Health effects of anabolic androgenic steroid use in male amateur athletes

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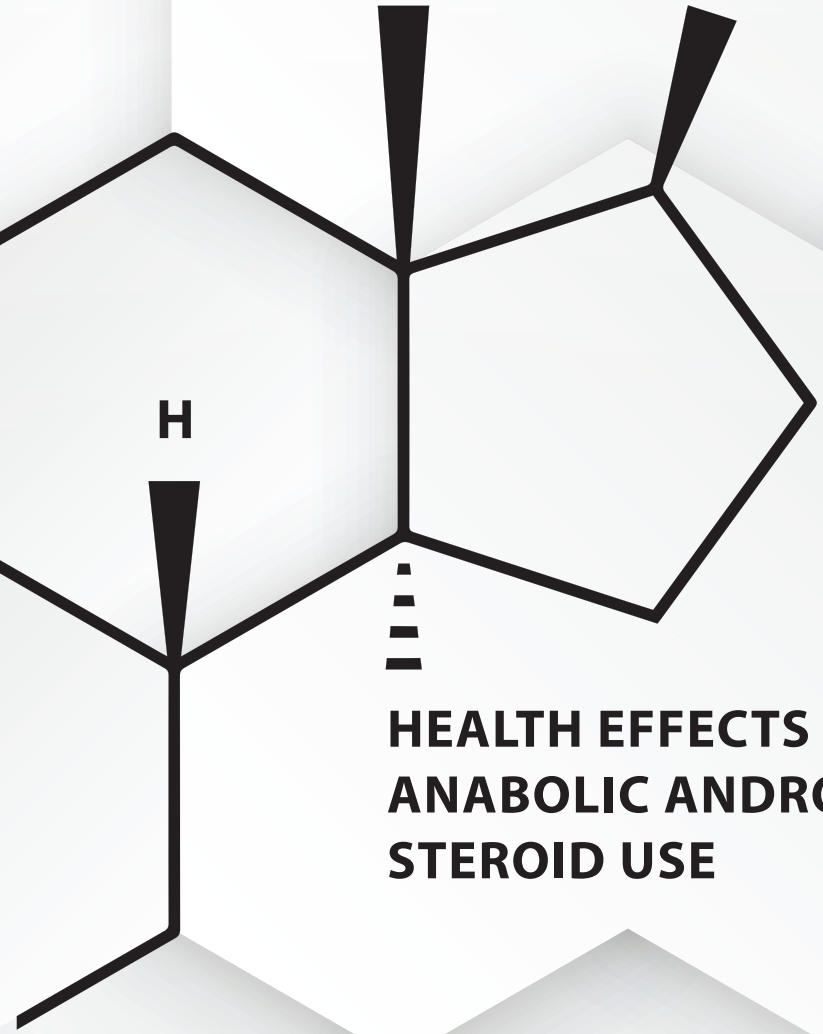

HEALTH EFFECTS OF ANABOLIC ANDROGENIC STEROID USE

HAARLEM study

HEALTH EFFECTS OF ANABOLIC ANDROGENIC STEROID USE

IN MALE AMATEUR ATHLETES

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Health effects of anabolic androgenic steroid use in male amateur athletes

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GENERAL INTRODUCTION

In the last decades the use of performance- and image-enhancing drugs (PIEDs) is on the rise. These drugs are substances used by people, mainly athletes, to change their physical appearance, enhance their sporting performance, or both. The most well-known example in this group of drugs is anabolic androgenic steroids (AAS), such as testosterone, trenbolone and nandrolone. Other common PIEDS are selective androgen receptor modulators (SARMs), beta-2-agonists (e.g., clenbuterol), stimulants (e.g., amphetamines), other hormones (e.g., growth hormone, insulin and thyroid hormone), and tanning agents (e.g., melanotan II).

The use of AAS has transitioned from a small group of male elite athletes in the previous century to widespread use among amateur athletes. These athletes typically do not engage in professional sporting events. More often they are ordinary visitors of the local gym; a subset of users take part in national and international bodybuilding competitions.[1] For the Netherlands, a survey in 2008 estimated that the prevalence of PIEDs use among frequenters of gyms is 8%.[2] This equates to approximately 160.000 people, of which 20.000 use AAS. This figure is now dated and considering the expanding popularity of the fitness industry, as well as the advent of social media in which physical appearance has become increasingly important, there is much reason to believe that currently even more men and women use PIEDs and AAS.

Definition of AAS

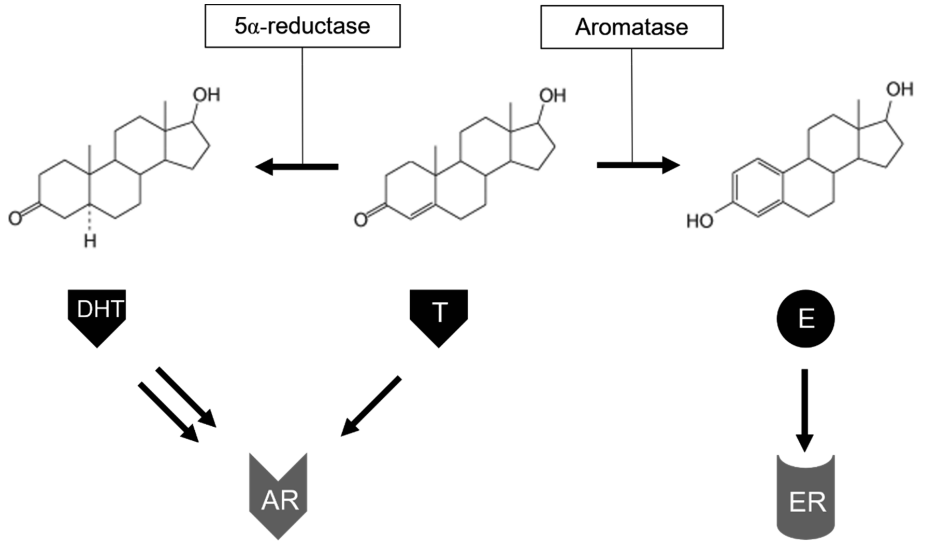
AAS comprise a group of compounds that are structurally similar to testosterone and have similar actions when administered in an appropriate dose. It has been long known, and much later also shown empirically, [3] that the administration of testosterone increases muscle mass and strength and that the effects of testosterone are dose-dependent. Strength training enhances the effects of AAS significantly. The term 'anabolic androgenic steroids' refers to the anabolic, i.e. muscle building, and androgenic, i.e. virilizing, effects of these compounds. The term has been criticized since all compounds included in this group bind and activate the androgen receptor, making them basically androgens, which, by definition, have muscle building and virilizing effects.[4]

Injectable testosterone esters are among the AAS most used. They have the advantage of bypassing first pass metabolism in the liver. Without modification, however, steroids rapidly enter the blood, result in high peak levels, and have a short plasma half-life. Therefore a fatty acid chain is attached to the steroid which improves pharmacokinetics and extends the half-life. The longer the fatty acid, the slower the release from the injected depot. The most common AAS administered by intramuscular injection are testosterone, trenbolone, drostanolone and nandrolone. Some AAS have been alkylated to prevent metabolization by the liver and render them suitable for oral administration. The most popular oral AAS are stanozolol, oxandrolone and methandienone.

Testosterone and many other androgens are converted to estradiol via the aromatase enzyme and converted to dihydrotestosterone via 5α-reductase (see *Figure 1*). Administration of high doses of testosterone thus results in increased levels of those hormones as well. Estradiol in men is essential

for modulating libido, erectile function and spermatogenesis, but elevated concentrations may stimulate breast tissue and cause gynecomastia. Dihydrotestosterone is the androgen that triggers adult male characteristics but its abundance may lead to male pattern baldness and increased body hair. By modifying androgens in a certain way, aromatization or 5α-reduction can be prevented (e.g., mesterolone), attempting to improve the benefit-to-harm ratio of the compound.

Figure 1. Testosterone (T) can bind directly with the androgen receptor (AR). Testosterone is irreversibly converted by the enzyme 5α-reductase to dihydrotestosterone (DHT), which binds with greater affinity to the AR, or by aromatase to estradiol (E), which binds to the estrogen receptor (ER).



AAS acquired through the black market

Production and trading of AAS without a license is prohibited in the Netherlands. Nevertheless it is easy to acquire AAS. It usually suffices to talk to – presumed – users of PIEDs in the gym in order to get in touch with local dealers. They are often quick to provide you with information and illegally obtained products on a short notice. Another more anonymous route of obtaining AAS is via the internet. A quick search online quickly reveals an abundance of websites that offer a great variety of AAS, largely imported from Eastern European or Asian countries.[5] Once the AAS are purchased by athletes, their use for personal purposes is legitimate. So unlike in other European countries, such as Belgium, Sweden or Denmark,[6] there are no doping controls in commercial gyms. Athletes in the Netherlands therefore do not risk a criminal prosecution or fine for the use or possession of AAS.

It should be no surprise that the quality of AAS acquired through the black market is very low. The production process is sloppy and usually carried out by self-taught chemists in secret basements or sheds with unprofessional equipment. Whether intentional or not, in many cases the products do not

contain the same type or dosage of AAS as is indicated on the label.[5,7] Despite this underdosing or sometimes even the lack of active ingredients, most products do contain AAS. And because several AAS products are used simultaneously, often in a – presumed – dose far exceeding a physiological replacement dose, desired effects of muscle growth and increased muscle strength are experienced by the user. One would also think that the careless production of AAS would pose the user at risk for bacterial infection due to contaminated products, but this appears to remain limited to incidental reports, and is probably much less common than with intravenous drug users.[8]

Patterns of AAS use

The most common way to use AAS is intermittently, i.e. in cycles. As serum androgen concentrations reach very high supraphysiological levels during the cycle, strength and muscle mass accordingly increase, sometimes even flagrantly. After the cycle, serum androgen concentrations slowly fall to physiological levels, and afterwards there is a variable period of relative testosterone deficiency,[9] referred to as post-AAS-hypogonadism. This is when symptoms of hypogonadism such as tiredness and lack of libido may occur, and muscle mass and strength rapidly decline. Many users employ post-cycle therapy (PCT), normally with anti-estrogens (e.g., tamoxifen) or aromatase inhibitors (e.g., anastrozol), after a cycle to speed up hormonal recovery and prevent symptoms of testosterone deficiency.

Multiple cycles are deemed necessary by many athletes to accumulate and maintain muscle mass. Some users adopt the so-called 'blast and cruise' strategy in which a maintenance dose of AAS is used in between cycles in order to further prevent muscle loss. Another subset of users stops cycling at some point but continues to use androgens as 'testosterone replacement therapy'. The routine of using AAS uninterruptedly for at least one year, such as with the 'blast and cruise' regimen or with a non-stop cycle, has become quite common and is a reason for concern.[10,11]

Decisions on how one is supposed to use AAS and which compounds are the most suitable depend largely on information acquired through the internet, from coaches and acquaintances, as well as on personal experience, goals and ambitions. Online there is a plethora of information about AAS and discussion forums make up the primary source for most users. This is a profound maze teeming with disinformation and pseudoscientific claims, and it is the staging area for gurus and self-proclaimed experts, tumbling over each other with advice and instructions.[12] As a result, many users think they are well-informed but oftentimes have wrong conceptions about AAS and their positive and negative effects.

Health risks of AAS use

There is little doubt regarding the harmfulness of AAS use. If considering the currently existing data in literature,[13,14] risks of AAS use on the short and long term slowly start to take shape (see *Table 1*). Many side effects are directly related to endocrine effects of testosterone or conversion to other hormones, such as estradiol and dihydrotestosterone. Examples are acne, male pattern baldness, gynecomastia and libido changes. Administration of exogenous testosterone leads to suppression of endogenous gonadotropin production, hence causing testicular atrophy and decreased sperm count. In addition, there is a number of discrete cardiovascular effects, such as hypertension and adverse change of lipid

profile. Much less data is available on the long term risks of AAS use, but these probably include prolonged post-AAS-hypogonadism[9], subfertility and cardiovascular disease.[15]

Table 1. Overview of the known health risks related to AAS use.[13,14]

Endocrine	**Psychological**
❖ Gynecomastia ❖ Testicular atrophy ❖ Libido changes ❖ Decreased sperm count ❖ Infertility or unwanted childlessness ❖ Menstrual irregularities (in women) ❖ Masculinization (in women)	❖ Addiction ❖ Mania ❖ Depression ❖ Aggression ❖ Mood swings
Cardiovascular	**Liver**
❖ Lipid profile changes ❖ Elevated blood pressure ❖ Decreased myocardial function ❖ Left ventricular hypertrophy ❖ Polycythemia ❖ Arrythmia	❖ Liver toxicity ❖ Hepatocellular adenoma ❖ Hepatocellular carcinoma
Musculoskeletal	**Dermatological**
❖ Risk of tendon tears ❖ Skeletal muscle injuries ❖ Intramuscular abscess	❖ Acne ❖ Male pattern baldness

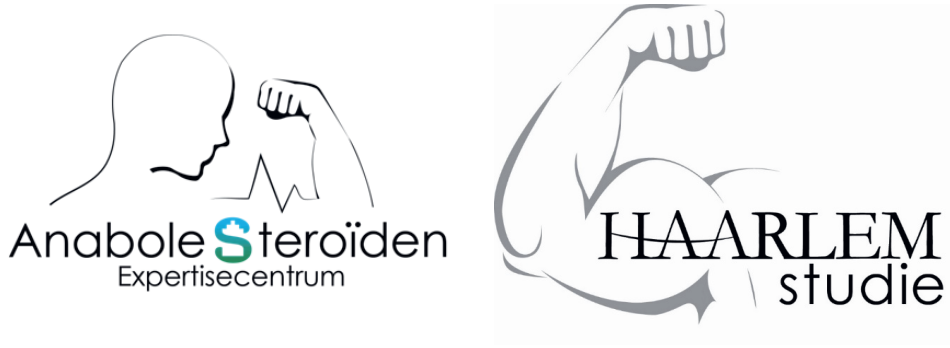
Nonetheless, reliable and precise data on the health risks of AAS use are scarce. This is, firstly, the result of low knowledge and awareness among physicians of the possibility of AAS use by patients. Physicians do not recognize symptoms related to AAS use, feel reluctant to inquire about possible AAS use even when they suspect it, or prematurely attribute certain health complaints to AAS use. Education about the unwanted somatic and psychological effects is not a routine part of the medicine curriculum in the Netherlands and users of AAS tend to be secretive about their use of AAS. They have no faith in the expertise of their treating physicians. By not bringing it up they widen the knowledge gap.[17]

Secondly, there is a sparsity of clinical research in the field of AAS. This is a consequence of the unfamiliarity with the topic among health professionals, which is necessary to spark the initiative to perform new studies. Also ethical issues hamper the carrying out of prospective trials. Problems here entail the illegal route through which the products are obtained, the absence of a registered indication for the use of supraphysiological doses of androgens, and the fact that most AAS have not been extensively studied for the use in humans. Most knowledge about the harmfulness of AAS is therefore

based on low level evidence, such as expert opinion, case reports and small observational studies. The Health Council of the Netherlands confirmed in 2010 that there is a necessity for more scientific evidence regarding the health risks of AAS use.[18] This evidence will be needed to improve education about AAS and to implement harm reduction strategies. This thesis will elaborate on the scientific knowledge about the risks of AAS use by amateur athletes that was gained from patients analyzed in the AAS clinic and from results obtained from the HAARLEM study.

***Figure 2*.** Logo of the Anabolic Steroids Center of Expertise (A) of the Spaarne Gasthuis in Haarlem and of the HAARLEM study used for marketing and publicity purposes.

A. **B.**



Outpatient AAS clinic

The 'Anabolenpoli' or outpatient AAS clinic for (former) users of AAS was established in 2011 in an attempt to gain more insight into the characteristics of AAS users, the methods of AAS use, and the health risk associated with AAS use. The clinic is the patient-oriented embodiment of the Anabolic Steroids Center of Expertise (see *Figure 2A*). Although initially the Spaarne Gasthuis in Haarlem was home to this clinic, from 2020 patients can also turn to the Elisabeth-TweeSteden hospital in Tilburg. It is the only center of expertise worldwide that focuses primarily on helping patients with health problems related to AAS. Patients need a referral to the clinic from their general practitioner or a medical specialist. The health care provided is fully covered by the Dutch health care insurance. An overview of all patients evaluated in the clinic between 2011 and 2016 is given in **CHAPTER 1**. Two remarkable cases from the clinic, namely an addicted AAS abuser who suffered from a spontaneous hemorrhage of a hepatocellular adenoma and a bodybuilder suffering from hypokalemic periodic paralysis due to liothyronine abuse, are extricated in **CHAPTER 2** and **CHAPTER 3**, respectively.

HAARLEM study

Besides ambulatory patient care the clinic also carries out scientific research on the topic of AAS. It receives financial support from the Spaarne Gasthuis Academy, a research funding organization affiliated with the hospital in Haarlem. The reputation of the clinic within the bodybuilding scene in the Netherlands creates a realizable route for subject recruitment. Therefore the clinic was able to successfully initiate a prospective observational study with a systematic approach, known as the HAARLEM-study, which is an acronym for **H**ealth risks of **A**nabolic **A**ndrogenic ste**R**oid use by ma**LE**

a**M**ateur athletes (see *Figure 2*). During this study a cohort of 100 men who intended to start a cycle of AAS on short notice was assembled. During a follow-up period of 1 year health was meticulously examined at four different time points, i.e. at the start of the cycle, at the end of the cycle, three months after the end of the cycle, and one year after the start of the cycle. Health analysis included a patient history, physical examination, blood, urine and semen analysis, psychological questionnaires, electrocardiography and, in a subgroup of subjects, echocardiography. Research questions addressed in the HAARLEM study are summarized in *Table 2*. The study was approved by the local institutional review board (registration M015-019, study number NH015.189) and subjects provided written informed consent.

Table 2. Main research questions addressed during the HAARLEM study.

Baseline characteristics – CHAPTER 4
❖ What are the sociodemographic characteristics of users of AAS?
❖ What are the reasons and motivations for AAS use?
❖ What are the methods of AAS use and cycle characteristics?
❖ What is the quality of AAS products used by subjects?
Positive and negative side effects – CHAPTER 5
❖ What is the incidence of self-reported side effects?
❖ What is the incidence of acne en gynecomastia?
❖ What is the incidence of kidney and liver toxicity?
❖ What are the psychological effects of AAS use?
Testicular function - CHAPTER 6
❖ What is the effect of AAS use on spermatogenesis during the cycle?
❖ What is the rate of recovery of serum testosterone and spermatogenesis after the cycle?
❖ What is the influence of post-cycle therapy on the recovery of testicular function?
Cardiovascular effects – CHAPTER 7
❖ What is the effect of AAS use on blood pressure, lipid profile and hematocrit?
❖ Are the cardiovascular effects of AAS use associated with cycle characteristics?
❖ Do oral AAS have a worse impact on lipid metabolism than injectable AAS?
Coagulation – CHAPTER 8
❖ What is the effect of AAS use on coagulation factor levels?
❖ Are the effects on coagulation factor levels associated with cycle characteristics?
❖ Do oral AAS have a greater impact on coagulation factor levels than injectable AAS?
Cardiac structure and function – CHAPTER 9
❖ What is the effect of AAS use on cardiac structure and function?
❖ Are the cardiac effects reversible after discontinuation of AAS use?
❖ Is there an association between these effects and cycle characteristics?

Patterns and determinants of AAS use – CHAPTER 10

- What are the patterns of AAS use after a single cycle?
- Which determinants predict AAS use in the second year?
- What is the incidence of serious adverse events?

The HAARLEM-study provided ample empirical data that form the core of this thesis. The study methodology and baseline characteristics of the cohort subjects and their methods of AAS use is reported in **CHAPTER 4**. Subsequently, the positive and negative side effects reported by the subjects during the first year of follow-up as well outcomes of kidney and liver parameters, and psychological questionnaires are summarized in **CHAPTER 5**. Specific and important organ functions and systems affected by AAS use are addressed in the following chapters. As such, **CHAPTER 6** zooms in on the endocrine sequelae caused by AAS, and consequently the disruption and recovery of endogenous testosterone production and spermatogenesis during and after a cycle. In **CHAPTER 7** the adverse effect of AAS on blood pressure, lipid profile and erythrocytosis are discussed in extensive detail. The way AAS influence the coagulation cascade and thrombosis risk is elaborated in **CHAPTER 8**. And lastly, **CHAPTER 9** describes the effect of AAS on cardiac structure and function.

After completion of the study, the follow-up period was extended to perform another, fifth, health analysis 2 years after subjects were enrolled. Subjects performed one cycle in the first year but could use AAS in the way of their choice during the second year. The longer follow-up created more insight into patterns and determinants of AAS use and the occurrence of serious adverse events. The results are condensed in **CHAPTER 10**. The thesis is concluded with a review of the data in **CHAPTER 11** and provides recommendations for general practitioners and medical specialists on dealing with users of AAS and their respective health issues.

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CHAPTER 1

OUTPATIENT CLINIC FOR USERS OF ANABOLIC ANDROGENIC STEROIDS: AN OVERVIEW

DL Smit, W de Ronde.

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ABSTRACT

Background

Anabolic androgenic steroids (AAS) are used by approximately 20.000 amateur athletes in the Netherlands. AAS are harmful but data are lacking as to how harmful they are precisely. An outpatient clinic for (former) users of AAS was established in 2011 to acquire more knowledge about the health risks associated with AAS abuse.

Methods

All case files of patients who visited the AAS clinic were reviewed retrospectively.

Results

180 patients visited the AAS clinic between May 2011 and May 2016. Patients were strength athletes (99% male, mean age 34 years, range 19-61) who had started AAS use at a median age of 23 years (range 16-53). 95% used AAS in cycles (median 4 cycles completed, median duration 10 weeks). Cycles consisted of a median of 3 different AAS, most commonly testosterone, nandrolone and trenbolone. Growth hormone was used by 34% in addition to AAS. Side effects occurred in 96% of patients, mainly acne (38%), gynecomastia (34%) and agitation (27%) during cycles; decreased libido (34%) and erectile dysfunction (20%) afterwards. Medications regularly used by patients to self-treat side effects were aromatase inhibitors, clomiphene citrate, human choriogonadotrophin, and tamoxifen.

