543-553.

Klein I, Ojamaa K. Cardiovascular manifestations of endocrine disease. J Clin Endocrinol Metab 1992; 75: 339-342.

Knuth UA, Nieschlag E. Endocrine approaches to male fertility control. Baillieres Clin Endocrinol Metab 1987; 1: 113-131.

Kochakian CD, Yesalis CE. Anabolic-androgenic steroids: a historical perspective and definition. in Anabolic steroids in sports and excercise Eds Yesalis CE 2nd eds. 2000 USA Human Kinetics

Korkia P, Stimson GV. Indications of prevalence, practice and effects of anabolic seroid use in Great Britain. Int J Sports Med 1997; 18: 557-562.

Kramhøft M, Solgaard S. Spontaneous rupture of the extensor pollicis longus tendon after anabolic steroids. J Hand Surg (British) 1986; 11: 87.

Krieg M, Smith K, Bartsch W. Demonstration of a specific androgen receptor in rat heart muscle: relationship between binding, metabolism, and tissue levels of androgens. Endocrinology 1978; 103: 1686-1694.

Laaksonen R, Riihimäki A, Laitila J, et al. Serum and muscle tissue ubiquinone levels in healthy subjects. J Clin Lab Med 1995; 25: 517-521.

Lai C, Schutzbach J. Dolichol induces membrane leakage of liposomes composed of phosphatidylethanolamine and phosphatidylcholine. FEBS Lett 1984; 169: 279-282.

Laseter JT, Russell JA. Anabolic steroid - induced tendon pathology: a review of the literature. Med Sci Sports Exerc 1991; 23: 1-3.

Lesna M, Taylor W. Liver lesion in BALB/C mice induced by an anabolic androgen (Decaduranabolin), with and without pretreatment with diethylnitrosamine. J Steroid Biochem 1986; 24: 449-453.

Lewis J, Spirito P, Pelliccia A, et al. Usefulness of doppler echocardiographic assesment of diastolic filling in distiguishing "athletes heart" from hypertrophic cardiomyopathy. Br Heart J 1992; 68: 296-300.

Lie JT. Pathology of the heart in acromegaly: anatomic findings in 27 autopsied patients. Am Heart J 1980; 100: 41-52.

Lim M, Barkan AL, Buda AJ. Rapid reduction of left ventricular hypertrophy in acromegaly after suppression of growth hormone hypersecretion. Ann Int Med 1992; 117: 719-726.

Lip GYH, Felmeden DC, Li-Saw-Hee FL, et al. Hypertensive heart disease. A complex syndrome or a hypertensive cardiomyopathy? Eur Heart J 2000; 21: 1653-1665.

Longcope C. Adrenal and gonadal androgen secretion in normal females. Clin Endocrinol Metab 1986; 15: 213-228.

Lukas SE. Current perspectives on anabolic-androgenic steroids abuse. Trends Pharmacol Sci 1993; 14: 61-68.

Lyngberg KK. Myocardial infarction and death of a body builder after using anabolic steroids. Ugeskrift for Laeger 1991; 153: 587-588.

Macintyre JG. Growth hormone in athletes. Sports Med 1987; 4: 129-142.

Marino M, Bruscalupi G, Manzi P, et al. Changes in plasma dolichol levels, transport, and hepatic delivery during rat liver regeneration. Metabolism 1994; 43: 677-680.

Maron BJ. Structural features of the athlete heart as defined by echocardiography. J Am Coll Cardiol 1986; 7: 190-203.

Maron BJ, Pelliccia A, Spirito P. Cardiac disease in young trained athletes. Insight into methods for distinguishing athlete's heart from structural heart disease, with particular emphasis on hypertrophic cardiomyopathy. Circulation 1995; 9: 1596-1601.

Maron BJ, Shirani J, Poliac LC, et al. Sudden death in young competitive athletes, clinical, demographic and pathological profiles. JAMA 1996; 276: 199-204.

Marsh JD, Lehmann MH, Ritchie RH, et al. Androgen reseptors mediate hypertrophy in cardiac myocytes. Circulation 1998; 98: 256-261.

Martikainen H, Alén M, Rahkila P, et al. Testicular responsiveness to human chorionic gonadotrophin during transient hypogonadotrophic hypogonadism induced by androgenic/anabolic steroids in power athletes. J Steroid Biochem 1986; 25: 109-112.

Mathews LS, Enberg B, Norstedt G. Regulation of rat growth hormone receptor gene expression. J Biol Chem 1989; 17: 9905-9910.