Conclusion

AAS abuse did not lead to critical health issues. However, the incidence of less severe side effects among AAS users appears high. Considering the large amount of abusers in the community, AAS abuse poses an important public health problem. A prospective study with a systematic approach is required to provide more reliable data regarding health risks of AAS abuse.

INTRODUCTION

AAS abuse in the Netherlands

In the Netherlands approximately 160.000 people use performance enhancing drugs (PED), of which 20.000 anabolic androgen steroids (AAS).[1] Production and trading of AAS without a license is prohibited in the Netherlands, yet AAS can be easily acquired illegally through local dealers or the internet. The majority of users do not engage in organized sporting events.[1]

Users of AAS most often use intermittently, i.e. in cycles, and their knowledge is based on information from acquaintances, trainers or the internet.[2] Among users, cycles differ greatly with respect to length, dosage as well as the number of different AAS used simultaneously or consecutively.[3] An earlier report showed that in about 50% of illegally obtained AAS the contents do not match the description on the label.[4] Therefore, it is difficult to attribute side effects to specific AAS or dosages.

Health concerns about AAS abuse

The Health Council of the Netherlands stated that AAS are harmful but data are lacking as to how harmful they are precisely.[5] Among physicians, there is low awareness of the possibility of AAS abuse by patients, and patients tend to be secretive about their use and do not rely on the physician's knowledge of AAS.[6] Knowledge of the unwanted somatic and psychological effects of AAS is limited because clinical research in the field of AAS is scarce. Prospective clinical trials among AAS users are hampered by ethical issues due to the fact that there is no registered indication for the use of supraphysiological doses of androgens, the products are mainly illegally obtained and most anabolic steroids are not registered for, nor extensively studied in, humans. As a result, most knowledge about the harmful effects of AAS is based on low level evidence, such as expert opinion, case reports or small observational studies.

Outpatient AAS clinic

An outpatient clinic for (former) users of AAS, the 'Anabolenpoli' or AAS clinic, was established in 2011 in the Spaarne Gasthuis in Haarlem in an attempt to gain more insight into the characteristics of AAS users, the methods of AAS use and the health risks associated with AAS abuse in the Netherlands. To our knowledge, it is the only clinic worldwide that focuses primarily on helping patients with health problems related to AAS. Patients need a referral to the clinic from their general practitioner or a medical specialist and the health care provided is fully covered by Dutch health care insurance. Haarlem is located centrally in the Netherlands with a maximum distance of 250 km to all country borders and is therefore readily reached by patients throughout the country.

The AAS clinic now exists for five years. This study provides an overview of all patients that were referred to the clinic and generates novel data regarding recreational AAS use in the Netherlands and related health issues.

MATERIALS AND METHODS

Consultation at AAS clinic

During consultation of each patient at the AAS clinic, the history of AAS use was assessed. Items discussed were the reasons for referral, the age at which the patient used AAS for the first time, the number of cycles completed, the number of years of active AAS use (every year with at least 1 cycle), and whether a patient had used continuously (defined as AAS use without interruptions for at least 1 year). Additionally, the types of AAS that a patient had used were inquired about as well as the use of other PED, medications to prevent or treat side effects, some of which are referred to as post-cycle therapy (PCT), with corresponding dosages. Patients were routinely asked which side effects were experienced during AAS use. All patients had a routine medical check including history and physical examination. If indicated, additional investigations were done, such as blood tests, urinalysis, semen analysis or electrocardiography.

Review of cases

For this study, all case files were reviewed for reasons for referral, patient and demographic characteristics, history and methods of AAS use, reported side effects, and use of other PED or medications. An overview was made of the investigations performed by referring physicians as well as those ordered by the clinic, the conclusions drawn and diagnoses established, and therapies employed. If available, follow-up data were recorded.

Descriptive analysis

Simple descriptive statistics were used to display quantitative data. If the variables were normally distributed, mean and standard deviation were calculated. If the distribution of a variable was skewed, a median is presented with range. Documentation of the variables analyzed was not complete in all case files. Missing data were frequent, for example for employment status, drug use or side effects. Therefore, percentages displayed in the results section (and tables) are not the percentages of all 180 patients but rather the percentage of patients from whom the data could be retrieved (numbers are indicated if applicable).

RESULTS

Patient characteristics

In total 180 patients visited the AAS clinic between May 2011 and May 2016. The number of patients referred annually was between 30 and 40. Patients came from municipalities throughout the Netherlands, and Belgium, as shown in *Figure 1*. Patient characteristics are displayed in *Table 1*. Patients had a mean age of 34 years (range 19-61) and 99% were male. The patients invariably engaged in physical strength sports, with 81% being (former) bodybuilder. 88% of patients were not on AAS at the time of consultation. The reasons for referring a patient to the AAS clinic are presented in *Table 2* and were related to symptoms occurring during or after a cycle (48%), suspected hypogonadism (10%) or abnormal blood tests (7%).

Table 1. Patient characteristics. Numbers of patients and percentages are displayed in the right column, "n" represents the number of subjects from whom the data could be retrieved,. SD = standard deviation.

Patient total	**n = 180**
Male	178 (99%)
Female	2 (1%)
Age (mean ±SD; range)	34 (±10,1; 19-61)
Occupational status	**n = 108**
Employed/working	72 (67%)
Student	12 (11%)
Unemployed	11 (10%)
Sick leave	7 (6%)
Disability benefit	3 (3%)
Welfare	3 (3%)
Recent drug use (other than nicotine/alcohol)	**n = 126**
XTC/speed	30 (24%)
Cocaine	28 (23%)
Cannabis	26 (21%)
GHB	23 (19%)
Sport (other than fitness/gym)	**n = 117**
Bodybuilding	95 (81%)
Combat sports (e.g. MMA, kickboxing, judo)	15 (13%)
Powerlifting	5 (4%)
Strongman athlete	2 (2%)

Current AAS use	**n = 180**
Not in cycle	158 (88%)
On cycle	4 (2%)
Bridging (i.e. AAS use in between cycles)	5 (3%)
Maintenance dose	13 (7%)

***Table 2*.** Referring physicians and reasons for referral. Some patients were referred for multiple symptoms. Numbers of patients and percentages are displayed in the right column.

Referring physician	**n = 174**
General practitioner	126 (72%)
Internist	20 (11%)
Self-referral	16 (9%)
Psychiatrist	4 (2%)
Urologist	2 (1%)
Sports medicine physician	1 (1%)
Dermatologist	1 (1%)
Correctional medicine physician	1 (1%)
Fertility specialist	1 (1%)
Rheumatologist	1 (1%)
Sexuologist	1 (1%)

Reasons for referral	**n = 180**
Symptoms during/after cycle	86 (48%)
- Decreased libido	20 (11%)
- Erectile dysfunction	16 (9%)
- Mood problems	13 (7%)
- Fatigue	13 (7%)
- Gynecomastia	12 (7%)
- Subfertility	5 (3%)
- Palpitations	4 (2%)
- Anxiety	3 (2%)
- Derealization	1 (1%)
- Acne	1 (1%)
- Wound infection	1 (1%)
- Other	14 (8%)
Abnormal blood tests	12 (7%)
- Polcythemia	6 (3%)
- Proteinuria	3 (2%)

- Abnormal liver biochemistry	2 (1%)
- Hyper-CK-emia	1 (1%)
Suspected post-AAS-hypogonadism	18 (10%)
Health check after AAS use	16 (9%)
Questions about AAS use	14 (8%)
Help/advise to abstain from AAS	12 (7%)
Other	22 (12%)

Methods of AAS use

Characteristics of AAS use by the patients and types of AAS used are summarized in *Table 3.* The patients had a median age of 23 years (range 16-53) when they first used AAS. 94% of patients used in cycles but 20% at some point used AAS uninterruptedly for more than 12 months. The number of cycles completed and the mean duration of a cycle per patient are shown in *Figure 2*. Most visitors had completed more than 3 cycles prior to their visit. The duration of cycles varied considerably. However, most cycles lasted between 6 and 18 weeks. Cycles mostly consisted of 2 or more different anabolic steroids. Products were used simultaneously or consecutively, and the dose, duration and combination of the different products used in the cycle differed substantially between cycles. Injectable testosterone esters were used by 80% of users, mostly combined with nandrolone, trenbolone, stanozolol and/or boldenone.

Other PED, medications and PCT

Other substances used in addition to AAS are shown in *Table 4*. Growth hormone was the most popular PED followed by clenbuterole and thyroid hormone. Medication used during or shortly after a cycle of AAS were aromatase inhibitors (anastrozole, exemestane and letrozole), anti-oestrogens (clomiphene citrate or tamoxifen), human choriogonadotrophin (hCG), isotretinoin, sildenafil, diuretics and finasteride. 94 (71%) patients always used PCT following AAS use for a mean period of 4 weeks; whereas 28 (21%) patients never used PCT. Agents used in PCT were mainly tamoxifen, hCG and clomiphene citrate.

Reported side effects

96% of patients reported at least 1 side effect attributed to the use of AAS *(Table 5)*. Particularly common were acne, decreased libido, testicular atrophy and gynecomastia. A general distinction could be made between side effects that occur during a cycle, i.e. gynecomastia, fluid retention and aggressiveness, and those occurring after a cycle, i.e. erectile dysfunction and decreased libido. Of the reported health issues none had led to hospital admission, except for a severe skin infection in 1 patient at an AAS injection site.

***Figure 1*.** Map of the Netherlands showing municipalities where patients lived when referred to the AAS clinic. The larger the green dot, the more patients come from the corresponding municipality.

Table 3. Characteristics of AAS use by the patients and types of AAS used. Medians with ranges and number of patients with percentages, respectively, are displayed in the right column.

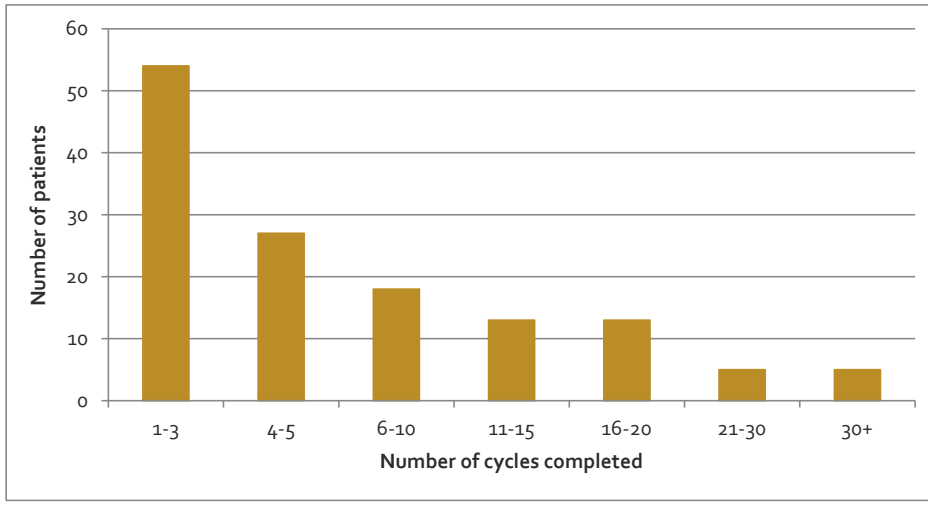
Characteristics of AAS use	**Median (range)**
Age of first AAS use	23 (range 16-53)
Number of years of active AAS use	4 (range 1-35)
Number of cycles completed (see also *Figure 2*)	4 (range 1-60)
Cycle length in weeks (see also *Figure 2*)	10 (range 2-48)
Number of AAS in cycle	3 (range 1-10)

Cycles or continuous use	**n = 170**
- Cycles (only)	135 (79%)
- Continous (only)	9 (5%)
- Both	26 (15%)

Types of AAS used	**n = 177**
Testosterone	142 (80%)
- Testosterone enanthate	101 (57%)
- Testosterone mixture (i.e. Sustanon)	54 (31%)
- Testosterone propionate	28 (16%)
- Testosterone cypionate	12 (7%)
Nandrolone	118 (67%)
Trenbolone	113 (64%)
Stanozolol	111 (63%)
Boldenone	71 (40%)
Oxandrolone	46 (26%)
Metenolone	43 (24%)
Drostanolone	33 (19%)
Oxymetholone	24 (14%)
Mesterolon	14 (8%)
Dihydromethyltestosterone	3 (2%)
Dihydroepiandrosterone	1 (1%)

***Figure 2*.** A. Bar chart of number of cycles completed by the patients. B. Bar chart of the mean cycle length (in weeks) performed by the patients.

A.



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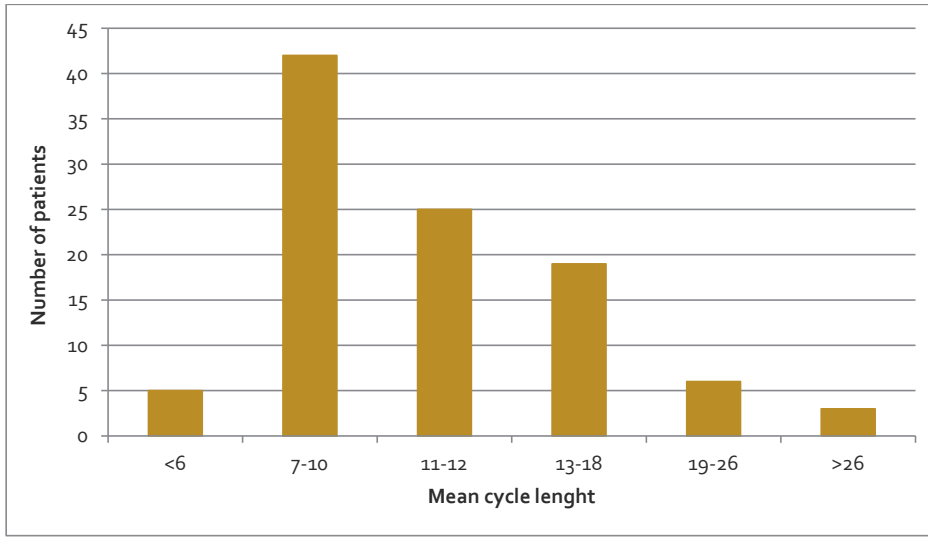

***Table 4*.** Other PED, medications and PCT used by patients. Numbers of patients and percentages are displayed in the right column.

Other PED	**n = 151**
Growth hormone	55 (36%)
Clenbuterol	46 (30%)
Thyroid hormone	35 (23%)
Efedrin	1 (1%)

Medications (on-cycle)	**n = 151**
Aromatase inhibitors	20 (13%)
Human choriogonadotrophin	19 (12%)
Clomiphene citrate	15 (10%)
Tamoxifen	14 (9%)
Isotretinoin	3 (2%)
Sildenafil	3 (2%)
Diuretics	2 (1%)
Finasteride	2 (1%)
Cabergolin	1 (1%)

Post-cycle therapy	**n = 151**
Tamoxifen	84 (56%)
Human choriogonadotrophin	62 (41%)
Clomiphene citrate	49 (32%)
Aromatase inhibitors	11 (7%)
Mesterolon	4 (3%)

Additional investigations

If appropriate, additional tests were performed to investigate the relationship between reported health issues and AAS use. The results of blood tests, urinalysis and semen analysis are summarized in *Table 6*. An elevated level of serum creatine kinase (CK) was observed in 45% of the tested patients. Of the 6 patients with a CK elevated 10 or more times the upper limit of the reference range, 5 used or recently finished using AAS. The CK restored to normal at repeat testing in the remaining patient. In one patient the liver biochemistry had been markedly abnormal during AAS use (7-8 times upper limit of ALAT reference range) but fully recovered when AAS were discontinued. An elevated plasma creatinine level (with a corresponding eGFR < 45 ml/min/1.73m^2 calculated by the MDRD-formula) was observed in 2 patients, of whom one had a previously diagnosed IgA nefropathy and the other used creatine ethyl esther (CEE) supplements daily. CEE is known to increase the plasma creatinine level, unrelated to kidney function.[7] After discontinuing the supplements, creatinine level returned to normal. Polycythemia (hematocrit > 55 %) occurred in 4 patients but was mild except for one patient who used a maintenance dose of Sustanon and required regular phlebotomy therapy.

***Table 5*.** Side effects reported. The number of patients with corresponding percentages in whom these side effects occurred are displayed in the right column.

Reported side effect	**n = 160**
Acne	60 (38%)
Decreased libido	60 (38%)
Gynecomastia	55 (34%)
Testicular atrophy	53 (33%)
Agitation	43 (27%)
Erectile dysfunction	32 (20%)
Mood problems	26 (16%)
Aggressiveness	18 (11%)
Fatigue	15 (9%)
Fluid retention	14 (9%)
Insomnia	10 (6%)
Diaphoresis	9 (6%)
Alopecia	8 (5%)
Infected site of injection	7 (4%)
Addictive behavior	6 (4%)
Subfertility	4 (3%)
Syncope	3 (2%)
Pruritus	2 (1%)
Sleep apnea	2 (1%)
Delusions	2 (1%)
Derealisation	1 (1%)
None	7 (4%)

Electrocardiography was performed in 24 patients and did not reveal any abnormalities except for one patient with positive voltage criteria for left ventricular hypertrophy. In one case, an MRI was executed to exclude a pituitary tumor as blood tests had revealed hyperprolactinemia. In another case, karyotyping was used to demonstrate the Klinefelter syndrome as blood tests had showed hypergonadotropic hypogonadism which is not expected after (previous) AAS use. Furthermore, in one case hepatitis virus serology and liver ultrasound was performed to analyze abnormal liver biochemistry. No explanation was found but a relationship with AAS use was less likely as the liver enzymes remained elevated after discontinuation of AAS.

***Table 6*.** Additional tests performed and corresponding results. The number of patients are displayed in the right column. Androgen deficiency was defined by a total and/or serum free testosterone level below the lower limit of the age-dependent reference range. Estimated glomerular filtration rate (eGFR) was calculated by the MDRD-formula. UL = upper limit of reference range.

Blood tests	**Definition**		**n = 152**
Elevated creatine kinase	CK > 170 U/l	1 – 3 x UL	34
		3 – 5 x UL	17
		5 – 10 x UL	11
		> 10 x UL	6
Abnormal liver biochemistry	ALAT > 45 U/l	1 – 2 x UL	27
		2 – 3 x UL	12
		3 – 4 x UL	5
		> 4 x UL	1
Androgen deficiency	< testosterone lower limit (variable)		53
Abnormal lipid profile	HDL-cholesterol < 0.9 mmol/l		32
Kidney damage	eGFR 45 – 60 ml/min/1.73m^2		7
	eGFR 30 – 45 ml/min/1.73m^2		1
	eGFR < 30 ml/min/1.73m^2		1
Polycythaemia	Hematocrit > 55%		4

Urine (dipstick) analysis		**n = 19**
No abnormalities		16
Proteinuria	+, ++, or +++	3

Semen analysis		**n = 9**
Normal fertility	Spermatozoa > 15 x 10^6/ml	3
Oligozoospermia	Spermatozoa < 15 x 10^6/ml	5
Azoospermia	Spermatozoa < 0,1 x 10^6/ml	1

Diagnoses and treatment

Treatment of symptoms usually consisted of patient education, reassurance and advice. The general recommendation was to not use AAS again. Gynecomastia was observed in 18 cases and treated with tamoxifen in 8 but usually relapsed after discontinuation. In total 6 patients with gynecomastia were referred to a plastic surgeon for extraction of breast tissue. Reduced fertility related to AAS use was diagnosed in 5 patients and treated temporarily with tamoxifen in 2 and hCG in 1. There is no follow-up data to confirm whether treatment was effective in these patients except for the patient treated with hCG who did not recover. Azoospermia due to AAS was not seen. One case of infertility was explained as posttraumatic obstructive azoospermia. Temporary and long-term (> 1 year) post-AAS-hypogonadism, defined as androgen deficiency due to AAS use, was established in 37 and 19 cases, respectively. Patients with long-term post-AAS-hypogonadism had a significantly longer history of AAS

use with a mean of 11 years compared to 6 years in the rest of the patient population (unpaired t-test, p = 0,001). In 17 patients with post-AAS-hypogonadism, tamoxifen was prescribed temporarily to enhance endogenous testosterone production. Testosterone substitution therapy was eventually instituted in 15 patients.

DISCUSSION

180 (former) AAS users visited the AAS clinic of the Spaarne Gasthuis in Haarlem, the Netherlands, between May 2011 and May 2016. The typical visitor of our clinic is a male, amateur strength athlete, who started using AAS in the 2nd decade of life. Anabolic steroids are mostly used in cycles with a duration between 6 and 18 weeks. The unproven rationale behind this strategy is to gain muscle mass and strength during a cycle, allowing the body to recover between cycles. Since muscle mass and strength start to decline after discontinuation of AAS, multiple cycles or continuous use are deemed necessary to maintain or further increase the gained muscle mass. Some have adopted the so-called "blast and cruise" strategy, in which cycles with multiple high dose AAS are alternated with a lower maintenance dose. The contents, dose, and duration of the cycles are mostly directed by advice from self-proclaimed experts and are based on unproven beliefs and personal experience. AAS cycles are rarely identical, which shows that, although most users have strong beliefs about which type, dose and combination of AAS should be optimal for their purpose, there are no widely accepted guidelines. Most AAS cycles contain a type of injectable testosterone ester, generally combined with nandrolone, trenbolone and/or boldenone esters. Our findings are in concordance with questionnaire studies performed among bodybuilders which found similar demographics and comparable characteristics of AAS use.[3,8] In addition to AAS, other substances are added, either to increase muscle mass (growth hormone) or to decrease fat mass (clenbuterole, thyroid hormone). Sometimes, medications are used to prevent or treat side effects during or after the cycle; aromatase inhibitors and tamoxifene (against gynecomastia and/or fluid retention), isotretinoin (to treat acne), human chorionic gonadotrophine (against testicular atrophy and/or reduced fertility), sildenafil (to treat erectile dysfunction) or finasteride (to prevent hair loss). As a result of prolonged AAS use, endogenous testosterone production and spermatogenesis are suppressed during and for weeks after the cycle. In an attempt to speed up hormonal recovery as well as to prevent symptoms of androgen withdrawal, most users take post-cycle therapy. PCT mostly consists of (a combination of) tamoxifen, clomiphene or hCG, usually taken for 2-4 weeks shortly after the end of the cycle with AAS. Although widely used, the efficacy of PCT remains to be determined.

Health risks of AAS abuse

There are several reasons why users of AAS may have an increased risk of health problems. Firstly, most of them fanatically engage in weight training which may lead to symptoms resulting from overburdened muscles, joints and tendons. Secondly, a considerable number of the visitors of our clinic admitted to using drugs, such as XTC, cocaine, cannabis and GHB. The high incidence of recent drug use has been reported previously. Survey studies showed that 23-33% of AAS users meet the criteria for substance dependence disorder compared to 11% of non-AAS users.[3,9-11] Most importantly, the use of high dose androgens and associated substances to treat side effects may have adverse effects. In our 180 patients no critical health issues occurred. The most severe complication was a serious skin

infection at the injection site. The large majority of our subjects nevertheless reported one or more side effects related to the use of AAS. The side effects and their frequency of occurrence correlate with those described in earlier studies.[8,12,13] Most users were familiar with common side effects such as acne, gynecomastia, testicular atrophy, fluid retention, agitation and fluctuations in libido and regarded them as inherent to the use of these substances. Most of these side effects were rated by them as mild or temporary and acceptable with respect to the perceived increase in muscle size and strength. A substantial proportion of the reasons for referral to our clinic were related to these side effects, especially when they persisted after discontinuation of the AAS. Side effects that may go undetected by the patient include high blood pressure, abnormal liver biochemistry, polycythemia, decreased HDL-cholesterol and decreased sperm count. Whereas high blood pressure and polycythemia were rare findings in our population, lower HDL-cholesterol was frequently encountered. Although low HDL-cholesterol is associated with an increased risk of cardiovascular disease in the general population, it is unclear if and to what magnitude this contributes to cardiovascular morbidity in AAS users, knowing that a decreased HDL-cholesterol may be transient. Mild elevation of ASAT and ALAT is a common finding in our subjects. Cholestatic liver damage is associated with use of oral AAS, since these compounds are alkylated to prevent extensive metabolism in the liver.[14] However, we believe that in most cases, mild elevation of ASAT and ALAT is not a sign of liver damage, but is due to muscle damage associated with extensive strength training. This is also suggested by a clearly elevated level of CK and normal levels of alkaline phosphatase and γ-glutamyl transferase in most subjects. Low sperm count results from suppression of the hypothalamo-pituitary-gonad axis during AAS use. Since supraphysiological doses of AAS are used, suppression of LH and FSH to levels below the limit of detection is inevitable. From male hormonal contraception studies it is known that it may take up to six months after the first injection of testosterone until sperm counts have decreased to 1 million/ml and that the magnitude of suppression may vary between men.[15] Both the extent of suppression and time to recovery of spermatogenesis will likely depend on the dose of AAS and cycle length. In our study we only performed semen analysis in the few cases in which reduced fertility was the reason for referral. Moreover, we did not have pre-cycle semen tests. Therefore we were unable to establish a causal relationship between AAS use and impaired semen quality.

Treatment of patients

Persisting side effects despite discontinuation of AAS were the most common reason for referral to the AAS clinic. Mostly, side effects were related to the (perceived) disturbance of male gonadal function, resulting in gynecomastia, erectile dysfunction, loss of libido and a range of less circumscribed symptoms that may be attributed to low testosterone levels. In most cases reassurance and advise to be patient for spontaneous relief of symptoms was sufficient. Persistent, painful gynecomastia was treated with tamoxifen, 20 mg daily. Although this relieved symptoms in the majority of cases, the chance of recurrence after cessation of therapy appeared to be high. Eventually, many patients chose surgery as a definitive treatment. Post-AAS-hypogonadism usually resolved without therapy within 6 months after the last injection of AAS. Testosterone substitution therapy was reserved for patients with long term post-AAS-hypogonadism who were motivated to lay off AAS indefinitely and had a significant symptom burden. It appeared that particularly the subjects with a high cumulative dose of AAS abuse were at increased risk of long term suppression of endogenous testosterone production. In these cases, endogenous testosterone levels were repeatedly slightly below or above the lower limit for young men and associated with symptoms such as erectile

dysfunction, loss of libido, fatigue and depressed mood. A particular drawback of testosterone treatment is that it stops recovery of the function of the hypothalamo-pituitary gonad axis. Especially when adequate spermatogenesis is important, a trial with tamoxifene 20 mg daily may be indicated instead. Tamoxifene acts as an estrogen antagonist on hypothalamus and pituitary and stimulates LH and FSH release by the pituitary. In our experience it mildly increases endogenous testosterone production and spermatogenesis. However, in most men testosterone levels decreased to its pre-treatment levels after stopping tamoxifene. When tamoxifene is not sufficient, hCG 1500 IU twice weekly mostly results in normalization of endogenous testosterone levels within weeks, followed by restoration of sperm concentration within months.

Limitations of the study

Our study has a few important limitations. Although our clinic is the only clinic dedicated to AAS in the Netherlands, it only provides care for a small minority of the total group of AAS users. It presents a selection of people that was (self-)referred because of side effects or problematic AAS abuse. Users without health issues would not consult the AAS clinic, therefore the estimated incidence of side effects in our study is probably exaggerated. Similarly, since only users with health problems were selected, their way of use may have been more hazardous. We were unable to verify the concentration and contents of the substances used by our patients. Evaluation, treatment and follow up of the subjects were not standardized. Patients were either past or current users, and the time between last AAS use and evaluation varied extensively. The current study is retrospective in nature and documentation was incomplete on many items. The documented percentages of types of AAS, PED and medications used as well as side effects reported are likely to be affected by recall bias and reporting bias. Additionally, we were unable to establish a causal relationship between AAS abuse and health issues or abnormal laboratory tests.

Nonetheless, this study is so far the most elaborate conducted in the Netherlands. Patients came from all over the country. Our experience with AAS users in the clinic and comparison with literature data have led us to believe that patient characteristics and the mode of AAS use as described in our study is representative for AAS abuse in general. We therefore believe this study gives a good insight into the practice of AAS abuse and its most common side effects and health risks.

Concluding remarks

This review of 180 patients referred to the AAS clinic suggests that AAS abuse does not structurally lead to severe health problems and critical side effects are limited to incidental cases as reported in literature. However, the incidence of side effects with a substantial symptom burden, reduced fertility, substance abuse dependence and potentially harmful concomitant use of other PED and medications among AAS users is high. Considering the large amount of users in the community, AAS abuse may be an important public health problem.[13] A prospective study with a systematic approach is required to provide more reliable data regarding short- and long term health risks of AAS abuse. Moreover, we need clinical trials to study the efficacy and long term effects of treatment.

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CHAPTER 2

SPONTANEOUS HEMORRHAGE OF HEPATIC ADENOMA IN A PATIENT ADDICTED TO ANABOLIC STEROIDS

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ABSTRACT

This case report describes a patient with a nearly fatal spontaneous haemorrhage of a hepatic adenoma that occurred in association with anabolic androgenic steroid (AAS) use. The patient was addicted to AAS and had been using exceptionally high dosages as well as growth hormone. After cessation of AAS use testosterone replacement therapy was started to prevent post-AAS-hypogonadism and consequent relapse.

INTRODUCTION

The use of anabolic androgenic steroids (AAS) by amateur strength athletes is widespread, with an estimated prevalence rate of 6%.[1] AAS are often used in cycles and are comprised of an injectable (intramuscular) testosterone ester and one or two other AAS types. Between cycles no AAS are used, allowing the pituitary-gonadal axis to recover. However, about 5% use AAS continuously. Besides AAS, other performance and image enhancing drugs (PIEDs) are commonly used, such as clenbuterol or growth hormone.[2]

Side effects are reported by virtually all users of AAS and include acne, gynecomastia, agitation, decreased libido, erectile dysfunction and depressed mood.[2] Illegally obtained medications are used to combat side effects, such as isotretinoin, tamoxifen and human chorionic gonadotrophin. Little is known about the long term negative health effects of AAS use, but these likely include cardiovascular toxicity[3] and hypogonadism,[4] We hereby present a case of a strength athlete who used extreme dosages of AAS and suffered a spontaneous haemorrhage of a hepatic adenoma.

CASE STUDY

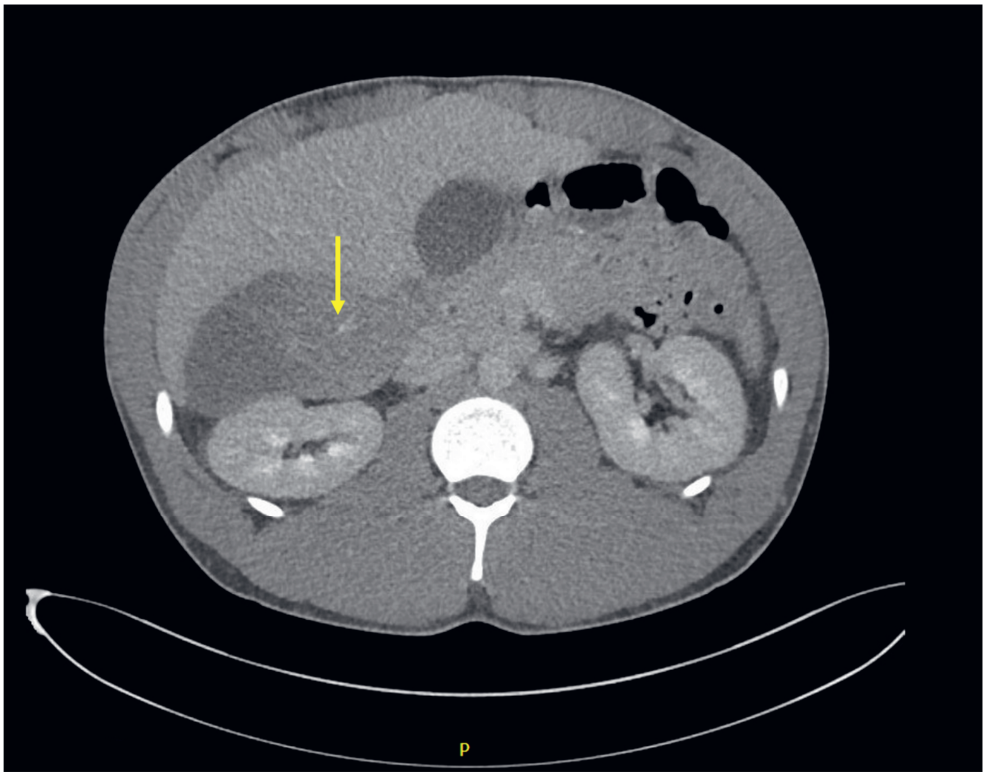
A 27 year old muscular male with an otherwise unremarkable medical history presented to the emergency room with sudden severe abdominal pain in the right upper quadrant. Computed tomography of the abdomen revealed a 15 cm large subcapsular liver hematoma in a lesion typical of adenoma. Two smaller adenomas were present in the liver. There was evidence of active bleeding (see *Figure 1*). Fluid resuscitation and blood transfusion was performed on the intensive care unit. A branch of the right hepatic artery was coiled to control the persistent bleeding and prevent multi organ failure as well as the need for emergency laparotomy. Continuous venovenous hemofiltration was necessary for several days because haemorrhagic shock had led to acute tubular necrosis.

The patient admitted he had been using AAS for the past 5 years. Before, he had struggled with cocaine, cannabis and alcohol addiction as well as depression. He started using a limited amount of AAS in cycles separated by several months. Afterwards, intervals shortened, the number of AAS types and dosages increased, and the last 3 years he had been alternating cycles with a high maintenance dose ('blast and cruise'). His cycle prior to the haemorrhage comprised more than 10 different AAS types. During the peak of this cycle he injected 60 ml of AAS per week, which is around 15.000 milligrams of testosterone equivalents. Besides AAS, he was concurrently using growth hormone (3,5 IU daily), clenbuterole, levothyroxin, tamoxifen, and anastrozole, next to protein, vitamin and mineral supplementations. The patient explained his escalated AAS use by a high level of ambitiousness and a deep-seated distortion of his self-image.

After the patient was transferred to the gastroenterology ward, he needed opiates for heavy abdominal pains due to pressure of the hematoma on the liver capsule. The hematoma became infected with *Staphylococcus aureus* after hematogenic spread from an intravenous catheter. He received treatment with clindamycin after flucloxacillin had caused Stevens-Johnson syndrome.

Antibiotic treatment was complicated by relapsing *Clostridium difficile*-associated diarrhoea for which he received vancomycin. The patient developed severe peripheral oedema caused by intermittent bleeding inside the hematoma that compressed the right atrium of the heart (see *Figure 2*). The oedema resolved after a percutaneous drain was inserted into the hematoma to release 8 litres of stale blood.

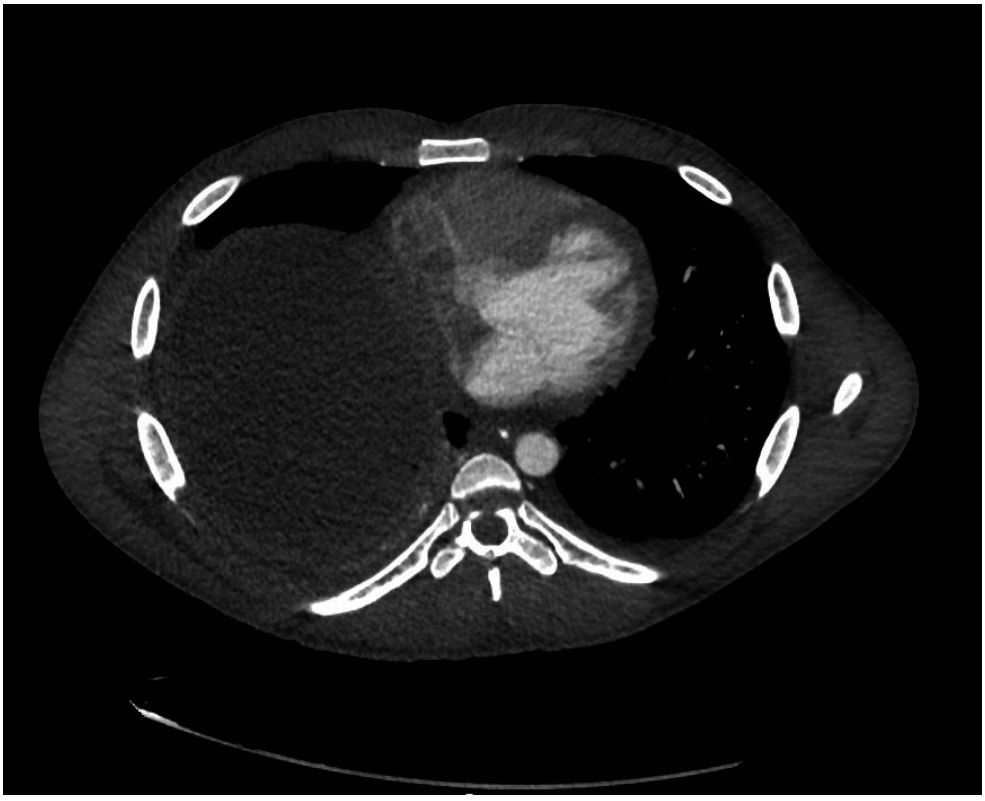
***Figure 1*.** Axial image of a computed tomography scan of the abdomen. A large subcapsular hematoma is visible in the right liver lobe originating from a round, well-demarcated, heterogenous lesion with nodular attenuation, most probably a hepatocellular adenoma. Contrast extravasation inside the hematoma (yellow arrow) is a sign of active bleeding. Note the large size of the iliopsoas and erector spinae muscles.



Almost 2 months after admission the patient was discharged. During hospital admission the patient did not use AAS and his testosterone concentration gradually declined from 195 nmol/l to 12 nmol/l. Gonadotropin levels were undetectable at all times. Replacement therapy with an injectable blend of testosterone esters was started on an outpatient basis when the testosterone concentrations became insufficient, with a limited issue of vials per prescription. The patient was followed-up alternately by an endocrinologist and addiction specialist at short intervals to survey hormone therapy, address the AAS addiction and muscle dysmorphia, and prevent relapse of AAS use. With magnetic resonance

imaging (MRI) the liver adenoma was not visible anymore. One year after the hospital admission the patient is in good health and has not used AAS or other illicit drugs again.

Figure 2. Axial image of a computed tomography scan of the chest. The liver hematoma compresses the right atrium of the heart obstructing venous return and causing peripheral oedema.



DISCUSSION

The occurrence of hepatic adenomas is very rare among men not using AAS, with an estimated incidence of less than 1 per million.[5] Spontaneous haemorrhage is a known complication of hepatic adenoma. The association between androgens and hepatic adenomas was noticed previously in patients treated for hereditary angio-oedema[6] and Fanconi anaemia[7] and has been reported in users of AAS as well.[8] The exact incidence of hepatic adenomas among users of AAS is unknown but the majority probably goes unnoticed. AAS presumably induce hepatic adenomas through the action of oestrogens derived from aromatization of AAS.[9] There appears to be a dose-dependent relationship between sex hormones and liver tumour occurrence, which may explain the size and spontaneous haemorrhage of the adenoma in our patient.

The presented case illustrates the possible dramatic course of AAS addiction. Although the patient started with separate AAS cycles, he eventually used AAS continuously. The applied dosages in the months before the liver haemorrhage were astronomical with 15.000 mg of testosterone equivalents per week, which by far is the most reported by an AAS user based on our experience from the AAS clinic – an outpatient clinic for past users of AAS in the Netherlands. In an earlier survey, the average dose of AAS equivalents used during a cycle was approximately 1000 mg per week, which is already a tenfold physiological amount.[10]

As much as 30% of AAS users at some point develop a certain degree of AAS addiction. Addiction to other recreational drugs and muscle dysmorphia were risk factors in our patient that predisposed to AAS addiction.[11] Treating a patient for AAS addiction should be a joint effort between endocrinologist and addiction specialist. In this patient, due to prolonged suppression of the pituitary-gonadal axis, recovery of normal endogenous testosterone was unlikely in the short term, i.e., there was a high likelihood of a permanent post-AAS-hypogonadism.[2] Symptoms of testosterone deficiency would increase the urge to use AAS again. Therefore we started testosterone replacement therapy as soon as hypogonadism occurred, which took several months due to the long half-life of certain testosterone esters, e.g. decanoate. Health parameters such as liver enzymes, haematocrit and cholesterol were monitored. An addiction specialist with an interest in AAS addiction applied cognitive behavioural therapy. Although this treatment was successful in our patient in the first year, the risk of relapse remains high.

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CHAPTER 3

A 29 YEAR OLD BODYBUILDER WITH LIOTHYRONINE-INDUCED THYROTOXIC HYPOKALEMIC PERIODIC PARALYSIS

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ABSTRACT

We describe a 29 year old male bodybuilder with recurrent attacks of myalgia and muscle weakness associated with hypokalemia and thyrotoxicosis due to abuse of liothyronine. The attacks quickly resolved after potassium supplementation and stopping liothyronine. We concluded that the patient suffered from thyrotoxic hypokalemic periodic paralysis (TPP). Although muscle weakness and hypokalemia are prominent symptoms of TPP, underlying thyrotoxicosis may be overlooked. Up to 25% of androgen abusers also abuse thyroid hormone. Lack of recognition of thyroid hormone abuse as a cause of hypokalemic periodic paralysis may result in unnecessary, potentially harmful medical investigations and improper treatment and advice.

CASE PRESENTATION

A 29-year old Caucasian male presented at the emergency room with progressive symptoms of myalgia and muscle weakness since three days. Over a period of six years prior to presentation, he had been experiencing six similar episodes of acute muscle weakness, with spontaneous recovery within hours. At previous emergency room presentations and during previous hospital admissions, laboratory tests were unremarkable except for varying serum potassium levels with incidental hypokalemia. Laboratory tests for the evaluation of thyroid function had not been performed previously. Extensive neurological evaluation, including spinal fluid analysis, magnetic resonant imaging of the spine and electro myogram had not shown abnormalities.

Patient was a heavyweight bodybuilder and admitted to use performance enhancing drugs, among others testosterone-enanthate, masterone-enanthate, metandienone and human growth hormone. He also admitted the use of liothyronine on a regular base in a dose between 50 and 100 micogram per day over the past 8 years. Physical examination at presentation showed a muscular young man (weight 128 kilogram, height 190 centimeters), with a blood pressure of 133/51 and a pulse of 110 BPM. Neurological examination revealed mild muscle weakness in legs and arms but was otherwise unremarkable. The patient did not report diarrhea and denied the use of diuretics.

Blood tests were consistent with the use of exogenous liothyronine an showed severe hypokalemia (table 1). Oral potassium supplementation was started and myalgia and muscle weakness recovered spontaneously over the course of a few hours. The patient was explicitly advised to stop using liothyronine and was discharged without further treatment.

Table 1. Lab results for potassium and thyroid function at first presentation and follow-up

	First presentation	After 2 weeks	After 3 weeks
Potassium (3.4-4.9 mmol/L)	2.7	1.5	4.3
FT3 (3.5-6.5 pmol/L)	15.4	2.1	4.5
FT4 (11.0-21.0 pmol/L)	3.3	3.1	11.2
TSH (0.35-5.0 mU/L)	0.01	0.01	2.8

Despite initial improvement, symptoms of muscle weakness persisted over subsequent weeks. Two weeks after initial presentation the patient was presented to the emergency unit because of rapid progression of muscle weakness accompanied with myalgia and the development of heavy chest pain. Blood tests revealed severe hypokalemia, suppressed concentrations of TSH and free T4, but a normal level of free T3 (table 1). Despite strict medical advice, the patient admitted to have used T3 since last presentation. Neurological examination provided no new symptoms in comparison to previous episodes.