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

Matsumoto AM, Paulsen CA, Hopper BR, et al. Human chorionic gonadotropin and testicular function: Stimulation of testosterone, testosterone precursors and sperm production despite high estradiol levels. J Clin Endocrinol Metab 1983; 56: 720-728.

Mauras N, Hayes V, Welch S, et al. Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength, and adiposity. J Clin Endocrinol Metab 1998; 83: 1886-1892.

Mayet J, Shahi M, McGrath K, et al. Left ventricular hypertrophy and QT dispersion in hypertension. Hypertension 1996; 28: 791-796.

Mänttäri M, Oikarinen L, Manninen V, et al. QT dispersion as a risk factor for sudden cardiac death and fatal myocardial infarction in a coronary risk population. Heart 1997; 78: 268-272.

Mäntysaari M, Karila T, Seppälä T. Cardiovascular findings in power athletes abusing anabolic androgenic steroids. Rev Int Serv Sante Forces Armees 2002; 75: 12-15.

McCarty MF. Suppression of dolichol synthesis with isoprenoids and statins may potentiate the cancer-retardant efficacy of IGF-I down-regulation. Med Hypoth 2001; 56: 12-16.

McCoughan G, Bilous M, Gallangher N. Long-term survival with tumor regression in androgen-induced liver tumors. Cancer 1985; 56: 2622-2626.

McKillop G, Ballantyne D. Lipoprotein analysis in bodybuilders. Int J Cardiol 1987; 17: 281-288.

McNutt RA, Ferenchick GS, Kirlin PC, et al. Acute myocardial infarction in a 22 year old world class weight lifter using anabolic steroids. Am J Cardiol 1988; 62: 164.

Melchert RB, Herron TJ, Welder AA. The effect of anabolic-androgenic steroids on primary myocardial cell cultures. Med Sci Sports Exerc 1992; 24: 266-212.

Melchert RB, Welder AA. Cardiovascular effects of androgenic-anabolic steroids. Med Sci Sports Exerc 1995; 27: 1252-1262.

Melkko J, Niemi S, Risteli J. Radioimmunoassay for the carboxyterminal propeptide of human type I procollagen (PICP). Clin Chem 1990; 36: 1328-1332.

Mendola C, Baker J. Lovastatin blocks N-ras oncogene-induced neuronal differentation. Cell Growth Differ 1990; 1: 499-502.

Mewis C, Spyridopoulos I, Kuhlkamp V, et al. Manisfestation of severe coronary heart disease after anabolic drug abuse. Clin Cardiol 1996; 19: 153-155.

Michna H. Organisation of collagen fibrils in tendon: changes induced by an anabolic steroid. Virchows Arch 1986; 52: 87-98.

Miller GJ, Miller NE. Plasma high density lipoprotein concentration and development of ischaemic heart disease. Lancet 1975; 1:16-20.

Mjos O, Thelle D, Forde O, et al. Family study of high density lipoprotein cholesterol. Relation to age and sex. The Tromso heart study. Acta Med Scand 1977; 201: 323-329.

Mohr D, Bowry VW, Stocker R. Dietary supplementation with coenzyme Q10 results in increased levels of ubiquinol-10 within circulating lipoproteins and increased resistance of human low-density lipoprotein to the initation of lipid peroxidation. Biochim Biophys Acta 1992; 1126: 247-254.

Morrison JA, Sprecher DL, Biro FM, et al. Sex hormones and lipoproteins in adolescent male offspring of parents with premature coronary heart disease and a control group. J Pediatr 1998; 133: 526-532.

Mowbray J, Davies JA, Bates DJ, et al. Growth hormone, cyclic nucleotides and the rapid control of translation in the heart muscle. Biochem J 1975; 152: 583-592.

Nakao J, Chang WC, Murota SI, et al. Testosterone inhibits prostacyclin production by rat aortic smooth muscle cells in culture. Atherosclerosis 1991; 39: 203-209.

Nass R, Huber RM, Klauss V, et al. Effect of growth hormone (hGH) replacement therapy on physical work capacity and cardiac and pulmonary function in patient with hGH defiency acquired in adulthood. J Clin Endocrinol Metab 1995; 80: 552-557.

NIDA Research Monograph Series 102. Anabolic Steroid Abuse. Lin GC, Erinoff L Eds. National Institute on Drug Abuse, Rockvilee. 1990.