The patient was admitted for intravenous potassium supplementation. Other causes for hypokalemia (i.e. dietary deficiencies, diarrhea, medication, alkalosis) were ruled out. With normalization of serum potassium levels, the muscle weakness resolved. From this point on, patient completely abstained of exogenous T3. Blood tests for thyroid function were repeated one week later, and showed normal thyroid function. Genetic screening did not show mutations in CACNA1S and SCN4A genes.

DISCUSSION

We present a patient with bouts of myalgia and muscle weakness associated with hypokalemia and misuse of thyroid hormone. After exclusion of other, more prevalent, causes of hypokalemia and muscle weakness, we concluded that the patient suffered from thyrotoxic periodic paralysis (TPP). This is a rare, but serious complication of thyrotoxicosis characterized by episodes of muscle paralysis and hypokalemia. An attack is characterized by recurrent, transient episodes of muscle weakness that range from mild weakness to complete paralysis. Patients may experience recurrent episodes of weakness that last from a few hours up to 72 hours, with complete recovery in between the attacks. There may also be prodromal symptoms of aches, cramps, and stiffness of the affected muscles.[1,2]

The symptoms are the result of a massive shift of potassium from the extracellular compartment into the intracellular compartment. This shift is facilitated by an increased number and activity of sodium-potassium pumps in the cell membrane of thyrotoxic patients. Attacks may be triggered by circumstances that induce intracellular influx of potassium, such as hyperinsulinemia, adrenergic activity and recovery after exercise.[1,2]

In Western countries, the incidence of this syndrome is low, probably around 0.1–0.2% in thyrotoxic patients. Patients of Asian descent are much more affected than Caucasians which suggests a genetic predisposition to the development of TPP. Mutations in the CACNA1S and SCN4A genes that are associated with familial hypokalemic periodic paralysis are normally absent.[2] Recently it was demonstrated that up to 33% of patients with TPP have a mutation in the gene that encodes for Kir 2.6, a potassium channel in muscle cells that is regulated by thyroid hormone.[3] Males are much more affected than females. The reason for this is unknown but suggests a role in the pathogenesis of TPP for androgens. TPP is normally seen in patients with thyroid dysfunction such as Graves disease and toxic goiter, which are the most prevalent causes of hyperthyroidism. However, TPP has been described in patients with thyrotoxicosis of all possible causes, including thyroid hormone abuse.[1,2]

The abuse of thyroid hormone is well established among androgen abusers for the purpose of reducing fat mass. In a recent survey in our clinic, 15% of androgen abusers also included thyroid hormone in their anabolic steroid regime[4] and 23% reported to have ever used thyroid hormone for non-medical reasons.[5] Adrenergic drugs, such as clenbuterol, ephedrine and caffeine are also used by a considerable number of androgen abusers to reduce fat mass or to improve training intensity.[4,5] In combination with strenuous exercise, all these factors may precipitate TPP.

In the acute care setting; rapid reversal of hypokalemia is the first step in treating TPP. However, since there is no actual body potassium deficit, massive potassium supplementation is contraindicated to prevent rebound hyperkalemia. Oral or intravenous administration of propranolol, has been advocated to rapidly reverse muscle weakness. Obviously, the cause of thyrotoxicosis should be established and treated. In the meantime, the patient should be instructed to avoid strenuous exercise and high carbohydrate diets. Oral propranolol may be effective to prevent attacks while thyrotoxicosis is being resolved.[2,3]

In conclusion, this case illustrates that in patients with recurrent episodes of muscle weakness and hypokalemia, TPP should be included in the differential diagnosis and thyroid function should be evaluated. Additionally, in young, muscular men, undisclosed use of thyroid hormone should be suspected.

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CHAPTER 4

BASELINE CHARACTERISTICS OF THE HAARLEM STUDY: 100 MALE AMATEUR ATHLETES USING ANABOLIC ANDROGENIC STEROIDS

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ABSTRACT

Background

The use of anabolic androgenic steroids (AAS) is common among visitors of fitness centers. Knowledge about health risks of AAS use is limited due to lack of clinical studies.

Methods

One hundred men, at least 18 years old, intending to start a cycle of AAS were recruited. Baseline demographical data and reasons for AAS use were recorded. Subjects provided samples of AAS for analysis with UPLC-QTOF-MS/MS.

Results

One hundred and eleven men were seen for a baseline visit. Nineteen percent had competed in bodybuilding competitions. Recent illicit drug use was reported by 56%. Seventy-seven percent of participants had used AAS in the past and 97% of them had experienced side effects. After exclusion, 100 men comprised the cohort for follow-up. The AAS cycle performed had a median duration of 13 weeks (range 2-52) and the average dose of AAS equivalents was 901 mg per week (range 250-3.382). Subjects used other performance and image enhancing drugs (PIEDs) such as growth hormone (21%). In total 272 AAS samples were analyzed and 47% contained the AAS indicated on the label. The principal reason for AAS use was gain of muscle mass (44%). Forty-eight percent self-reported to being addicted to AAS.

Conclusion

The HAARLEM-study cohort shows that strength athletes use AAS in a wide variety of cycles and often also use illicit drugs and other potentially harmful PIEDs. The quality of the AAS used is strikingly low. Follow-up of the cohort will provide novel data regarding health risks of AAS use.

INTRODUCTION

It is estimated that 1-6% of regular visitors of fitness centers use anabolic androgenic steroids (AAS),[1-5] but prevalence may be as high as 30%,[6,7] depending on country, region and type of fitness centre. Although production and trading of AAS without a license is prohibited in most countries, AAS can be easily acquired illegally through local dealers or the internet. Discussion forums on the internet are an accessible way for users of AAS to seek and share information and become more acquainted with methods of AAS use.[8] These forums are usually teemed with anecdotal evidence ('broscience') and pro-AAS information impelling and justifying the use of AAS.[9]

AAS are harmful but data are lacking as to how harmful they are precisely. Knowledge of the unwanted somatic and psychological effects of AAS is based on low-level evidence, such as expert opinion, case reports and cross-sectional or retrospective studies. Furthermore, official pharmacological data of testosterone derivates concern patients as opposed to athletes, clinical dosages are a fraction of dosages used by athletes, and athletes use mostly unofficial delivery and production lines of AAS. There is, however, reasonable evidence indicating that AAS use is associated with injuries such as tendon ruptures,[10] cardiovascular toxicity,[11,12] psychopathology such as mood and anxiety disorders,[13] and hypogonadism after cessation of use – so-called post-AAS-hypogonadism.[14-17]

An outpatient clinic for (former) users of AAS, the 'Anabolenpoli' or AAS clinic, in Haarlem, the Netherlands, attempts to gain more insight into health risks associated with AAS use. An overview of 180 patients referred to the clinic between 2011 and 2016 was published recently.[16] This analysis was informative about current methods of AAS use and side effects, but data were retrospective in nature, documentation was incomplete on many variables, and selection bias was an important issue.

Therefore, a prospective observational study with a systematic approach was initiated to provide more reliable data regarding health risks of AAS abuse: the HAARLEM-study (acronym for Health risks of Anabolic Androgenic steRoid use by maLE aMateur athletes). We assembled a cohort of 100 men intending to start a cycle of AAS. Health analysis comprising e.g. blood, urine and semen analysis, will be performed before, during and after the cycle. Follow-up will continue up to 1 year after the start of the cycle. This report focuses only on the baseline data of the HAARLEM-study. The aim is to provide a reliable overview of sociodemographic characteristics of users of AAS, reasons for AAS use, methods of AAS use with AAS cycle characteristics, and quality of AAS used. Data of the health analysis during follow-up will be described in a future report.

MATERIALS AND METHODS

Subjects

Men eligible for participation in the HAARLEM-study were at least 18 years old and intending to start a self-initiated cycle of AAS on short notice, i.e., within two weeks after signing informed consent. The AAS had to be acquired through their usual channels. The scheduled cycle had to be at least 6 weeks, comprise at least two different types of AAS (or one type of AAS that contained different AAS esters), and average a weekly dose of 200 mg or more. These criteria were selected because lower doses are highly unusual among AAS users and probably have a low risk of causing significant side effects. An exclusion criterion was AAS use 3 months prior to informed consent. Men were also refused to participate in the study if within 6 months prior to inclusion a new chronic or psychiatric illness had been diagnosed, treatment for a chronic illness had been started or altered significantly, or a psychiatric or somatic illness other than trauma had led to admission to a psychiatric institution or hospital, respectively.

Recruitment

The study was approved by the local Medical Ethical Committee (MEC registration M015-019, study number NH015.189) in October 2015 after which inclusion of subjects started. Awareness of the HAARLEM-study was created on a large scale by presenting its concept on national television and in regional newspapers. The study was promoted in online messages published by several websites known to be visited frequently by men that engage in strength sports (e.g. bodybuilding discussion forum, news website for health and fitness). Subjects did not receive any reward or reimbursement for participating.

Data collection

All data were imputed into a research database anonymously. Demographic variables were documented, as well as medical history, current medication use, family history, (recent, i.e. <3 months) drug abuse, sports history, and present training frequency. Historic AAS use was registered in detail, including side effects experienced. A semi-structured interview was performed addressing reasons for taking part in the study, as well as reasons and immediate cause for AAS use. Important issues with regard to AAS use such as approval by friends and family, addictiveness, financial burden and future plans were explored. The AAS cycle performed by subjects during follow-up was recorded in detail, with duration and dosage of each type of AAS used, in addition to use of other performance and image enhancing drugs (PIEDs), medication and post-cycle therapy (PCT). Cumulative dose of the AAS cycle was calculated by adding up the total used mg of AAS equivalents of every cycle week, using the product label information of the AAS.

Analysis of AAS

Subjects were requested to bring the AAS products of their scheduled cycle to the intake i.e. enrollment session. Of every liquid AAS a 1 ml sample was taken along with 1-2 pills of every oral AAS. Brand, AAS subtype, and dosage in mg or mg/ml were recorded. Samples were shipped in batches to the National Institute for Public Health and the Environment in Bilthoven, the Netherlands, which

carried out and funded this analysis. Qualitative analysis of the AAS was performed using ultra-high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS/MS). The aim was to analyze at least 200 AAS samples. Results were not disclosed to the investigator before the last subject was enrolled and not to subjects before the end of the study. Subjects did not receive any compensation for the delivered samples.

Statistical analysis

Simple descriptive statistics were used to display quantitative data. If the variables were normally distributed, mean and standard deviation were calculated. If the distribution of a variable was skewed, a median is presented with range. Multivariable linear regression analysis (using Stata/SE 14.1 for Windows) was used to assess the relationship between sociodemographic variables (age, body mass index, education, social status, recent use of ≥2 drug types, competitive bodybuilding, weekly training time, and previous AAS use) and AAS cycle characteristics (cycle duration, average weekly dose, cumulative dose, number of AAS types). Chi-square test (χ^2) was used to compare the quality of AAS samples obtained through dealers and/or friends versus the internet.

RESULTS

Between October 2015 and May 2018, a total of 220 men signed up for participation in the HAARLEM-study. Many were not eligible because they had recently used AAS, were still using, or had already started a cycle (16 subjects), had a cycle that did not meet the inclusion criteria (19 subjects; e.g. a cycle with only one type of AAS), did not agree with the study design (10 subjects; e.g. requesting to receive health or AAS analysis results straight away) or were not intending to start a cycle on short notice (25 subjects). Others changed their minds about participation (7 subjects; e.g. too much effort), never answered to an invitation after initial registration (27 subjects) or did not show up at the intake appointment (5 subjects). No subjects were ineligible based on health criteria. To reach a sample size of 100, a total of 111 subjects visited the AAS clinic for an intake session, as 11 would later turn out to either not have timely started a cycle after the intake (3 subjects) or have withdrawn consent before the end of their cycle due to various – in particular not medical – reasons (8 subjects).

Demographic data

Data of all 111 initially included subjects were available. The average age was 31 years (standard deviation, SD ± 8.4) at time of inclusion (see *Table 1*). Average body mass index (BMI) was 27.0 kg/m^2 (SD ± 3.2). 17% of subjects reported a history of psychiatric illness, of which attention deficit (and hyperactivity) disorder (7%) and past depression (3%) were most common. Eighty-nine percent had completed or attended at least a vocational education and 96% was currently employed or student. Forty-nine lived together with a partner, 93% considered himself to be heterosexual, and 24% was father of at least one child. Recent (<3 months) illicit drug use was reported by 56%, mainly ecstasy/amphetamines (34%), cocaine (23%) and cannabis (23%). At time of inclusion, 99% actively engaged in strength sports, averaging 306 minutes of training per week at the gym. Nineteen percent had been a contestant in a bodybuilding competition. Besides fitness, 43% of subjects was or had been active in combat sports.

Table 1. Characteristics of the 111 initially included subjects as recorded at baseline. SD, standard deviation.

General	**n (%), median (SD, ranges)**
Male	111 (100%)
Age	31 (±8,4; 19-67)
Height (cm)	182 (±7,0; 166-199)
Weight (kg)	88 (±12,8; 60-139)
Body mass index (BMI, kg/m^2)	27 (±3,2; 20,8-41,5)

Educational level	**n (%)**
Primary school	1 (1%)
Pre-vocational secondary education	7 (6%)
Senior general secondary education	4 (4%)
Secondary vocational education	51 (46%)
Higher professional education	32 (29%)
University education	16 (14%)

Occupational status	**n (%)**
Student	16 (14%)
Employed	91 (82%)
Unemployed	4 (4%)

Social status	**n (%)**
Living with parents	17 (15%)
Single	40 (36%)
Living together (non-marital)	41 (37%)
Married or civil partnership	13 (12%)
Father (of at least 1 child)	27 (24%)

Recent drug use (<3 months)	**n (%)**
Nicotine	36 (32%)
Alcohol	78 (70%)
Ecstasy/amphetamines	38 (34%)
Cannabis	26 (23%)
Cocaine	26 (23%)
Gamma-hydroxybutyric acid (GHB)	23 (21%)
Ketamine	3 (3%)
LSD, heroin, opiates	0 (0%)

No drugs (except alcohol/nicotine)	49 (44%)
≥2 types of drugs (except alcohol/nicotine)	31 (28%)

Current sport	**n (%)**
Fitness/bodybuilding	110 (99%)
Competitive bodybuilding	21 (19%)
Weight lifting	3 (3%)
Strongman athlete	3 (3%)
Combat sports (e.g. kickboxing, karate, judo)	48 (43%)

Current fitness schedule	**Median (SD, ranges)**
Number of training sessions (/week)	4 (±1,4; 0-7)
Duration of training sessions (minutes)	73 (±18; 30-120)
Time weekly spent at gym (minutes)	306 (±120; 60-720)

History of AAS use

Eighty-six (77%) of 111 initially included participants had used AAS in the past. Median age of first AAS use in this group was 24 years (range 14-49; see *Supplementary table 1*). The median number of cycles performed was 4, the median length of an AAS cycle was 12 weeks, and a median of 3 different AAS types were used each cycle. Post-cycle therapy (PCT) had been performed by 81% of subjects after at least 75% of cycles, using a median number of 2 PCT agents for a median duration of 4 weeks. AAS were obtained through dealers/acquaintances or internet by 90% and 23% of subjects, respectively. The most common types of AAS used were testosterone (95%), stanozolol (79%) and boldenone (71%).

Ninety-four percent of subjects had used proteins and/or various legal nutritional supplements (e.g. vitamins, minerals) and 79% had been using creatine. Of the subjects that had used AAS before, 36%, 53% and 33% had also used growth hormone, clenbuterol and thyroid hormone, respectively (see *Supplementary table 2*). Medications most frequently used, either to treat side effects or as PCT, were tamoxifen (71%), human chorionic gonadotropin (59%), clomiphene citrate (55%) and aromatase inhibitors (49%).

Desired effects of AAS use, mainly increased muscle mass and increased strength, were reported by 94% subjects who had used AAS (see *Table 2*). Other desired effects were decreased fat mass (81%), increased energy (76%) and enhanced concentration (56%). Side effects were reported by 97% of subjects, especially fluid retention (79%), acne (58%) and agitation (47%) during the cycle, and decreased libido after the cycle (58%). Increased libido was experienced by 76 (88%), but whether this was a desired effect or side effect differed among subjects.

Cycle characteristics

The 100 subjects that were followed-up performed an AAS cycle with a median duration of 13 weeks (range 2-52) and used a median of 4 types of AAS (range 1-11; see *Table 3*). The median dose of

(equivalents of) AAS was 901 mg per week and the median cost of an AAS cycle (including other PIEDs) was €400, which converts to an average of €30 per week. Eighty-one percent of subjects retrieved their AAS through local dealers and/or acquaintances. Testosterone was used by 96% of subjects, followed by trenbolone (52%) and drostanolone (39%).

***Table 2*.** Desired effects and side effects of AAS use experienced by 86 of 111 included subjects that had used AAS in the past.

Desired effect	**n (%)**
Increased muscle mass	81 (94%)
Increased strength	81 (94%)
Decreased fat mass	70 (81%)
Increased energy	65 (76%)
Improved concentration	48 (56%)

Side effects	**n (%)**
Increased libido*	76 (88%)
Fluid retention	68 (79%)
Testicular atrophy	61 (71%)
Acne	50 (58%)
Decreased libido**	50 (58%)
Agitation	40 (47%)
Increased fat mass**	39 (45%)
Gynecomastia	36 (42%)
Aggressiveness	35 (41%)
Striae	34 (40%)
Severe snoring	34 (40%)
Erectile dysfunction**	33 (38%)
Palpitations	30 (35%)
Painful injection site	29 (37%)
Addictive behaviour	23 (27%)
Alopecia	22 (26%)
Persistent erections	21 (24%)
Fever	16 (19%)
Pain during micturition	9 (10%)
Hyperhydrosis	7 (8%)
Subfertility	7 (8%)
Insomnia	6 (7%)
Depressed mood**	3 (3%)
Syncope	2 (2%)

Tendon rupture	1 (1%)
Fatigue	1 (1%)
Jaundice	1 (1%)
Increased hair growth	1 (1%)
None	3 (3%)

* Increased libido was sometimes appreciated as a positive effect.

** Side effect (usually) occurred after and not during AAS cycle.

Table 3. Details of AAS cycles and PCT of the 100 subjects included in follow-up.

Characteristics of AAS cycle	**Median (ranges)**
Cycle duration (weeks)	13 (2-52)
Types of AAS used	5 (1-11)
Cumulative cycle dose (mg)	13.200 (700-74.410)
Average weekly dose (mg)	901 (250-3.382)
Cost of AAS cycle (€)	400 (50-6.500)
Average weekly cost (€)	30 (4-300)

Routes of obtaining AAS	**n (=%)**
Dealers/acquaintances	81
Internet	19
Pharmacy abroad	2
Personal trainer	1

Types of AAS used (as indicated on label)	**n (=%)**
Testosterone	96
Trenbolone	52
Drostanolone	39
Boldenone	38
Nandrolone	33
Stanozolol	29
Methandienone	25
Oxandrolone	23
Mesterolon	19
Metenolone	17
Oxymetholone	15
Dehydrochlorotestosterone	2
19-Norandrostenedione	1

Trestolone	1
Fluoxymesterone	1
Methylepitiostanol	1

Post-cycle therapy (PCT)	**n (=%), median (range)**
Use of PCT	80
Length of PCT (weeks)	4 (1-8)
Number of PCT agents	2 (1-4)

Other PIEDs were used by 57 subjects (see *Table 4*), most often growth hormone (21) and clenbuterol (19). Medications to prevent or treat side effects during the cycle were used by 55 subjects, mainly hCG (26), tamoxifen (23) and anastrozol (22). PCT was performed by 80 subjects for a median duration of 4 weeks (range 1-8). The usual agents were tamoxifen (56), clomiphene citrate (54) and hCG (44).

***Table 4*.** Use of appearance and performance-enhancing drugs (PIEDs) – other than legal nutritional supplements such as creatine, proteins and vitamins – and medications during the cycle by the 100 subjects included in follow-up, and PCT medication used by the 80 subjects that performed PCT.

Other PIEDs	**n (=%)**
Growth hormone	21
Clenbuterol	19
Thyroid hormone	15
Insulin	7
ECA-stack (ephedrine, caffein, aspirin)	7
Insulin-like growth factor 1 (IGF-1)	3
Ephedrine	3
Growth hormone releasing hormone (GHRH)	2
Amphetamine	2
Sibutramine	1
Selective androgen receptor modulator (SARM)	1
Dinitrophenol (DNP)	1
L-carnitine (intramuscular)	1

Medication during cycle	**n (=%)**
Human chorionic gonadotropin (hCG)	26
Tamoxifen	23
Anastrozole	22
Exemestane	9
Isotretinoin	6
Diuretics	4

Clomiphene citrate	4
Bromocriptine	3
Finasteride	3
Cabergolin	3
Letrozole	3
Placental growth factor (PGF-)peptide	1
Modafinil	1

Medication as PCT	**n (%)**
Tamoxifen	56 (70%)
Clomiphene citrate	43 (54%)
Human chorionic gonadotropin (hCG)	35 (55%)
Anastrozole	4 (5%)
Mesterolone	4 (5%)
Exemestane	2 (3%)
Human menopausal gonadotropin (HMG)	2 (3%)
Selective androgen receptor modulator (SARM)	1 (1%)

In a multivariable linear regression model assessing the relationship between sociodemographic variables and AAS cycle characteristics, there was a positive correlation between previous AAS use and longer AAS cycles (β = 5,78, 95%-confidence interval [CI] 0,15-11,4, p = 0,04), higher average weekly dose (β = 334, 95%-CI 76,2-592, p = 0,01), higher cumulative AAS dose (β = 11.821, 95%-CI 3539-20.102, p < 0,01) and higher number of AAS types (β = 1,77, 95%-CI 0,15-3,38, p = 0,03). Age, BMI and level of education were not independently associated with cycle duration and dose. Competitive bodybuilding was only associated with a higher number of AAS types (β = 3,51, 95%-CI 1,90-5,12, p < 0,01).