NIDA Research Report - Steroid abuse and addiction: NIH Publication No. 00-3721. April, 2000.

Nieminen MS, Rämo MP, Viitasalo M, et al. Serious cardiovascular side effects of large doses of anabolic steroids in weight lifters. Eur Heart J 1996; 17: 1576-1583.

Nilsson S, Baigi A, Marklund B, et al. The prevalance of the use of androgenic anabolic steroids by adolescents in a country of Sweden. Eur J Public Health 2001; 11: 195-197. (2001a)

Nilsson S, Baigi A, Marklund B, et al. Trends in the misuse of androgenic anabolic steroids among boys 16-17 years old in a primary health care area in Sweden. Scand J Prim Health Care 2001; 19:181-182. (2001b)

O'Connor JS, Baldini FD, Skinner JS, et al. Blood chemistry of current and previous anabolic steroid users. Mil Med 1990; 155: 72-75.

Oikarinen A, Autio P, Vuori J, et al. Systemic glucocorticoid treatment decreases serum concentrations of carboxyterminal propeptide of type I procollagen and aminoterminal propeptide of type III procollagen. Br J Derm 1992; 126: 172-178.

Olson RE, Rudney H. Biosynthesis of ubiquinone. Vitam Horm 1983; 40: 1-43.

Oparil S. Pathogenesis of ventricular hypertrophy. J Am Coll Cardiol 1985; 5: 57-65.

O'Sullivan AJ, Kennedy MC, Casey JH, el al. Anabolic-androgenic steroids: medical assessment of present, past and potential users. Med J Australia. 2000; 173: 323-327.

Palatini P, Giada F, Garavelli G, et al. Cardiovascular effects of anabolic steroids in weight-trained subjects. J Clin Pharmacol 1996; 36: 1132-1140.

Palokangas H, Kovanen V, Duncan A, et al. Age-related changes in the concentration of hydroxypyridinium crosslinks in functionally different skeletal muscles. Matrix - Coll Rel Res 1992; 12: 291-296.

Pardridge WM. Serum bioavailability of sex steroid hormones. Clin Endocrinol Metab 1986; 15: 259-278.

Pärssinen M, Kujala U, Vartiainen E, et al. Increased premature mortality of competitive powerlifters suspected to have used anabolic agents. Int J Sports Med 2000; 21: 225-227.

Pärssinen M, Seppälä T. Steroid use and long term risk to health in former athletes. Sports Med 2002; 32: 83-94.

Pearson AC, Schiff M, Mrosek D, et al. Left ventricular diastolic function in weight lifters. Am J Cardiol 1986; 58: 1254-1259.

Pelkonen R. Hypotalamus, aivolisäke ja käpylisäke. in Kliininen Endokrinologia. Lamberg B-A, Koivisto V, Pelkonen R eds. Duodecim, Jyväskylä. 1992; 34-85.

Pelliccia A, Maron BJ, Spataro A, et al. The upper limit of physiologic cardiac hypertrophy in highly trained elite athletes. N Engl J Med 1991; 324: 295-301.

Pelliccia A, Spataro A, Caselli G, et al. Absence of left ventricular wall thickening in athletes engaged in intense power training. Am J Cardiol 1993; 72: 1048-1054 .

Perkiömäki JS, Ikäheimo MJ, Pikkujämsä SM, et al. Dispersion of QT interval and autonomic modulation of heart rate in hypertensive men with and without left ventricular hypertrophy. Hypertension 1996; 28: 16-21.

Polderman KH, Stehouwer CDA, van Kamp GJ. Influence of sex hormones on plasma endothelin levels. Ann Intern Med 1993; 118: 429-32.

Pope HG, Katz DL. Psychiatric and medical effects of anabolic-androgenic steroid use. A controlled study of 160 athletes. Arch Gen Psychiatry 1994; 51: 375-382.

Pope HG, Kouri EM, Hudson JI. Effects of supraphysiologic doses of testosterone on mood and aggression in normal men: A randomized controlled trial. Arch Gen Psychiatry 2000; 57: 133-140.

Porcerelli JH, Sandler BA. Anabolic-androgenic steroid abuse and psychopathology. Psychiatr Clin North Am 1998; 21: 829-833.

Post WS, Larson MG, Levy D. Impact of left ventricular structure on the incidence of hypertension. The Framingham heart study. Circulation 1994; 90: 179-185.

Problems with growth hormone. Med Lett Drugs Ther 1985; 27: 57-60.