Quality of AAS used

All subjects provided AAS samples. A total of 272 AAS samples from 46 different brands, supplied by the first 55 enrolled subjects, were analyzed by UPLC-QTOF-MS/MS (see *Table 5*). The specific AAS subtype as declared on the label was detected in 129 (47%) samples. Forty-eight of all samples were bought online, and of these 34 (71%) contained the AAS subtype declared on the label (χ^2 12,81, p < 0,01)**.** In 184 (68%) samples undeclared AAS were found. A hundred and thirty-four (49%) samples contained a higher number of AAS than declared. In only 35 (13%) of samples we identified solely the declared AAS. No AAS brand showed consistent results. Furthermore, 60 (22%) of all received AAS samples were a duplicate (i.e. different subjects delivering the same AAS subtype from the same brand) and in only 20 (33%) the analysis result was the same. In 6% of samples, no AAS were found at all.

Undeclared non-AAS pharmacologically active compounds were found in 39 (14%) of analyzed samples. In 37 samples estrogens or progestogens were detected. Other non-AAS compounds detected were diuretics (2), anaesthetics (2), corticosteroids (1), anti-androgens (1) and anti-estrogens (1).

Table 5. Test results of the AAS samples delivered by the 111 initially included subjects. Analysis was performed with ultra-high performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS/MS).

Qualitative AAS sample analysis	**n (%)**
Number of samples analyzed	272 (from 46 brands)
Median number of AAS found in a single sample	2 (SD ±1,4; range 0-7)
Samples containing:	
- Declared AAS only	35 (13%)
- Declared and undeclared AAS	94 (35%)
- Undeclared AAS only	128 (47%)
- No AAS	15 (6%)

Other pharmaceuticals identified	**n (%)**
No other pharmaceuticals indentified	233 (85%)
Estrogens/progestogens	37 (14%)
Diuretics	2 (1%)
Anesthetics	2 (1%)
Corticosteroids	1 (0%)
Anti-androgens	1 (0%)
Anti-estrogens	1 (0%)

Reasons for and issues of AAS use

Data of all 111 initially included subjects were available. Gain of muscle mass was the reported reason for AAS use in 44% (see *Supplementary table 3*). Twenty-six percent of subjects were convinced they could not improve their current level of strength and needed AAS to reach a new level in their training. Aesthetics were also a motivation for AAS use, with reasons such as trying to achieve a more perfect or ideal body (18%) or becoming more attractive (3%). Fifteen percent of subjects needed AAS to be a significant competitor in their sports, mainly bodybuilding.

The immediate cause for first AAS use was brought about by friends who were familiar with using AAS (35%), a perceived necessity to compete (8%), advice from personal trainers (7%), or appealing results found on the internet or published by the media (5%). Thirty-two percent of subjects took the initiative to start using AAS entirely by themselves and usually had carried out extensive research beforehand. Twenty-nine of subjects have friends that use AAS or acquaintances at the gym, 8% encounter many users in their sports circuit, and 7% have a partner or family member that uses AAS. Thirty-eight of subjects are reluctant to disclose to others that they use AAS. When they do reveal their AAS use, significant others disapprove (36%), seem indifferent (32%), and rarely encourage (5%) AAS use.

Forty-eight percent of subjects that had used AAS before self-reported to being addicted. Positive physical and psychological effects of an AAS cycle turned out to be most compelling, as reported by

41% and 32% of this group, respectively. Twelve percent of all subjects indicated to recognize some signs of dependency but claim to be still entirely in control of their use. Forty-one percent do not perceive themselves to be addicted at all – 31% of this group reported they could certainly imagine others being addicted. Ninety percent do not find AAS expensive; 3% quit hobbies or other activities to be able to afford regular AAS use.

DISCUSSION

The HAARLEM-study aims to assess the negative health effects of AAS use. A cohort of 100 men was assembled after launching the concept of the study by the media on a national level and promoting the study on popular websites where many users of AAS are known to gather. This method of selection is expected to result in a group of AAS users representative of those in the general population and subjects from all over the Netherlands were included. The data show that the typical user of AAS is an educated and working male who is active as an amateur strength athlete, and spends about 5 hours per week at the gym. About 1 in 3 is active in bodybuilding competitions. The demographic characteristics of our cohort are similar to patients seen in the AAS clinic[16] and in earlier survey studies.[18,19] Contrary to the results of an earlier survey study,[20] the number of homosexual subjects was small. As expected based on previous reports,[19,21-25] recent illicit drug use was common and may indicate a higher level of sensation-seeking behavior by AAS users.[26]

Characteristics of AAS use – in the past and during the study – compare well to patients at our AAS clinic[16] and previous reports[23,25] with onset of AAS use occurring in the 3rd decade of life. An AAS cycle is usually composed of a testosterone ester as a 'core', and other AAS types are added in an attempt to increase efficiency, pursue a certain body shape, and/or due to a supposed synergism. Multiple AAS cycles are generally performed to gain muscle mass and allow the body to recover in between. Over time, athletes may perform longer and heavier AAS cycles, as becomes apparent from the association of previous AAS use with these AAS cycle characteristics.

Other PIEDs such as growth hormone, insulin-like growth factor 1 (IGF-1) and growth hormone releasing hormone (GHRH) are added to further boost muscle mass,[27] or to maintain physical strength in between cycles, as gained muscle mass is ordinarily lost almost entirely after AAS are discontinued. Substances such as clenbuterol, thyroid hormone or ephedrine are believed to decrease fat mass. A recent report describing a body builder who received stem cell transfusions from his trainer is an attest to the fact that some athletes go to great lengths to improve their physical shape and strength.[28]

Besides clearly positive reported effects on muscle mass, strength, fat mass and energy levels, nearly all AAS users report side effects.[16,27] Various medications are used to prevent or combat these side effects, such as aromatase inhibitors and tamoxifen (against gynecomastia and/or fluid retention), isotretinoin (to treat acne) and hCG (against testicular atrophy and/or reduced fertility). Many users employ post-cycle therapy (PCT) after a cycle to speed up hormonal recovery and prevent symptoms of testosterone deficiency. PCT is continued for several weeks and usually consists of a combination of

tamoxifen, clomiphene citrate and/or hCG. Although PCT is deemed imperative by many AAS users, its effectiveness has never been proven.

Unreliability of black market AAS

Analysis of AAS delivered by subjects of the HAARLEM-study revealed a strikingly low quality of circulating AAS products and confirms the results of earlier reports.[29,30] Only about one half of analyzed AAS samples contained the AAS type as indicated on the label and more often than not the samples contained AAS types not indicated on the label. The results underline the careless production process of AAS at the black market.

Although illicitly obtained AAS are very unreliable, most products do contain AAS – in most cases in a lower dose than indicated on the label[30] – and desired effects will be experienced by the user. Contamination of AAS with other harmful compounds appears negligible. In 6% of samples we were unable to detect an active substance, but as most users of AAS concurrently use multiple AAS types, this probably goes unnoticed. Attempting to circumvent untrustworthy AAS products by buying from a seemingly 'reliable' brand or dealer – an ever-intriguing topic to users of AAS – appears trifling as no brand showed consistent reliability. The results demonstrate that careful assembly of different AAS types by users in an attempt to create more effective cycles is hardly to any avail. In addition, online information regarding AAS types and products is based on personal experience and thus inherently fallible.

Background of AAS use

The semi-structured interview of subjects of the HAARLEM-study generated valuable insights into reasons for AAS use and social, financial and dependency issues. Firstly, reasons to use AAS are diverse and not only concern a straightforward desire to increase muscle mass and strength, resembling the findings of a previously performed interview study.[18] Certain domains of sport, especially bodybuilding, coerce participants to use AAS in order to be able to compete. Certain fitness or appearance-focused media can also pressure men into using AAS, an effect that has been described before.[31] Others felt the pressure of adapting to the male beauty ideal of society. A few specifically reported that their AAS use was a result of a distorted body image, a condition known to affect users of AAS.[32] Contrary to popular belief, subjects rarely indicated that they use AAS specifically to be found more attractive.

Secondly, a majority of users have friends or acquaintances at the gym that use AAS. Indeed, more than one-third of subjects reported that they started AAS together with or after a friend that was more experienced. Outside their network of AAS users, subjects prefer to keep their AAS use concealed, even if someone makes a point-blank inquiry. If they do tell significant others, they are frequently confronted with disapproval but this usually does not affect their usage.

Thirdly, more than half of subjects admitted to be addicted to AAS at least to some extent. AAS dependency is a well-described syndrome that poses a serious public health problem.[24] The cause of AAS addiction appears twofold, as it is not only the result of a rewardingly increase in muscle mass and strength, and positive effects on mood and self-esteem, but also the despondency and deplorable loss

of gained muscle mass that follow a cycle which are remediable by starting a new cycle of AAS or maintenance dose. The low cost of AAS use, in this cohort averaging €30 per week, hardly poses a barrier to further usage. Accordingly, only 90% reported that spending money on AAS was not a significant financial burden.

Limitations

The most important limitation of the HAARLEM-study cohort is that the design of the study required subjects to completely discontinue using AAS after their cycle. It is known that a small proportion of users (~5%) uses AAS more or less continuously.[16] They do not use AAS in a cycling pattern and/or adopt a 'blast-and-cruise' policy in which a maintenance dose of AAS is applied in between cycles to prevent muscle loss and symptoms of testosterone deficiency. This type of user was not included in the HAARLEM-study because health measurements after a cycle were supposed to assess recovery and during continuous AAS use no or incomplete recovery occurs. This group of users may use AAS more hazardously and the cumulative dose of AAS is usually larger.

Another selection bias is introduced by the fact that the study provided subjects with the opportunity to monitor health variables – for free – even though the results were disclosed only after they completed their cycle. This could mean the cohort is composed of generally more careful and wary subjects who use AAS more moderately. Conversely, it may also have posed an occasion for subjects to perform a heavier AAS cycle because health was checked anyway. Comparison of past and current AAS cycles, however, does not show a relevant difference in terms of cycle length and types of AAS used. Average doses of past cycles were not recorded as recall bias would have been too substantial, so comparison on this level was not possible. A previous American survey study among AAS users reported that 60% used at least 1000 mg of AAS (equivalents) per week,[27] which was only 38% in this cohort, but this may rather be due to regional differences than selection bias.

Furthermore, calculated cumulative AAS cycle dose was based on label information of the supplied products. AAS product content oftentimes did not match label information and occasionally no active ingredients were found. Due to practical and financial limitations we were unable to test all AAS used by the subjects in our study. As a result, cumulative dose presented in the results only serves as an estimate of total androgen exposure.

A final limitation is that this study was restricted to men in order to be able to study a rather homogenous and relatively easily accessible group of athletes. Only 1% of visitors of the AAS clinic was female,[16] so women presumably constitute a small minority of AAS users. The health effects of AAS use amongst females remain underrepresented in scientific literature.

PERSPECTIVE

The HAARLEM study assembled a cohort of 100 male amateur strength athletes intending to use AAS on short notice. Baseline characteristics show that users are exposed to health risks not only by the low quality AAS that they use, with side effects occurring in virtually all users, but also by their frequent

concomitant use of illicit drugs, PIEDs and other medications. Follow-up of the cohort with repeated health analysis will provide novel prospective data about the precise health risks of AAS use. Better understanding of these risks may assist in mitigating against AAS use and curtailing possible long-term hazards. Dissuading athletes from using AAS is important as its negative health effects may become an increasingly serious public health issue.

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CHAPTER 5

POSITIVE AND NEGATIVE EFFECTS OF ANDROGEN ABUSE. THE HAARLEM STUDY: A ONE-YEAR PROSPECTIVE COHORT STUDY IN 100 MEN

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ABSTRACT

Background

An estimated 4-6% of fitness center visitors uses anabolic androgenic steroids (AAS). Reliable data about adverse reactions of AAS are scarce.

Methods

The HAARLEM study included 100 men (≥18 years) who intended to start an AAS cycle on short notice. Clinic visits took place before (T_0), at the end (T_1), and three months after the end of the AAS cycle (T_2), and one year after the start of the cycle (T_3), and comprised a medical history, physical examination, laboratory analysis and psychological questionnaires.

Results

Four subjects reported a serious adverse event, i.e. congestive heart failure, acute pancreatitis, suicidal ideation and exacerbation of ulcerative colitis. All subjects reported positive side effects during AAS use, mainly increased strength (100%), and every subject reported at least one negative health effect. Most common were fluid retention (56%) and agitation (36%) during the cycle, and decreased libido (58%) after the cycle. Acne and gynecomastia were observed in 28% and 19%. Mean alanine transaminase (ALT) and creatinine increased 18.7 U/l and 4.7 µmol/l, respectively. AAS dose and cycle duration were not associated with the type and severity of side effects. After one year follow-up (T_3), the prevalence of observed effects had returned to baseline. There was no significant change in total scores of questionnaires investigating wellbeing, quality of life and depression.

Conclusions

All subjects experienced positive effects during AAS use. Four subjects experienced a serious adverse event. Other side effects were mostly anticipated, mild and transient.

INTRODUCTION

The use of anabolic androgenic steroids (AAS) is not uncommon among regular visitors of fitness centers. The prevalence for men is estimated to be 4-6%.[1,2] Production and trading of AAS without a license is prohibited in most countries. Nevertheless AAS can be easily acquired illegally through local dealers or the internet. The quality of AAS on the black market is remarkably poor and many contain undeclared ingredients.[3,4]

Current knowledge of the side effects of AAS is based on rather low-level evidence, such as case reports, cross-sectional studies, and a few larger retrospective studies. Common side effects associated with androgen abuse in men are acne, gynecomastia, low HDL cholesterol, kidney and liver toxicity, disruption of the hypothalamic-pituitary-gonadal axis[5] and psychiatric effects such as mood disorders, agitation and anxiety.[6]

Due to the lack of prospective observational studies, however, it is not well known what the incidence and severity of particular side effects is, whether these effects are reversible, and if there is a clear relationship with androgen dose or duration of use. Therefore we initiated a prospective observational study, the HAARLEM study (acronym for Health risks of Anabolic Androgenic steRoid use by maLE aMateur athletes). We meticulously registered the effects of androgen exposure in a cohort of 100 volunteer strength athletes during one year follow-up: before, during and after a cycle of AAS. In this paper we will focus on the incidence of self-reported effects, acne and gynecomastia, kidney and liver toxicity, and psychological effects. Endocrine and cardiovascular effects will be described in separate papers.

METHODS

A detailed description of the methods of subject recruitment are described in a previous report.[3] In short, subjects included in the HAARLEM-study were men of at least 18 years old intending to start an AAS cycle on short notice (i.e. within two weeks). Subjects were required to not have used AAS for at least three months prior to inclusion. Men were also refused to participate in the study if, within six months prior to inclusion: 1) a new chronic or psychiatric illness had been diagnosed, 2) treatment for a chronic illness had been started or altered significantly, 3) or a psychiatric or somatic illness other than trauma had led to admission to a psychiatric institution or hospital.

The AAS cycle performed by subjects during follow-up was recorded in detail, with duration and dosage of each type of AAS used, in addition to use of other performance and image–enhancing drugs (PIEDs), medication, and post-cycle therapy (PCT). Cumulative dose of the AAS cycle was calculated by adding up the total used mg of androgens of every cycle week, using the product label information of the AAS. The study took place in the outpatient AAS clinic in Haarlem, the Netherlands, after the study was promoted on national television and in regional newspapers. The study was approved by the local institutional review board (IRB) and subjects provided written informed consent.

Clinic visits

Subjects visited the clinic four times during a one year study period. A medical investigation was performed at baseline i.e. within two weeks before the initiation of the AAS cycle (T_0), comprising medical history, physical examination, laboratory analysis and psychological questionnaires. Investigations were repeated in the last week of the AAS cycle (T_1), three months after the end of the cycle (T_2), and one year after inclusion (T_3).

Subjects were asked about health complaints, positive and negative side effects, and recent doctor and hospital visits. We inquired whether a subject changed or stopped the AAS cycle due to symptoms. Subjects underwent a routine physical examination. Skin was visually examined for acne and scored from 0 to 4 according to James & Tisserand.[7] Breast tissue was visually and manually evaluated for gynecomastia and classified from 0 to 4.[8]

Laboratory analysis

Subjects visited the hospital laboratory for collection of blood after an overnight fast. Blood was drawn by venipuncture by a qualified laboratory assistant. Lithium heparin samples were analyzed on the Abbott ARCHITECT system for routine biochemical markers. Parameters examined were urea, creatinine, bilirubine, alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), creatine kinase (CK) and prostate-specific antigen (PSA). Estimated glomerular filtration rate (eGFR) was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[9] Urinalysis was performed with automated dipstick test (Clinitek Advantus Siemens).

Questionnaires

A total of five different questionnaires were filled out by the subjects. Current subjective psychological well-being was assessed with the 5-item WHO well-being index (WHO-5, range 0-100 points). The 26-item WHO Quality of Life Scale (WHOQOL-BREF, range 0-100 points) was used to measure quality of life. This questionnaire explores physical, psychological and social dimensions, and environment.[10]

The presence, severity and depth of depression symptoms were rated with the 21-item Beck Depression Inventory (BDI, range 0-39 points). Mild and moderate depression were classified as 14 to 19 points or ≥20 points, respectively. Aggression was measured with the 29-item Buss & Perry Aggression Questionnaire (BPAQ, range 29-145). Subcategories were physical aggression, verbal aggression, anger and hostility.[11] A higher score indicates a higher level of aggression. The Body Dysmorphic Disorder Modification of the Yale-Brown Obsessive Compulsive Scale (BDD-YBOCS, range 0-48) determined presence and severity of symptoms of body dysmorphic disorder.[12] Suspected BDD was classified as ≥15 points, with moderate BDD being 15-22 points and severe BDD ≥23 points.

Analysis

Simple descriptive statistics were used to display quantitative data. As measurements were clustered within patients, linear and logistic mixed models were used for continuous and dichotomous variables, respectively, to calculate mean differences, 95%-confidence intervals (CI) and relative risks (RR), as well as P values. This analysis takes missing data into account.[13]

Multivariable logistic and linear regression analysis assessed the confounding role of AAS cycle dose and length, number of AAS used, and the use of post-cycle therapy (PCT). The analysis also accounted for the use of PIEDs and other medication that were used most commonly by subjects during follow-up, i.e. growth hormone (GH), clenbuterol, thyroid hormone, human chorionic gonadotropin (hCG), tamoxifen, and aromatase inhibitors. To correct for multiple testing, only interactions with a P value <.01 were considered statistically significant. Stata software (for Windows, version 15, StataCorp, 2017) was used for analysis.

RESULTS

One hundred men were included in the HAARLEM-study cohort and follow-up took place between October 2015 and May 2018. The median age was 31 years (range 19-67). Seventy-nine subjects had a history of AAS use. During follow-up subjects performed an AAS cycle with a median duration of 13 weeks (range 2-52) and 4 types of AAS (range 1-11). The mean androgen dose was 898 mg per week (range 250-3382). Eighty subjects carried out post-cycle therapy (PCT). Demographic data, historic AAS use, and details of the AAS cycles and PIEDs used during follow-up period are described elsewhere.[3]

The course of follow-up with respect to AAS use and clinic visits of the 100 subjects is shown in *Table 1*. The median follow-up period of 11.9 months (range 8-17) was completed by 98 subjects. One subject withdrew consent and one subject emigrated, both shortly after completion of the cycle. Both stayed in contact and in the remaining part of the year after inclusion no serious adverse events were reported. T_1 took place in the last week of the AAS cycle after a median of 3.5 months after inclusion. T_2 took place after a median of 3.2 months after T_1 and reflects recovery after the cycle.

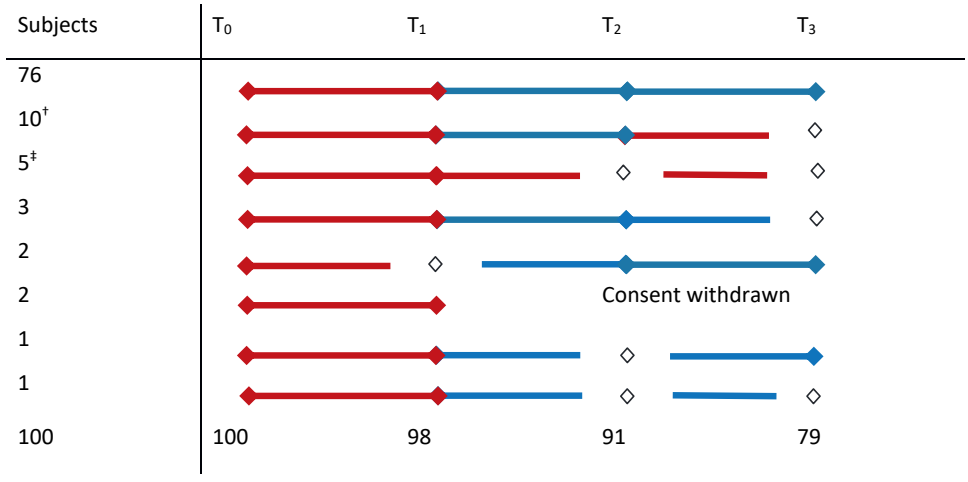
Of the initially planned 400 clinic visits, 12 (3%) were missed. Reasons for the missed visits were various but mainly concerned not being able to attend the clinic due to obligations for work or personal circumstances. None of the visits was missed due to health reasons. Fifteen subjects used AAS continuously or started a second cycle of AAS during the follow-up of the study. In these subjects, T_2 and/or T_3 measurements were not useful for analysis of recovery.