Pye M, Quinn AC, Cobbe SM. QT-interval dispersion: a non-invasive marker of susceptibility to arrhythmia in patients with sustained ventricular arrhythmias? Br Heart J 1994; 71: 511-514.

Rämö P. Anabolic steroids alter the hemodynamic response of the canine left ventricle. Acta Physiol Scand 1987; 130: 209-217.

Rich JD, Dickinson BP, Feller A, et al. The infectious complications of anabolic-androgenic steroid injection. Int J Sports Med 1999; 20: 563-566.

Rip J, Rupar C, Ravi K, et al. Distribution, metabolism and function of dolichol and polyprenols. Prog Lipid Res 1985; 24: 269-309.

Risteli J , Niemi S, Trivedi P, et al. Rapid equilibrium radioimmunoassay for the aminoterminal propeptide of human type III procollagen. Clin Chem 1988; 34: 715-718.

Risteli J, Elomaa I, Niemi S, et al. Radioimmunoassay for the pyridinoline cross-linked carboxyterminal telopeptide of type I collagen: A new serum marker for bone resorption. Clin Chem 1993; 39: 635-640.

Rogol AD, Yesalis CE. Anabolic-androgenic steroids and athletes: What are the issues? J Clin Endocrinol Metab 1992; 74: 465-469.

Rosenfield RL. Role of androgens in growth and development of the fetus, child, and adolescent. Adv Pediatr 1972; 19: 172-213.

Rossi E, Zuppi P, Pennestri F, et al. Acromegalic cardiomyopathy: left ventricular filling and hypertrophy in active and surgically treated disease. Chest 1992; 102: 204-208.

Ruokonen A, Alén M, Bolton N, et al. Response of serum testosterone and its precursor steroids, SHBG and CBG to anabolic steroid and testosterone self-administration in man. J Steroid Biochem 1985; 23: 33-38.

Sachtleben TR, Berg KE, Elias BA, et al. The effects of anabolic steroids; on myocardial structure and cardiovascular fitness. Med Sci Sport Exec 1993; 25: 1240-1245.

Sader MA, Griffiths KA, McCredie RJ, et al. Androgenic anabolic steroids and arterial structure and function in male bodybuilders. J Am Coll Cardiol 2001; 37: 224-230.

Sahn DJ, DeMaria A, Kisslo J, et al. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978; 58: 1072-1083.

Santos E, Nebreda A. Structural and functional properties of ras proteins. Fed Am Soc Exp Biol J 1989; 3: 2151-2163.

Sarna S, Sahi T, Koskenvuo M, et al. Increased life expectancy of world class male athletes. Med Sci Sports Exer 1993; 25: 237-244.

Schiller NB, Shah PM, Crawford M, et al. Recommendations for quantition of the left ventricle by two-dimensional echocardiography. J Am Soc Echocard 1989; 2: 358-367.

Schurmeyer T, Knuth UA, Belkien E, et al. Reversible azoospermia induced by the anabolic steroid 19-nortestosterone. Lancet 1984; 1: 417-420.

Scow RO, Hagan SN. Effect of testosterone propionate and growth hormone on growth and chemical composition of muscle and other tissues in hypophysectomized male rats. Endocrinology 1965; 77: 852-858.

Seidman SN, Araujo AB, Roose SP, et al. Testosterone level, androgen receptor polymorphism, and depressive symptoms in middle-aged men. Biol Psychiatry 2001; 50: 371-376.

Seppälä M, Laatikainen T. Gynekologinen endokrinologia. in Kliininen Endokrinologia. Lamberg B-A, Koivisto V, Pelkonen R eds. Duodecim, Jyväskylä. 1992; 413-438.

Shahidi NT. A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids. Clin Ther 2001; 23: 1355-1390.

Shapiro CM, Smith RC. Effects of training on left ventricular structure and function. An echocardiographic study. Br Heart J 1983; 50: 534-539.

Shapiro LM. Physiological left ventricular hypertrophy. Br Heart J 1984; 52: 130-135.

Sheridan PJ. Androgen receptors in the brain: what are we measuring? Endocr Rev 1983; 4: 171-178.

Shozawa Z, Yamada H, Mabuchi C, et al. Superior sagittal sinus thrombosis associated with androgen therapy for hypoplastic anemia. Ann Neurol 1982; 12: 580-587.

Signorello LB, Tzonou A, Mantzoros CS, et al. Serum steroids in relation to prostate cancer risk in a case-control study. Cancer Causes Control 1997; 8: 632-636.

Silver MD. In Cardiovascular Pathology, 2nd ed. Churchill Livingstone, New York, NY. 1991.

Siperstain M. Role of cholesterogenesis and isoprenoid synthesis in DNA replication and cell growth. J Lipid Res 1984; 25: 1462-1468.

Small M, Beastall GH, Semple CG, et al. Alteration of hormone levels in normal males given anabolic steroid stanatzolol. Clin Endocrinol 1984; 21: 49-55.

Smallridge RC, Rajfer S, Davia J, et al. Acromegaly and the heart. An echocardiographic study. Am J Med 1979; 66: 22-27.

Smit JW, Janssen YJ, Lamb HJ, et al. Six months of recombinant human GH therapy in patients with ischemic cardiac failure does not influence left ventricular function and mass. J Clin Endocrinol Metab 2001; 86: 4635-4637.

Spirito P, Pelliccia A, Proschan MA, et al. Morphology of the "athlete's heart" assessed by echocardiography in 947 elite athletes representing 27 sports. Am J Cardiol 1994; 74: 802-806.

Stocker R, Bowry VW, Frei B. Ubiquinol-10 protects human low density lipoprotein more efficiently against lipid peroxidation than does a-tocopherol. Proc Natl Acad Sci USA 1991; 88: 1646-1650.

Stolt A, Karjalainen J, Heinonen OJ, et al. Left ventricular mass, geometry and filling in elite female and male endurance athletes. Scand J Med Sci Sports 2000; 10: 28-32.

Stolt A, Kujala UM, Karjalainen J, et al. Electrocardiographic findings in female endurance athletes. Clin J Sport Med 1997; 7: 85-89.

Strawford A, Barbieri T, Van Loan M, et al. Resistance exercise and supraphysiologic androgen therapy in eugonadal men with HIV-related weight loss: A randomized controlled trial. JAMA 1999; 281: 1282-1290.

Sullivan ML, Martinez CM, Gallagher EJ. Atrial fibrillation and anabolic steroids. J Emer Med 1999; 17: 851-857.

Suominen J, Vierula M. Semen quality in Finnish men. Br Med J 1993; 306: 1579.

Swerdloff RS, Wang C, Hines M, et al. Effect of androgens on the brain and other organs during development and aging. Psychoneuroendocrinology 1992; 17: 375-383.

Systat for Windows: Statistics, version 5. Systat inc, Evanston, IC. 1992; 750.

Tagarakis CVM, Bloch W, Hartmann G, et al. Anabolic steroids impair the exercise-indused growth of the cardiac capillary bed. Int J Sports Med 2000; 21: 412-418.

Taimela S, Seppälä T. Voimaharjoittelussa käytettavien anabolisten steroidien haitat. Suomen Lääkärilehti 1994: 49: 2051-2061.

Tapanainen JS, Aittomäki K, Min J, et al. Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. Nat Genet 1997; 15: 205-206.

Terney R, McLain L. The use of anabolic steroids in high school students. Am J Dis Child 1990; 144: 99-103.

Thompson PD, Sadaniantz A, Cullinane EM, et al. Left ventricular function is not impaired in weight lifters who use anabolic steroids. J Am Coll Cardiol 1992; 19: 278-282.

Thorisdottir H, Evans JA, Schwartz HJ, et al. Some clotting factors in plasma during danazol therapy: free and total protein S, but not C4b-binding protein, are elevated by danazol therapy. J Lab Clin Med 1992; 119: 698-701.

Tokgözoglu SL, Erbas T, Aytemir K, et al. Effects of otreotide on left ventricular mass in acromegaly. Am J Cardiol 1994; 74: 1072-1074.

Tomoda H. Effect of Oxymetholone on left ventricular dimensions in heart failure secondary to idiopathic dilated cardiomyopathy or to mitral or aortic regurgitation. Am J Cardiol 1999; 83: 123-125.

Torres-Calleja J, Gonzalez-Unzaga M, DeCelis-Carrillo R, et al. Effect of androgenic anabolic steroids on sperm quality and serum hormone levels in adult male body builders. Life Sci 2001; 68: 1769-1774.

Trifunovic B, Norton GR, Duffield MJ, et al. An androgenic steroid decreases left ventricular compliance in rats. Am J Physiol 1995; 268: 1096-1105.