Serious adverse events

In four subjects a serious adverse event occurred during the follow-up period. One subject was hospitalized three weeks after start of the AAS cycle with fatigue and dyspnea. He was diagnosed with congestive heart failure and hypertrophic cardiomyopathy. With medication he made an excellent

recovery over the course of several weeks. A second subject experienced an exacerbation of ulcerative colitis six weeks into his cycle. He was treated with prednisolone and attained durable remission with mesalazine and adalimumab. He had a history of ulcerative colitis but the illness had been stable for over a year prior to inclusion. A third subject was admitted to a hospital because of acute relapsing pancreatitis that occurred in his 40th cycle week. It was his third episode of pancreatitis with the previous two also taking place during AAS use. He was treated with intravenous fluids and pain medication, and was discharged from the hospital in good health after five days. A fourth subject had suicidal thoughts four months into his cycle after suffering from an increasingly depressed mood, which was reportedly triggered by a relationship breakup several months prior to inclusion. The subject sought and received ambulatory help and mood gradually stabilized with sertraline and quetiapine. All four subjects terminated their AAS cycle after the serious adverse event happened and did not use AAS again for the remainder of the follow-up.

Table 1. Overview of follow-up period of the 100 subjects included in the HAARLEM study. Red lines correspond to subjects using AAS, whereas blue lines correspond to subjects not using AAS. Colored diamonds are performed clinic visits, whereas white diamonds are missed clinic visits or clinic visits without useful data due to AAS use not according to protocol.



†Subjects who started a new cycle after T_2. ‡Subjects who did not discontinue AAS after T_1.

Self-reported positive and negative side effects

Side effects, both positive and negative, self-reported by subjects during the clinic visits are presented in *Table 2*. Results of the multivariable regression analysis are summarized in *Supplementary table 1*. An increase in muscle mass and in strength was reported by 95% and 100% of users at the end of the AAS cycle (T_1), respectively. Eighty percent of subjects indicated to experience an increased libido, but this was not consistently reported as positive or negative. One year after inclusion (T_3) the prevalence of self-reported positive side effects had returned to baseline.

Table 2. Positive and negative side effects reported by subjects during the clinic visits. Effects were included if experienced by the subject between current and previous clinic visit (in case of T_0, in the prior 3 months). T_0 = at the start of the cycle, T_1 = in the last week of the cycle, T_2 = 3 months after the cycle, T_3 = 1 year after the start of the cycle. RR = relative risk, CI = 95% confidence interval. *P=.01-.05, **P<.01. †Steroid cough: dry cough that arises within seconds to minutes after injecting AAS and resolves quickly.

	T_0 (n=100)	**T_1 (n=98)**	**T_2 (n=91)**	**T_3 (n=79)**
Positive side effects	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Increased muscle mass	17 (17%)	93 (95%)**	20 (22%)	8 (10%)
Increased strength	17 (17%)	98 (100%)**	21 (23%)	10 (13%)
Decreased fat mass	13 (13%)	72 (73%)**	23 (25%)*	9 (11%)
Increased energy	8 (8%)	44 (45%)**	12 (13%)	4 (5%)
Improved concentration	8 (8%)	28 (29%)**	10 (10%)	3 (4%)
Negative side effects	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Testicular atrophy	8 (8%)	57 (58%)**	10 (11%)	1 (1%)
Fluid retention	7 (7%)	55 (56%)**	10 (11%)	6 (8%)
Acne	10 (10%)	51 (52%)**	26 (29%)**	11 (14%)
Agitation	1 (1%)	35 (36%)**	4 (4%)	0 (0%)
Severe snoring	5 (5%)	29 (30%)**	4 (4%)	0 (0%)
Gynecomastia	7 (7%)	25 (26%)**	8 (9%)	4 (5%)
Decreased libido	18 (18%)	22 (22%)	53 (58%)**	19 (24%)
Aggressiveness	1 (1%)	23 (23%)**	3 (3%)	2 (3%)
Palpitations	2 (2%)	21 (21%)**	10 (11%)*	1 (1%)
Increased fat mass	21 (21%)	20 (20%)	39 (43%)**	26 (33%)
Painful injection site	0 (0%)	20 (20%)*	0 (0%)	0 (0%)
Hyperhidrosis	1 (1%)	17 (17%)**	3 (3%)	0 (0%)
Addictive behaviour	8 (8%)	15 (15%)*	4 (4%)	4 (5%)
Erectile dysfunction	8 (8%)	12 (12%)	13 (14%)	1 (1%)
Striae	6 (6%)	12 (12%)	4 (4%)	3 (4%)
Alopecia	2 (2%)	12 (12%)**	8 (9%)	4 (5%)
Persistent erections	2 (2%)	10 (10%)*	0 (0%)	1 (1%)
Fatigue	0 (0%)	16 (16%)*	8 (9%)*	3 (4%)
Insomnia	0 (0%)	10 (10%)*	3 (3%)	0 (0%)
Tendon injuries	1 (1%)	7 (7%)	7 (8%)	2 (3%)
Painful micturition	0 (0%)	6 (6%)	0 (0%)	0 (0%)
Headache	1 (1%)	6 (6%)	1 (1%)	0 (0%)
Increased hair growth	0 (0%)	5 (5%)	0 (0%)	0 (0%)

Dyspnea	0 (0%)	5 (5%)	1 (1%)	0 (0%)
'Steroid cough'[†]	0 (0%)	3 (3%)	0 (0%)	0 (0%)
Depressed mood	0 (0%)	3 (3%)	5 (6%)	1 (1%)
Mood swings	0 (0%)	3 (3%)	5 (6%)	1 (1%)
Any	39 (39%)	98 (100%)[**]	77 (84%)[**]	38 (44%)

All subjects experienced at least one negative health effect during the cycle. The most common negative side effects were testicular atrophy (58%), fluid retention (56%), acne (52%), agitation (36%) and severe snoring (30%) during the cycle, and decreased libido (58%) after the cycle. Acne persisted after the cycle and was reported by 29% of subjects three months after the AAS cycle (T_2).

Other negative side effects that were reported during AAS use were gynecomastia (26%), aggressiveness (23%), palpitations (21%), hyperhidrosis (17%) and alopecia (12%). There was no significant difference in the reported prevalence of erectile dysfunction before (8%), during (12%) and after the cycle (14%). One year after the start of the cycle (T_3), the prevalence of reported negative side effects had returned to baseline values. Of all the reported side effects, only hair loss was positively associated with cycle length. Weekly steroid dose was not positively associated with any side effect. The reported incidence of palpitations was higher in users of oral AAS; severe snoring was reported more often in users of GH.

The occurrence of negative side effects led to an earlier than planned termination of the AAS cycle in an additional four subjects, not including the four subjects with a serious adverse event. The reasons for stopping AAS were: fatigue, gynecomastia, erectile dysfunction and shoulder injury. Negative side effects led to major alterations in the AAS cycle in another five subjects. One developed persistent singultus and reduced the dose of AAS, one stopped taking stanozolol due to palpitations, one stopped trenbolone due to agitation, one stopped nandrolone due to dizziness, and one reduced the dose of the AAS due to hyperhidrosis.

There was no correlation between the use of other PIEDs or self-administered medications and most reported side effects. Users of clenbuterol less frequently reported acne and striae during the cycle (T_1), but reported tendon injuries and hyperhidrosis more frequently three months after the cycle (T_2). During the cycle (T_1), hyperhidrosis was reported less often by users of GH, gynecomastia was associated with tamoxifen use, and striae were reported more often by subjects using thyroid hormone.

Physical examination

Findings of the physical examination are summarized in *Table 3.* Mean body weight increased by 4.9 kg (CI 4.0 to 5.7; P<.01) during the cycle and remained 0.8 kg (CI 0.5 to 2.4; P<.01) above baseline at the end of follow up (T_3). At T_1, two subjects had a cardiac murmur and one subject had hepatomegaly, but both were no longer present at T_2 and T_3. Fifty subjects had a tattoo.

Table 3. Findings of physical examination during clinic visits. T_0 = at the start of the cycle, T_1 = in the last week of the cycle, T_2 = 3 months after the cycle, T_3 = 1 year after the start of the cycle. CI = 95%-confidence interval. CI and P values were calculated with mixed models and compare visit T_1, T_2 and T_3 to T_0. *P=.01-.05, **P<.01.

	T_0 (n=100)	**T_1 (n=98)**	**T_2 (n=91)**	**T_3 (n=79)**
Clinical parameter	**x̄ [CI]**	**x̄ [CI]**	**x̄ [CI]**	**x̄ [CI]**
Height	181.8 [180.4-183.2]	182.0 [180.4-183.2]	181.8 [180.4-183.2]	181.9 [180.3-183.1]
Weight	88.8 [86.2-91.4]	93.7** [91.0-96.3]	90.3** [88.4-93.7]	89.6* [87.6-92.9]
Body mass index (BMI)	26.8 [26.2-27.5]	28.2** [27.6-28.9]	27.3** [26.8-28.1]	27.0** [26.6-27.9]
Clinical parameter	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Acne	13 (13%)	28 (29%)**	21 (23%)	8 (10%)
- Grade 1	9 (8%)	21 (21%)	13 (14%)	7 (9%)
- Grade 2	3 (3%)	2 (2%)	6 (7%)	1 (1%)
- Grade 3	1 (1%)	5 (5%)	2 (2%)	0 (0%)
Gynecomastia	7 (7%)	19 (19%)**	6 (7%)	4 (5%)
- Grade 1	6 (6%)	16 (16%)	5 (6%)	3 (4%)
- Grade 2	1 (1%)	3 (3%)	0 (%)	0 (0%)
- Grade 3	0 (0%)	0 (0%)	1 (1%)	1 (1%)

Acne was detected in 29 subjects at the end of the cycle (RR 2.2, CI 1.2-4.0, P<.01) compared to 13 at baseline (T_0). Acne grade 2 and higher was found in seven subjects at T_1 compared to four at baseline. There was a negative association between AAS cycle length and the prevalence of acne three months after the cycle (T_2).

The prevalence of gynecomastia increased significantly from 7% at baseline (T_0) to 19% at the end of the cycle (RR 2.8, CI 1.2-6.3, P<.01). Gynecomastia occurred more frequently in subjects with a history of AAS use. Gynecomastia grade 2 and higher was observed in three subjects at T_1 and one of them continued to have gynecomastia grade 3 which was still present at the end of follow up (T_3). Excluding this subject, the prevalence of gynecomastia returned to baseline at the end of follow up (T_3).

Laboratory analysis

Details of laboratory analysis are displayed in *Table 4*. Before the start of the AAS cycle (T_0), concentrations of ALT, AST, LDH and CK were elevated in 39%, 41%, 11% and 82% of subjects, respectively. During androgen exposure (T_1), mean levels for these variables increased significantly, returning to baseline three months after the cycle (T_2). Mean concentrations of bilirubin, GGT and ALP decreased slightly, but significantly, during androgen exposure (T_1), returning to baseline after three months (T_2). The eGFR was within the reference range for 99% of subjects at baseline (T_0), decreased

slightly during androgen exposure (T_1) and returned to baseline three months after stopping AAS (T_2). The urea concentration was slightly elevated in 62% of subjects at baseline (T_0) and followed a similar pattern as eGFR. The mean PSA concentration increased slightly but significantly during androgen exposure (T_1), but only two subjects had a PSA level above the reference range. Albuminuria emerged or increased in 16 (16%) subjects during androgen exposure.

AAS weekly dose, cycle length and the use of oral AAS were not associated with the extent of the derangements, nor were other PIEDs or self-administered medications used during or after the cycle. At the end of follow up (T_3), none of the laboratory parameters was significantly different compared to baseline (T_0), except for eGFR which was 3 ml/min/1.73 m^2 higher (CI 0.3 to 5.0; P=.03).

Table 4. Results of laboratory analysis during clinic visits. T_0 = at the start of the cycle, T_1 = in the last week of the cycle, T_2 = 3 months after the cycle, T_3 = 1 year after the start of the cycle. eGFR = estimated glomerular filtration rate. CI = 95%-confidence interval. RR = reference range. N (%) = number and percentage of subjects that deviated from the reference range. CI and P values were calculated with mixed models and compare visit T_1, T_2 and T_3 to T_0. *P=.01-.05, **P<.001. †Dipstick results are classified as 0 = none, 1 = trace, 2 = +, 3 = ++, 4 = +++.

	T_0 (n = 100)		T_1 (n=98)		T_2 (n=90)		T_3 (n=79)	
Blood analysis (unit, RR)	x̄ [CI]	n (%)	x̄ [CI]	n (%)	x̄ [CI]	n (%)	x̄ [CI]	n (%)
Urea (mmol/l. 2.5-6.4)	6.9 [6.6-7.2]	62 (62%)	6.2** [5.9-6.5]	39 (40%)*	6.7 [6.4-7.0]	48 (53%)	6.7 [6.3-7.0]	42 (53%)
Creatinine (µmol/l. 64-104)	93.1 [90.1-96.1]	21 (21%)	97.8** [94.8-101]	33 (33%)*	93.6 [90.6-96.7]	18 (20%)	90.0* [86.9-93]	11 (14%)
eGFR (ml/min/1.73 m^2)	95.1 [92-98]	1 (1%)	90.1** [87-93]	3 (3%)	94.0 [91-97]	2 (2%)	97.7* [94-101]	0 (0%)
Bilirubin (µmol/l. 3-20)	12.9 [11.8-14.0]	10 (10%)	11.3** [10.1-12.4]	6 (6%)	12.4 [11.2-13.6]	5 (6%)	12.7 [11.4-13.9]	6 (7%)
ALP (U/l. 0-115)	74.0 [70.2-77.7]	3 (3%)	66.4** [62.6-70.2]	3 (3%)	72.2 [68.3-76.1]	3 (3%)	72.7 68.7-76.6]	2 (1%)
γ-GT (U/l. 0-55)	25.6 [23.3-28.0]	3 (3%)	21.8** [19.5-24.2]	1 (1%)	26.5 [24.1-29.0]	4 (4%)	25.1 [22.5-27.7]	1 (1%)
ALT (U/l. 0-45)	49.2 [43.6-54.7]	39 (39%)	67.9** [62.3-73.5]	75** (77%)	51.9 [46.1-57.8]	47* (52%)	47.2 [41.0-53.5]	32 (41%)
AST (U/l. 0-35)	35.9 [32.4-39.5]	41 (41%)	51.2** [47.6-54.8]	71 (72%)**	37.4 [33.7-41.1]	36 (40%)	36.8 [32.9-40.8]	30 (38%)
LDH (U/l. 0-248)	202 [193-211]	11 (11%)	235** [227-244]	32 (33%)**	200 [191-209]	8 (9%)	196 [187-206]	6 (7%)
Creatine kinase (U/l. 0-171)	472 [321-623]	82 (82%)	987** [835-1139]	92 (94%)*	502 [344-660]	78 (87%)	607 [439-775]	63 (80%)
PSA (µg/l. 0-2.0)	0.71 [0.61-0.81]	0 (0%)	0.93** [0.83-1.02]	2 (2%)	0.74 [0.64-0.84]	1 (1%)	0.77 [0.66-0.87]	2 (2%)

Urine analysis (RR)	x̄ [CI]	n (%)	x̄ [CI]	n (%)	x̄ [CI]	n (%)	x̄ [CI]	n (%)
Hemoglobin (<1)†	0.13 [0.02-0.38]	9 (9%)	0.26** [0.15-0.38]	14 (14%)	0.17 [0.05-0.29]	11 (12%)	0.12 [0.00-0.24]	5 (6%)
Albumin (<1)†	0.08 [0.00-0.18]	7 (7%)	0.30** [0.20-0.41]	18 (18%)*	0.08 [-0.03-0.18]	11 (12%)	0.16 [0.04-0.27]	8 (10%)

Psychological questionnaires

Findings of the psychological questionnaires are displayed in *Table 5*. No significant changes were observed in the mean total scores of the WHO wellbeing index and WHOQOL-BREF. The subcategories of the WHOQOL-BREF showed a significant decline in the scores on 'physical health' of 2.5 points (CI -4.6 to -0.4, P=.02) during androgen exposure (T_1), and a significant increase in 'psychological health' of 2.0 points (95%CI 0.0 to 4.1, P=.05) three months after exposure (T_2) and 2.8 points (CI 0.7 to 4.9, P=.01) at the end of follow up (T_3).

The prevalence of mild or moderate depression, as scored by the BDI, was 10% at baseline (T_0). There was no significant change in the mean total score of the BDI during follow up, thus the prevalence of mild and moderate depression was not significantly different between clinic visits. This score showed a positive correlation with weekly androgen dose, indicating that users of a higher androgen dose were more likely to be depressed. The mean total score of the BPAQ did not show a significant change during androgen exposure (T_1), but was slightly lower compared to baseline after androgen exposure (2.3 points (CI 0.2 to 4.2; P=.03) at T_2 and 2.4 points (CI 0.1 to 4.3; P=.04) at T_3). This was mainly due to a significant reduction in the mean score on the subcategory 'physical aggression'.

***Table 5*.** Average scores of psychological questionnaires filled in during clinic visits. The subcategories of questionnaires are shown, if applicable. T_0 = at the start of the cycle, T_1 = in the last week of the cycle, T_2 = 3 months after the cycle, T_3 = 1 year after the start of the cycle. WHOQOL-BREF = Abbreviated WHO Quality of Life Scale, BDD-YBOCS = Body Dysmorphic Disorder Modification of the Yale-Brown Obsessive Compulsive Scale, CI = 95%-confidence interval. CI and P values were calculated with mixed models and compare visit T_1, T_2 and T_3 to T_0 *P=.01-.05, **P<.01.

	T_0 (n=100)	**T_1 (n=98)**	**T_2 (n=91)**	**T_3 (n=79)**
Questionnaire (score range)	**x̄ [CI]**	**x̄ [CI]**	**x̄ [CI]**	**x̄ [CI]**
5-item WHO wellbeing index (0-100)	67.5 [64.0-70.9]	66.0 [62.5-69.5]	68.1 [64.5-71.6]	70.7 [67.0-74.4]
WHOQOL-BREF (0-100)	75.1 [72.8-77.2]	75.0 [72.7-77.2]	76.1 [73.8-78.4]	75.9 [73.6-78.3]
- General health	81.7 [79.1-84.3]	82.0 [79.4-84.6]	83.2 [80.5-85.8]	82.0 [79.2-84.8]
- Physical health	77.3 [74.7-78.0]	74.8* [72.2-77.5]	76.5 [73.7-79.2]	76.2 [73.4-79.0]
- Psychological health	68.8 [66.0-71.6]	70.0 [67.2-72.8]	70.8* [68.0-73.7]	71.6* [68.7-74.5]
- Social relationships	71.5 [68.1-74.9]	71.1 [67.7-74.5]	71.3 [67.8-74.5]	71.4 [67.8-75.0]
- Environment	76.2 [73.9-78.5]	77.0 [74.7-79.3]	78.6** [76.3-80.9]	78.3* [76.0-80.7]

Beck Depression Inventory (0-39)	5.3 [4.2-6.4]	5.0 [3.9-6.1]	4.9 [3.7-6.0]	4.3 [3.1-5.5]
- Mild depression (≥14)	6 (6%)	3 (3%)	3 (3%)	3 (4%)
- Moderate depression (≥20)	4 (4%)	2 (2%)	3 (3%)	2 (3%)
Aggression Questionnaire (29-145)	68.9 [66.4-71.3]	69.6 [67.1-72.0]	66.7* [64.1-69.2]	66.7* [64.0-69.3]
- Physical aggression	22.5 [21.3-23.6]	22.3 [21.2-23.4]	21.8 [20.7-22.9]	21.4** [20.3-22.5]
- Verbal aggression	13.6 [13.1-14.0]	13.6 [13.1-14.1]	13.5 [13.0-14.0]	13.7 [13.1-14.2]
- Anger	16.4 [15.5-17.3]	17.2* [16.3-18.1]	15.3* [14.4-16.3]	15.7 [14.8-16.7]
- Hostility	16.5 [15.6-17.4]	16.4 [15.6-17.3]	16.0 [15.1-16.9]	16.1 [15.1-17.0]
BDD-YBOCS (0-48)	11.7 [10.5-13.0]	11.8 [10.5-13.0]	9.7** [8.4-11.0]	8.8** [7.4-10.1]
- Obsession	4.0 [3.4-4.5]	4.2 [3.6-4.7]	3.2** [2.6-3.7]	3.1** [2.5-3.6]
- Compulsion	6.1 [5.3-6.8]	6.1 [5.3-6.8]	4.9** [4.2-5.6]	4.4** [3.6-5.1]
- Insight	1.7 [1.3-2.0]	1.5 [1.2-1.8]	1.6 [1.3-2.0]	1.3 [1.0-1.7]
- Suspected BDD (≥15)	32 (32%)	32 (32%)	17** (19%)	13** (16%)
- Moderate BDD (15-22)	22 (22%)	24 (24%)	14 (15%)	11 (13%)
- Severe BDD (≥23)	10 (10%)	8 (8%)	3* (3%)	2* (2%)

According to the BDD-YBOCS, 32 subjects had suspected BDD at baseline (T_0), of which 10 subjects classified as having severe BDD. Mean total scores on the BDD-YBOCS did not change during androgen exposure, but were significantly lower three months after the cycle (T_2) and at the end of follow up (T_3), indicating less concerns with body appearance. Indeed, the number of subjects that were classified as having signs of BDD at the end of follow up (T_3) was 16% compared to 32% at baseline.

DISCUSSION

The HAARLEM-study is the first large-scale trial to prospectively analyze side effects of AAS use. Analysis was performed in 100 male amateur athletes during four clinic visits – before, during and twice after a cycle of AAS. The cohort is likely to be representative of AAS users in the general population because recruitment occurred on a nationwide level and it was not necessary to decline athletes based on exclusion criteria. The cycles performed contained very high supraphysiological doses of androgens.[3]

Serious adverse events

Four subjects experienced a serious adverse event during the study period, i.e. heart failure, acute pancreatitis, exacerbation of ulcerative colitis, and suicidal ideation. Of these, only the association between AAS and congestive heart failure[14-16] and suicide[17,18] have been reported previously. Our study does not allow us to draw conclusions regarding causality between these events and AAS use. For strong conclusions, very large prospective trials or well-designed case control studies are needed. The two largest case control studies to date, including 545 and 409 subjects, respectively, showed significantly increased morbidity and mortality amongst users of AAS compared to nonusers.[19,20] Most of the excess morbidity was explained by well-known side effects of AAS such as erectile dysfunction,

gynecomastia, acne and heart disease. The incidence of pancreas illnesses was not different between cases and controls, and the incidence of bowel disease was not mentioned.

Self-reported positive and negative effects

Besides the serious adverse events in a few, all subjects reported side effects during the study. At baseline, 39% of subjects reported various health issues, presumably reflecting the general prevalence of these symptoms and possibly the result of a carryover effect of previous AAS abuse (at least three months prior to inclusion). During androgen exposure, a large proportion of subjects reported positive side effects; increased muscle mass and strength, decreased fat mass, increased libido, improved energy and concentration. This clearly explains the popularity of anabolic steroids among bodybuilders and weightlifters. These effects disappeared within three months after stopping AAS urging users to start a new cycle. This may explain why 79% of the subjects in our study were past users and 48% of users considered themselves to be addicted.[3] As expected, all users experienced at least one negative side effect during androgen exposure, such as testicular atrophy, fluid retention, acne, agitation, snoring, palpitations and gynecomastia. However, these were mostly considered mild, anticipated and transient. As a result, only 13% of the subjects stopped or altered the intended AAS cycle due to side effects.

Fifty-three percent of subjects reported loss of libido in the three months after the end of the cycle, which is not unexpected knowing that androgen abuse results in suppression of endogenous testosterone production. Surprisingly, other symptoms that are frequently associated with this "steroid dip", such as erectile dysfunction, mood swings or depressed mood, were not reported more frequently compared to baseline. The type and frequency of desired effects and side effects during and after androgen exposure were similar to those recollected by experienced users from previous cycles.[3] The fact that 79% of our cohort had past experience with AAS suggests that for most users the benefits of androgen abuse clearly outweigh the side effects. It also indicates that users of AAS are well aware of the possible side effects of androgen abuse. Therefore, educating them about these side effects will probably not be a viable strategy to withhold them from future use.

Physical examination and laboratory analysis

Besides moderate weight gain, acne and gynecomastia, physical examination during or after androgen exposure was mostly unremarkable. The prevalence of acne and gynecomastia as identified by physical examination during the AAS cycle was lower compared to the prevalence as reported by the participants. This was probably because physical examination reflected a point prevalence at the end of the cycle whereas reported prevalence concerned gynecomastia or acne at some time during the cycle. Gynecomastia is a well-known side effect of androgen abuse and was frequently self-medicated with tamoxifen to prevent or treat it. Furthermore, subjects sometimes regarded a few comedones on the back and shoulders as acne, but in fact it was too limited to be classified as a skin condition. Acne and gynecomastia were mild and reversible in most cases, although 7% of participants already had grade 1 or 2 gynecomastia at baseline and previous AAS use was strongly associated with gynecomastia at baseline and the development of gynecomastia during androgen exposure. Only one subject developed severe gynecomastia which did not recover during follow-up.

Kidney

Androgen abuse has been associated with kidney damage, although direct toxic effects of AAS on renal function have not been well characterized in humans. There are several potential factors that may cause or contribute to kidney damage in AAS users. Muscular AAS users engaged in heavy weightlifting may suffer from rhabdomyolysis, sometimes with massive elevations of serum creatine kinase levels leading to myoglobinuria, decreased glomerular filtration, and occasional progression to acute renal failure. Androgen abuse has been associated with elevated blood pressure which may contribute to renal damage. It has been suggested that AAS abuse, or the increased body mass that results from it, may lead to focal segmental glomerulosclerosis in susceptible individuals.[21] In our study, laboratory analysis showed a small but reversible increase in mean creatinine concentrations during AAS use and new or progressive proteinuria (as measured by urine dipstick) in 16% of subjects. Of note, estimation of kidney function may be unreliable as creatinine is a muscle breakdown product and muscle mass increases substantially during AAS use. At the end of follow up, all kidney parameters were similar or slightly improved compared to baseline. We conclude from these findings that androgen abuse, even in high doses, does not result in acute kidney damage.

Liver

Liver toxicity has been described mainly in association with the use of 17-alkylated oral androgens.[22] Although clinical application has been largely abandoned, oral AAS are still widely used. Hepatotoxicity caused by oral AAS is characterized by high bilirubin levels and causes pronounced jaundice.[23] Of our cohort, 67% reported the use of oral steroids. Contrarily, mean levels of bilirubin, ALP and GGT decreased slightly but significantly during androgen exposure, returning to baseline within three months. In most subjects a rise in the concentrations of ALT, AST and LDH occurred. Although this may suggest liver damage, we assume this to be the result of frequent heavy workouts, underlined by the steep rise in CK concentrations during androgen exposure in almost all subjects. From these findings we conclude that (sub)acute liver damage is a rare finding among androgen abusers, even though oral anabolic steroids are widely used.

Psychological impact of AAS

AAS exposure has been associated with manic or hypomanic behavior, irritability, aggression and even psychosis, whereas androgen abstinence has been associated with depression and anhedonia. In our cohort, one subject stopped using androgens due to suicidal ideation. In the other subjects, AAS did not induce relevant psychological disturbances based on the results of psychological questionnaires. Although a significant proportion of our subjects self-reported increased aggression and agitation during androgen exposure, the aggression questionnaire did not reveal an elevated physical aggression index. A previous study documented similarly elevated levels of irritability in association with AAS use without a rise in acts of physical violence.[24] Mean scores on wellbeing and quality of life questionnaires did not change during androgen exposure, although a significant proportion of the participants self-reported improved concentration and energy during the androgen cycle. Most importantly, the recovery period was not associated with an increased prevalence of depressive symptoms. The results

of our study indicate that androgen exposure and withdrawal will not lead to a significant disruption of psychological health in the vast majority of users.

Moderate to severe body dysmorphic disorder, according to the BDD-YBOCS questionnaire, was present in 32% of subjects at baseline, and this disorder may be a driving force behind AAS use in a subgroup of users.[25] Scores remained unchanged during androgen abuse despite the increase in muscle size and strength, indicating the disengagement of body image from true muscularity. Remarkably, the scores decreased towards the end of follow up, even though muscle strength and muscle mass are rapidly lost after androgen exposure. The decrease in symptoms of body dysmorphic disorder during follow-up may reflect a relatively high baseline score, when subjects are caught up in their self-image while preparing for the AAS cycle, as well as a decline after the cycle when physical appearance may become a lower priority after the cycle.

Determinants of the risks of side effects

Our estimate of cycle dose did not accurately reflect actual androgen exposure. It was based on the declared and not actual concentration of the abused products, which may be quite different.[4] The estimated weekly androgen dose and cumulative dose varied considerably among study subjects; the highest weekly dose was more than tenfold the lowest dose and the highest cumulative dose was more than hundredfold the lowest cumulative dose.[3] We are therefore quite confident that we were able to classify an AAS cycle as “high” or “low” dose. Remarkably, our data showed that reported weekly or cumulative dose could not be reliably used to predict the chance, type or severity of side effects. Higher androgen exposure was not necessarily associated with a higher risk of self-reported side effects and side effects such as gynecomastia, acne, liver and kidney damage, degree of aggression or depression. More importantly, lower dose steroid cycles cannot be considered safe. Of note, we were unable to link particular side effects to particular abused substances, as declared AAS types often do not match the actual content.[3]

Study limitations

Several limitations of the HAARLEM study are described elsewhere[3] and include the exclusion of athletes who use AAS continuously and the introduction of selection bias by offering medical check-ups for free. Moreover, AAS cycles were greatly heterogeneous and, as mentioned, calculation of androgen dose was unreliable. Multivariable analysis cannot fully adjust for confounding factors and unmeasured or unknown confounders were not taken into account.

Furthermore, only one clinic visit took place during the AAS cycle. The used questionnaires reflect on the past one or two weeks and psychological changes that occurred earlier in the cycle may have been missed. Some recall bias may still have led to underreporting of certain side effects, but was minimized by using checklists during clinic visits. Also, the questionnaires are not validated and may not be sensitive to detect effects typically resulting from androgen exposure. Another shortcoming is that the severity of the self-reported side effects was not recorded systematically. However, only 13% of subjects considered side effects severe enough to stop or adjust the intended AAS cycle. Obviously, the results of this study do not exclude potential long term side effects of androgen abuse. The design

of the study also precludes convincing statements about causality of the rare but serious adverse effects that were encountered.

PERSPECTIVE

The HAARLEM study provides a detailed and reliable overview of side effects of AAS use in a prospectively analyzed and representative cohort of 100 amateur male athletes during one year follow-up. All users experience positive side effects such as an increase in muscle strength and size. All users encountered negative side effects as well, most prominently gynecomastia and acne, but these were usually anticipated, mild and transient. Acute or subacute effects on liver and kidney function were absent or completely reversible and unrelated to the use of oral androgens. In the vast majority of users, androgen exposure and withdrawal will not lead to clinically relevant psychological effects. In a few subjects, a serious adverse event occurred. Cycle dose and duration cannot be used to predict the chance to suffer side effects, nor its severity. For most users the benefits of AAS use outweighed the side effects, supported by the observation that side effects rarely led to termination of AAS use. Solely informing AAS users about side effects will probably not be effective in preventing athletes from using AAS. Future studies should therefore focus on the long-term health risks of AAS use and on how to reduce harm in athletes using AAS.

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CHAPTER 6

DISRUPTION AND RECOVERY OF TESTICULAR FUNCTION DURING AND AFTER ANDROGEN ABUSE. THE HAARLEM STUDY

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Human Reproduction
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ABSTRACT

Study question: What is the speed and extent by which endogenous testosterone production and spermatogenesis recover after androgen abuse?

Summary answer: Testosterone concentrations normalized within 3 months after discontinuation of androgen abuse in most subjects but recovery of spermatogenesis took longer – approximately 1 year.

What is known already: An estimated 4-6% of amateur strength athletes uses androgens. Abuse of supraphysiological doses of androgens completely suppresses endogenous testosterone production and spermatogenesis.

Study design, size, duration: Prospective and observational cohort study in which 100 male amateur athletes participated for 1 year.

Participants/materials, setting, methods: Subjects (≥18 years) were included if they had not used androgens for at least 3 months and intended to start an androgen cycle within 2 weeks. Clinic visits took place before (T_0), at the end (T_1), and 3 months after the end of the cycle (T_2), and 1 year after start of the cycle (T_3), and included blood test for gonadotropins , sex hormones and semen analysis.

Main results and the role of chance: During androgen abuse, 77% of subjects had a total sperm count (TSC) below 40 million. Three months after the end of the cycle (T_2), total (-1.9 nmol/l, CI -12.2 to 8.33, P=.71) and free (-38.6 pmol/l, CI -476 to 399, P=.86) testosterone concentrations were not different compared to baseline, whereas mean TSC was 61.7 million (CI 33.7 to 90.0; P<.01) lower. At the end of follow-up (T_3), there was no statistically significant difference for total (-0.82 nmol/l, CI -11.5 to 9.86, P=.88) and free (-25.8 pmol/l, CI -480 to 428, P=.91) testosterone compared to baseline, but there was for TSC (-29.7 million, CI -59.1 to -0.39, P=.05). In 9 (11%) subjects, however, testosterone concentrations were below normal at the end of follow-up (T_3), and 25 (34%) subjects still had a TSC below 40 million.

Limitations, reasons for caution: The follow-up period (after the cycle) was relatively short, especially considering the long recovery time of spermatogenesis after discontinuation of androgens.

Wider implications of the findings: Endogenous testosterone production and spermatogenesis recover in the vast majority of users. Nevertheless not all users achieve a normalized testicular function. This may especially be the case for athletes with a high past exposure to androgens.

INTRODUCTION

The use of androgens is not uncommon among regular visitors of fitness centres. The prevalence for men is estimated at 4-6%.[1,2] The abuse of androgens is harmful but exact data about the frequency, severity and reversibility of side effects associated with androgen abuse are scarce. Knowledge of the unwanted effects of androgens is based on low-level evidence, such as case reports and cross-sectional studies, and a few larger retrospective studies.

One of the well-known effects of androgen abuse is complete suppression of gonadotropins, endogenous testosterone production and spermatogenesis.[3] After cessation of androgens it takes time before endogenous testosterone production and spermatogenesis recover. The speed of recovery of endogenous testosterone production presumably depends primarily on the type and dose of the androgens used in the last phase of the cycle. Androgens are mostly injected as an intramuscular depot. Depending on the type of fatty acid chain attached to the steroid, the plasma half-life after injection may be days to weeks. Taking into consideration that the administered doses are many times the natural endogenous production, it may take several weeks before exogenous androgen levels are low enough to allow endogenous testosterone production to resume.

Male contraceptive studies provide valuable information about the recovery of gonadal function after exposure to a variety of testosterone formulations, albeit mostly combined with progestins.[4] However, results obtained in these studies may not be extrapolated because androgen abusers typically use much higher doses and a variety of androgens. Most of these androgens, such as trenbolone, boldenone and drostanolone, are not registered for human use and the effects on gonadal function are therefore not well studied. Because of this, exposing individuals to these agents in the extreme doses typically used by androgen abusers is considered unethical. Therefore, most of the current evidence derives from case reports, retrospective studies and cross-sectional investigations.[5] Recent case control studies attempted to quantify the recovery of testosterone production and spermatogenesis after androgen abuse with conflicting results.[6,7]

Anti-oestrogens, such as tamoxifen and clomiphene citrate, have been shown to moderately stimulate gonadotrophin and testosterone production in eugonadal, obese and hypogonadal men.[8,9] Consequently, these substances are frequently used following androgen exposure, based on the unproven assumption that they will speed up recovery of the male hypothalamic-pituitary-gonadal axis.[10,11] In addition, human choriogonadotropin (hCG) is used during or following the cycle to boost endogenous testosterone production and spermatogenesis. Although frequently advocated, there is no evidence that these strategies, commonly referred to as post cycle therapy (PCT), are effective, and claimed effects from non-controlled studies may well reflect natural recovery.

We initiated a prospective observational study to provide more reliable data regarding health risks associated with androgen abuse: the HAARLEM-study (acronym for Health risks of Anabolic Androgenic steRoid use by maLE aMateur athletes). We assembled a cohort of 100 men and performed semen and hormone analysis before, during and after a cycle of androgens in order to answer the following

questions: 1) what is the effect of androgen abuse on spermatogenesis, 2) do testosterone concentrations and total sperm count reach normal laboratory values after androgens are discontinued, 3) are cycle dose and duration associated with the extent of recovery, and 4) what is the influence of PCT on these outcomes?

METHODS

A detailed description of the methods of subject recruitment are described in a previous report (Smit *et al.*, 2019). In short, the 100 subjects included in the HAARLEM-study were men of at least 18 years old intending to start an androgen cycle on short notice (i.e. within 2 weeks). The scheduled cycle had to be at least 6 weeks in duration, comprise at least two different androgen types, and average a weekly dose of 200 mg or more. Subjects were required to not have used androgens for at least 3 months prior to inclusion. The study took place in the outpatient anabolic steroids clinic in Haarlem, the Netherlands, after the study was promoted on national television and in regional newspapers, as well as on social media and bodybuilding forums where many androgen abusers are known to gather. Androgens, performance and image-enhancing drugs (PIEDs) and medication were acquired by subjects through their usual channels and were not prescribed by the clinic, nor did they receive any advice regarding their cycles. The study was approved by the local institutional review board (IRB) and subjects provided written informed consent.

Clinic visits

Subjects visited the study clinic 4 times during a 1 year study period. A medical investigation was performed at baseline i.e. within 2 weeks before the initiation of the androgen cycle (T_0), comprising blood and semen analysis. Testicular size was measured with an orchidometer. Mean weekly androgen dose was based on label information and, because the exact content of androgens was unknown, all androgen types were considered equivalent (e.g. 1 mg testosterone = 1 mg nandrolone = 1 mg stanozolol). Investigations were repeated in the last week of the cycle (T_1), 3 months after the end of the cycle (T_2), and 1 year after the start of the cycle (T_3).

Laboratory analysis

Subjects visited the hospital laboratory for collection of blood in the morning after an overnight fast. Blood was drawn by venepuncture by a qualified laboratory assistant. Lithium heparin and serum samples were analysed on the Abbott ARCHITECT system. Parameters measured were luteinizing hormone (LH), follicle stimulating hormone (FSH), 17-β-oestradiol, total testosterone and sex-hormone binding globulin (SHBG). Free testosterone was calculated from SHBG, albumin and total testosterone.[12]

Semen was collected on the same morning by masturbation in a designated room in the hospital and analysed according to World Health Organization (WHO) standards.[13] Subjects were instructed to avoid ejaculation 72 hours prior to this analysis. Total sperm count (TSC) was based on semen volume and concentration.

Statistical analysis

Simple descriptive statistics were used to display quantitative data. As measurements were clustered within patients, linear and logistic mixed models were used for continuous and dichotomous variables, respectively, to calculate mean differences, 95%-confidence intervals (CI), odds ratios (OR) and P values. This analysis takes missing data into account.[14] For laboratory parameters the mean with CI was calculated, as well as the number of subjects that had a result outside the reference range, and the percentage change of the parameter compared to baseline for subjects individually. The analysis was repeated in the subgroup of subjects without gonadal dysfunction at baseline, defined as a having a normal total testosterone (≥9.0 nmol/l, lower limit of reference range in our laboratory) and TSC (≥40 million).

Multivariable regression analysis was performed with mixed models as well and assessed the confounding role of weekly androgen dose and cycle duration, number of androgens used, the use of post-cycle therapy (PCT), the use of human chorionic gonadotropin (hCG), and recent history of androgen use (<12 months). To correct for multiple testing, a Bonferroni adjustment to P values for significance was employed. Stata software (for Windows, version 15, StataCorp, 2017) was used for analysis.

RESULTS

Between October 2015 and May 2018, 220 subjects signed up for the HAARLEM study, 163 subjects were assessed for eligibility, and 111 men signed informed consent and performed a baseline clinic visit. Of these subjects, 100 would remain in the study for follow-up (see *Figure* 1). The median age of the subjects in this group was 31 years (range 19-67). All but one subject engaged in strength sports and the average amount of time spent in the gym was 306 minutes per week. Seventy-nine subjects had a history of androgen abuse but 20 of them had not used androgens in the year prior to inclusion. During follow-up subjects performed a cycle with a median duration of 13 weeks (range 2-52) and 5 types of androgens (range 1-11). The median dose of androgens was 901 mg per week (range 250-3382) of which 97 mg were oral androgens. Androgens most commonly used – based on product label information – were testosterone (96%), trenbolone (52%) and drostanolone (39%). Only one subject used testosterone only; all other subjects used testosterone combined with other androgens. Of all the injectables in our study, the distribution of esters as declared on the label was as follows: enanthate 43%; propionate 19%; decanoate 11%; undecylenate 10%; acetate 8%; cypionate 4%; and mixed esters (Sustanon) 6%. Eighty subjects carried out post-cycle therapy (PCT). The median duration of PCT was 4 weeks (range 1-8) and subjects used a median of 2 PCT agents, mostly tamoxifen (70%), clomiphene citrate (54%) and hCG (55%). Further demographic data of subjects and additional details of the androgen cycles and PIEDs used during follow-up are described elsewhere.[15]

Two subjects withdrew consent after T_1, of whom 1 due to emigration, and no data were available from T_2 and T_3 (see *Figure 1*). There were 5 subjects who did not terminate the cycle after T_1 and 10 who started a second cycle of androgens during follow-up; these subjects were excluded from analysis for the remaining clinic visits. Another 9 clinic visits were missed (twice T_1, thrice T_2, four times T_3) due to obligations for work or personal circumstances. Six subjects did not have semen analysed because

of previous operative sterilization. One subject was not able to produce semen at T_1 due to lack of libido. In *Table 1* and *Table 2* for each clinic visit the number of subjects is displayed from whom data were available for analysis.

***Table 1*.** Results of laboratory analysis during clinic visits. The mean (x̄) of every parameter with 95%-confidence interval (CI) and number of subjects (n) with a result outside the reference range (RR) are displayed. T_0 = before the start of the cycle, T_1 = in the last week of the cycle, T_2 = 3 months after the cycle, T_3 = 1 year after the start of the cycle. CI, OR and P values were calculated with mixed models and compare visit T_1, T_2 and T_3 with T_0. For the number of times subjects deviated from the RR only significant P values are shown without odds ratio. †P=.01-.05, ‡P<.01.

	T_0 (n = 100)	**T_1 (n=98)**	**T_2 (n=90)**	**T_3 (n=79)**
Blood analysis (unit, RR)	**x̄ [CI]**	**x̄ [CI]**	**x̄ [CI]**	**x̄ [CI]**
	n (%)	**n (%)**	**n (%)**	**n (%)**
LH (U/l, 1-12)	3.0 [2.7-3.4]	0.1‡ [-0.2-0.5]	2.8 [2.4-3.2]	3.1 [2.7-3.5]
	9 (9%)	93‡ (95%)	9 (10%)	4 (5%)
FSH (U/l, 1-12)	3.9 [3.4-4.5]	0.1‡ [-0.5-0.6]	3.2† [2.6-3.7]	4.1 [3.5-4.6]
	13 (13%)	94‡ (96%)	10 (11%)	5 (5%)
17-β-oestradiol (pmol/l, 40-162)	85 [22-149]	350‡ [286-415]	79 [11-145]	81 [10-154]
	2 (2%)	51‡ (52%)	2 (2%)	0 (0%)
Testosterone, total (nmol/l, 9.0-37.3)	16.6 [9.3-23.9]	79.8‡ [72.4-87.1]	14.8 [7.0-22.3]	15.6 [7.6-24.0]
	21 (21%)	74‡ (76%)	17 (19%)	9 (11%)
SHBG (nmol/l, 14-71)	29.9 [27.8-32.0]	12.5‡ [10.2-14.5]	26.4‡ [24.0-28.4]	29.2 [26.4-30.9]
	12 (12%)	62‡ (63%)	9 (10%)	5 (6%)

The median follow-up period was 11.9 months (range 8.0-17.0). T_1 took place in the last week of the cycle after a median of 3.5 months (range 0.5-11.7) after inclusion. T_2 and T_3 took place after a median of 3.2 (range 2.1-8.1) and 9.4 months (range 3.7-12.1) after T_1, respectively, and reflected recovery after the cycle. Details of blood and semen analysis are displayed in *Table 1* and *Table 2,* respectively. Box and whisker plots of total testosterone and TSC during clinic visits are shown in *Figure 2*.