Turner TT, Jones CE, Howards SS, et al. On the androgen microenvironment of maturing spermatozoa. Endocrinology 1984; 155: 1925-1932.

Urhausen A, Hölpes R, Kindermann W. One- and two-dimensional echocardiography in bodybuilders using anabolic steroids. Eur J Appl Physiol 1989; 58: 633-640.

Urhausen A, Kindermann W. Sport-specific adaptations and differentiation of the athlete's heart. Sports Med 1999; 28: 237-244.

Uzych L. Anabolic-androgenic steroids and psychiatric-related effects: A review. Can J Psychiatry. 1992; 37: 23-28.

Valtersson C, van Duijn A, Verkleij T et al: The influence of dolichol, dolichol esters, and dolichyl phosphate on phospholipid polymorphism and fluidity in model membranes. J Biol Chem 1985; 260: 2742-2751.

Vandekerckhove P, Lilford R, Vail A, et al. Androgens versus placebo or no treatment for idiopathic oligo/asthenospermia. Cochrane Database Syst Rev. 2000; (2): CD000150.

Vanderschueren D, Bouillon R. Androgens and bone. Clac Tissue Int 1995; 56: 341-346.

Vierula M, Niemi M, Keiski A, et al. High and unchanged sperm counts of Finnish men. Int J Androl 1996; 19: 11-17.

Vogel W, Klaiber EL, Broverman DM. A comparison of the antidepressant effects of a synthetic androgen (mesterolone) and amitriptyline in depressed men. J Clin Psychiatry 1985; 46: 6-8.

Webb OL, Laskarzewski PM, Glueck CJ. Severe depression of high-density lipoprotein cholesterol levels in weight lifters and body builders by self-admistered exogenous testosterone and anabolic-androgenic steroids. Metabolism 1984; 33: 971-975.

Wichstrom L, Pedersen W. Use of anabolic-androgenic steroids in adolescence: Winning, looking good or being bad? J Stud Alcoh 2001; 62: 5-13.

Wight JN, Salem D. Sudden cardiac death and the "athlete`s heart". Arch Internal Med 1995; 155: 1473-1480.

Wilson J. Androgen abused by athletes. Endocr Rev 1988; 9: 81-199.

Windsor R, Dumitru D: Prevalence of anabolic steroid use by male and female adolescents. Med Sci Sports Exerc 1989; 21: 494-497.

Winters SJ. Androgens: Endocrine physiology and pharmacology. in Anabolic steroid abuse, research monogeraph 102. Lin GC, Erinoff L eds. NIDA, Rockville. 1990; 113-131.

Wood TO, Cooke PH, Goodship AE. The effect of exercise and anabolic steroids on the mechanical properties and crimp morphology of the rat tendon. Am J Sports Med 1988; 16: 153-158.

World Antidoping Agency. www.wada-ama.com. 2002.

World Health Organisation task force on methods for the regulation of male fertility. Contraceptive efficancy of testosterone-indused azoospermia in normal men. Lancet 1990; 336: 955-959.

World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction. Cambridge University Press, Cambridge, UK.

Yasugi E, Oshima M. Sequential microanalyses of free dolichol, dolichyl fatty acid ester and dolichyl phosphate levels in human serum. Biochim Biophys Acta 1994; 1211: 107-113.

Yeater R, Reed C, Ullrich I, et al. 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 1996; 30: 11-14.

Yesalis CE. Anabolic steroids in sport and exercise. Human Kinetics Publishers, Champaign, IL, USA. 1993.

Yesalis CE, Bahrke MS. Doping among adolescent athletes. Clin Endocrinol Metab 2000; 14: 25-35.

Yesalis CE, Barsukiewicz CK, Kopstein AN, et al. Trends in anabolic-androgenic steroid use among adolescents. Arch Pediatr Adolesc Med 1997; 151: 1197-1206.

Yesalis CE, Wrigth JE, Bahrke MS. Epidemiological and policy issues in the measurements of long term health effects of anabolic-androgenic steroids. Sports Med 1989; 8: 129-138.

Zabel M, Portnoy S, Franz MR. Electrocardiographic indexes of dispersion of ventricular repolarization: an isolated heart validation study. J Am Coll Cardiol 1995; 25: 746-752.

Zahm SH, Fraumeni JF. The epidemiology of soft tissue sarcoma. Semin Oncol 1997; 24: 504-514.

Zimmerman HJ, Lewis JH. Drug-induced cholestasis. Med Toxicol 1987; 2: 112-160.