Baseline values

At baseline, 37 subjects showed signs of gonadal dysfunction based on low total testosterone concentration (<9.0 nmol/l) or low sperm count (TSC <40 million). Characteristics of these subjects are shown in *Supplementary table 1*. The cumulative exposure to androgens in the past was higher in this group compared to the 63 subjects without gonadal dysfunction at baseline (91 versus 46 weeks, P=.02). Twenty-five subjects in this group of 37, including 4 with azoospermia, had self-reported androgen abuse 3 to 6 months prior to inclusion. Six had reportedly used androgens 6 to 12 months

prior to inclusion and 4 subjects 12 months or longer. Two subjects had no history of androgen abuse and only showed a mildly decreased TSC.

***Table 2*.** Results of semen analysis (including testicular volume) during clinic visits. The mean ($\bar{x}$) of every parameter with 95%-confidence interval (CI), as well as the number of subjects (n) had a result outside the reference range (RR) are displayed. T_0 = before the start of the cycle, T_1 = in the last week of the cycle, T_2 = 3 months after the cycle, T_3 = 1 year after the start of the cycle. CI and P values were calculated with mixed models and compare visit T_1, T_2 and T_3 with T_0. †P=.01-.05, ‡P<.001.

	T_0 (n = 94)	**T_1 (n=91)**	**T_2 (n=84)**	**T_3 (n=73)**
Semen analysis (units, RR)	**$\bar{x}$ [CI]**	**$\bar{x}$ [CI]**	**$\bar{x}$ [CI]**	**$\bar{x}$ [CI]**
	n (%)	**n (%)**	**n (%)**	**n (%)**
Semen volume (ml, ≥2.0)	3.1 [2.8-3.4]	2.4‡ [2.1-2.8]	2.7† [2.3-3.0]	2.7† [2.3-3.0]
	29 (31%)	42† (45%)	107 31 (37%)	107 21 (29%)
Concentration ($x10^6$/ml, ≥15)	46.8 [40.1-53.6]	11.7‡ [5.0-18.7]	36.4† [28.6-42.8]	44.0 [35.8-50.9]
	24 (26%)	62‡ (68%)	28 (33%)	21 (29%)
Total sperm count ($x10^6$, ≥40)	145 [124-174]	30.0‡ [4.9-55.2]	87.9‡ [62.0-114]	120† [92.5-147]
	27 (28%)	70‡ (77%)	34 (40%)	25 (34%)
Progressive motility (%, ≥32%)	47.1 [42.7-50.5]	32.8‡ [28.0-36.3]	43.5 [39.0-47.1]	46.7 [41.7-50.2]
	16 (18%)	36‡ (48%)	24† (29%)	15 (21%)
Mean testicular volume (ml, ≥12)	17.4 [16.6-18.2]	13.2‡ [12.4-14.0]	16.7† [15.9-17.4]	17.5 [16.6-18.3]
	11 (12%)	28 (31%)‡	10 (12%)	102 6 (8%)

When comparing all subjects with a history of androgen abuse in the cohort (n=79) to those without (n=21), there was no statistically significant difference in mean total (-2.8 mmol/l, CI -20.5 to 14.9, P=.76) and free testosterone (-51.1 pmol/l, CI -8.03 to 701, P=.89) at baseline. However, a history of androgen abuse was associated with significantly lower mean testicular volume (-3.14 ml, CI -4.99 to -1.3, P<.01).

Of the 111 subjects who performed a baseline clinic visit (see *Figure 1)*, 25 subjects had no history of androgen abuse. They can be considered a non-user control group. The median age of this group was 30 years, all of them engaged in strength sports, and the average weekly time spent in the gym was 331 minutes. Their mean total testosterone was 18.5 nmol/l (SD 5.3) and mean TSC was 189 million (SD 138).

Figure 1. Flow chart showing the disposition and follow-up of study subjects. Twenty-five of the 111 included subjects did not have a history of androgen abuse and could be considered a non-user control group.

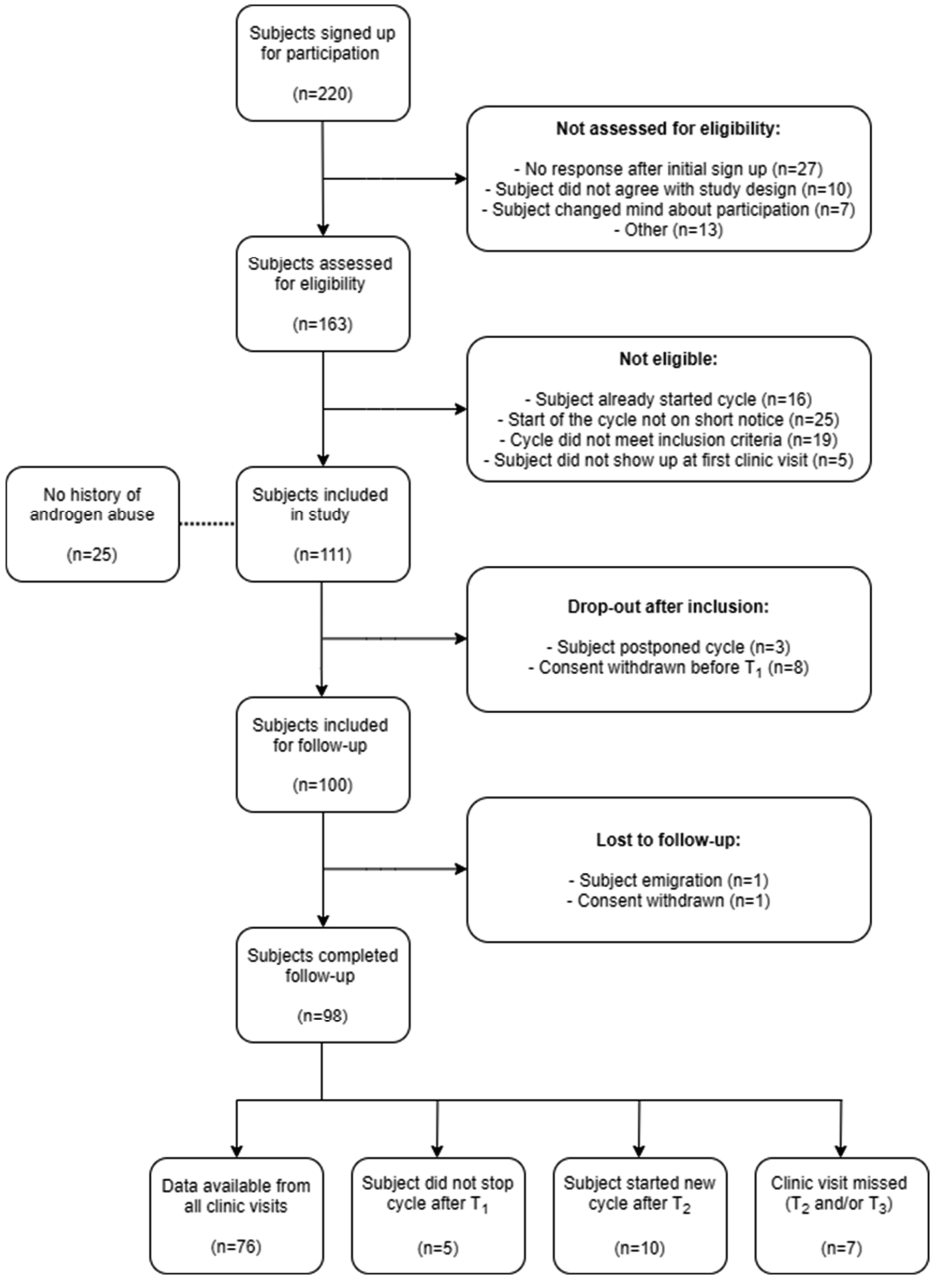
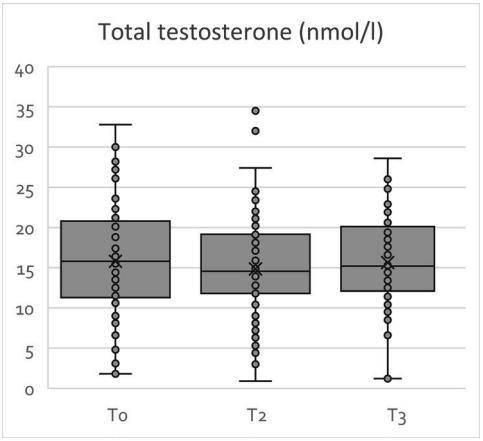
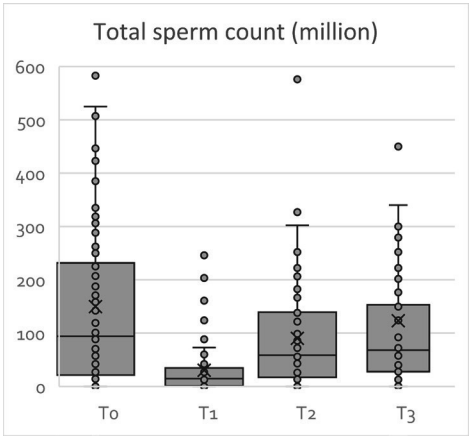


Figure 2. Box and whisker plots of (A) total testosterone, (B) total sperm count and (C) testicular volume at the clinic visits. The box portion is defined by the 25th and 75th percentile. The median appears as a horizontal line inside the box portion whereas the mean is shown with an X. For the sake of clarity, clinic visit T_1 is left out in the diagram of total testosterone. T_0 = before the start of the cycle, T_1 = in the last week of the cycle, T_2 = 3 months after the cycle, T_3 = 1 year after the start of the cycle.

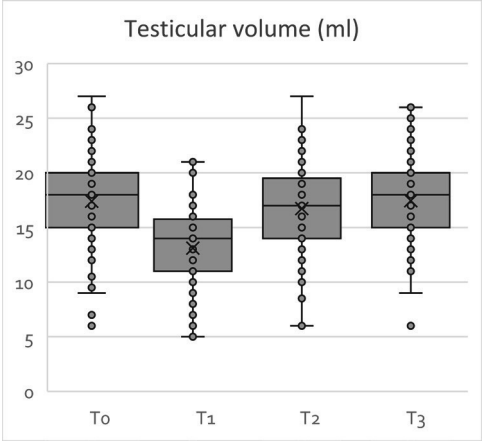
A.



B.



C.



Changes during androgen abuse

Between T_0 and T_1, mean levels of FSH and LH declined and mean concentrations of total and free testosterone increased significantly (see *Table 1* and *Figure 2*). The increase of total and free testosterone was positively correlated with cycle duration and average weekly dose. The mean concentration of SHBG declined significantly during androgen exposure; the rate of decline was not

associated with cycle duration or average weekly dose. The mean concentration of 17-β-oestradiol increased significantly during androgen exposure.

Testicular volume decreased 4.3 ml (CI 3.7 to 4.8; P<.01; see *Table 2* and *Figure 2*) and the decrease was smaller in subjects with a history of androgen abuse, who already had a lower testicular volume at baseline. Mean TSC decreased by 120 million (CI 92.3 to 147; P<.01) or 61.8% (95%CI -93.8.0 to -29.8, P<.01) between T_0 and T_1. During androgen exposure, 70 subjects (77%) had a TSC below 40 million, of whom 21 subjects (23%) had azoospermia.

Recovery after androgen abuse

After the cycle, at both T_2 and T_3, mean concentrations of LH, 17-β-oestradiol, total and free testosterone were not significantly different compared to baseline (T_0); see *Figure 2* and *Table 1*. Mean concentrations of FSH and SHBG were lower compared to baseline 3 months of the end of the cycle (T_2) with -0.73 U/l (CI -1.31 to -0.15, P=.01) and -3.68 nmol/l (CI -5.83 to -1.53, P<.01), respectively.

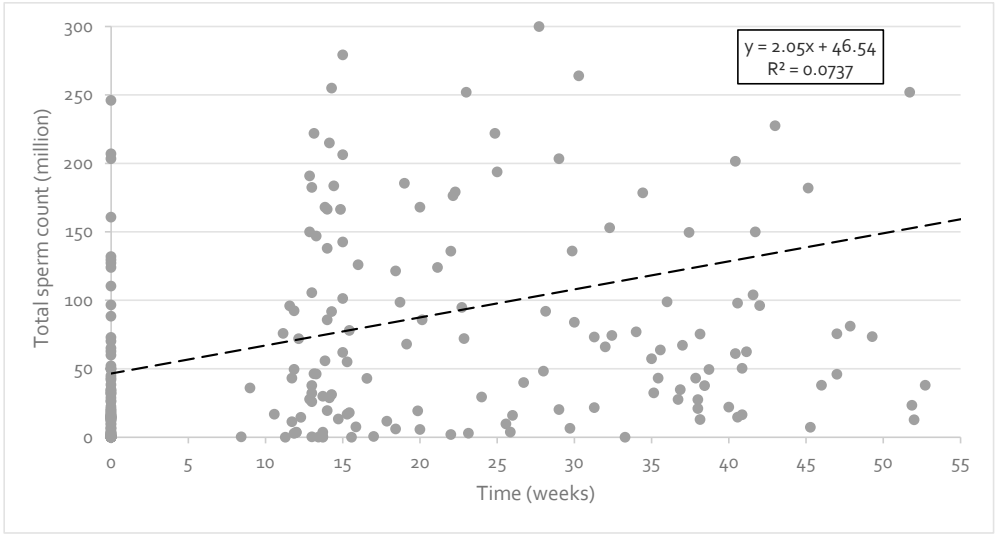
Mean testicular volume, semen volume and concentration were significantly lower 3 months after androgen exposure (T_2) compared to baseline. Mean TSC was 61.7 million (CI 33.7 to 90.0, P<.01) lower at this point compared to baseline. Of the 34 subjects (40%) with a TSC below the reference range at T_2, 8 (11%) had azoospermia. At the end of follow-up (T_3), the parameters that were still significantly different compared to baseline were semen volume (-0.4 ml, CI -0.8 to -0.1; P<.01), and TSC (-29.7 million, CI -59.1 to -0.39, P=.05).

The first recovery visit (T_2) was fixed at 3 months after the end of the cycle, whereas the second recovery visit took place 12 months after inclusion. As the duration of the androgen cycle varied considerably between subjects, the interval between visits T_2 and T_3 also varied, inversely in relation to cycle length. All measurements of TSC at visits T_1, T_2 and T_3 are plotted in *Figure 3A* relative to the elapsed time since the end of the cycle (T_1). Recovery of TSC showed a positive correlation with time (β=2.05, CI 1.14 to 2.96, P<.01). Based on the regression equation, time to complete recovery of TSC, i.e. the time needed for the equation to reach the mean TSC at baseline, can be estimated to be 48 weeks (CI 33-86). If the mean TSC of the 25 controls without a history of AAS use is taken as a reference, this estimate would be 69 weeks (CI 48-124).

There were 9 subjects with a total testosterone below normal at the end of follow-up (T_3). They all had a low testosterone at baseline with low to low-normal LH and FSH, a history of recent androgen abuse prior to inclusion (range 3-7 months), and a high cumulative past exposure to androgens (108 months on average). Their cycle performed during follow-up was longer (25 vs. 17 weeks, P=.04) and had a higher average weekly dose (1.448 vs. 977 mg, P=.01) compared to the rest of the cohort. The regression equation of recovery of testosterone between T_2 and T_3 for this group showed a non-significant negative slope (β=-0.05, CI -0.23 to 0.12, P=.53).

Figure 3. Scatter plots and linear regression of the recovery of total sperm count (TSC) after androgen exposure. For this analysis, T_1 was considered as baseline (time = 0). Separate plots are shown for the entire cohort (A) and the subgroup of 63 subjects without gonadal dysfunction at baseline (B). The calculated slopes were 2.05 (CI 1.14 to 2.96, P<.01) and 2.75 (CI 1.43 to 4.08, P<.01), respectively.

A.



B.

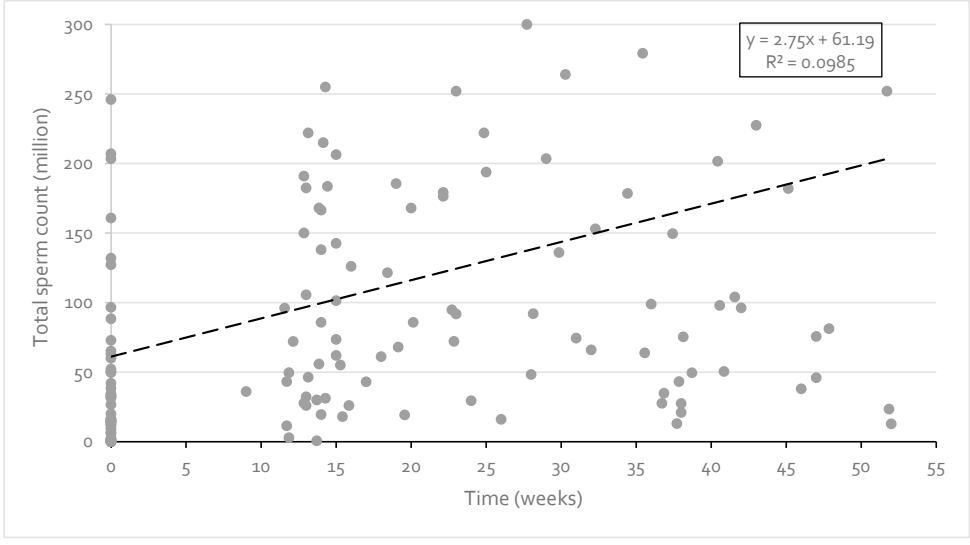


Table 3. Results of laboratory analysis during clinic visits of the 63 subjects with normal gonadal function at baseline. The mean ($\bar{x}$) of every parameter with 95%-confidence interval (CI), as well as the number of subjects (n) had a result outside the reference range (RR) are displayed. T_0 = before the start of the cycle, T_1 = in the last week of the cycle, T_2 = 3 months after the cycle, T_3 = 1 year after the start of the cycle. CI and P values were calculated with mixed models and compare visit T_1, T_2 and T_3 with T_0. †P=.01-.05, ‡P<.01.

	T_0 (n = 63)	**T_1 (n=63)**	**T_2 (n=59)**	**T_3 (n=52)**
Blood analysis (unit, RR)	**$\bar{x}$ [CI]**	**$\bar{x}$ [CI]**	**$\bar{x}$ [CI]**	**$\bar{x}$ [CI]**
	n (%)	**n (%)**	**n (%)**	**n (%)**
LH (U/l, 1-12)	3.1 [2.7-3.5]	0.2‡ [-0.2-0.6]	3.0 [1.6-2.4]	3.1 [2.7-3.5]
	0 (0%)	59 (94%)	3 (5%)	2 (4%)
FSH (U/l, 1-12)	4.0 [3.5-4.5]	0.1‡ [-0.4-0.7]	3.1‡ [2.6-3.6]	3.9 [3.4-4.5]
	0 (%)	63 (100%)	3 (8%)	1 (2%)
17-β-oestradiol (pmol/l, 40-162)	83.4 [40.6-126]	308‡ [266-351]	80.5 [36-125]	83 [36-130]
	0 (0%)	34 (54%)	2 (3%)	0 (0%)
Testosterone, total (nmol/l, 9.0-37.3)	17.8 [10.6-25.2]	73.7‡ [66.2-81.3]	15.9 [8.4-23.7]	17.1 [9.2-25.5]
	0 (0%)	62 (98%)	6 (10%)	0 (0%)
SHBG (nmol/l, 14-71)	34.0 [31.6-36.4]	13.6‡ [11.2-16.0]	28.9‡ [26.4-31.3]	32.1 [29.5-34.7]
	0 (0%)	41 (60%)	4 (6%)	0 (0%)

Subjects without gonadal dysfunction at baseline

In order to monitor recovery of gonadal function without potential carryover effects of previous androgen abuse and/or pre-existent gonadal dysfunction we excluded the 37 subjects that had gonadal dysfunction at baseline, i.e. low testosterone concentration or TSC. Details of blood and semen analysis of clinic visit T_0 through T_3 of the remaining 69 subjects are shown in *Table 3* and *Table 4*. In this group, as a result of androgen exposure, mean testicular volume decreased with -4.3 ml (CI -5.0 to -3.6, P<.01). Mean TSC declined 167 million (CI 128 to 207, P<.01). Forty-one subjects (71%) had a TSC below 40 million during androgen exposure (T_1) of whom 10 (16%) had azoospermia.

For the group of subjects without gonadal dysfunction at baseline, recovery of total testosterone, LH, FSH and TSC is shown as box and whisker plots in *Figure 4*. Mean concentrations of FSH and SHBG were still significantly lower at T_2 compared to baseline by -0.89 U/l (CI -1.45 to -0.30, P<.01) and -5.26 nmol/l (CI -7.92 to -2.52, P<.01), respectively. Six (10%) subjects had a total testosterone concentration below the reference range at T_2 but none of the subjects had low testosterone at the end of follow up (T_3). TMC was reduced by 82.0 million (42.0 to 122, P<.01) 3 months after androgen exposure (T_2) and by 45.9 million (CI 4.32 to 87.5, P=.03) at the end of follow up (T_3). The number of subjects in this group with a TMC below 40 million and azoospermia were 8 (15%) and 8 (15%) at T_2, and 10 (20%) and 0 (0%) at T_3, respectively. *Figure 3B* shows the time-dependent recovery of TMC after androgen exposure.

Recovery time for this subgroup can be estimated at 56 weeks (CI 38-108) with the linear regression equation. The estimated recovery time is 47 weeks (CI 31-89) if the control group of 25 subjects without a history of AAS use is taken as a reference.

Table 4. Results of semen analysis (including testicular volume) during clinic visits of the 63 subjects with normal gonadal function at baseline. The mean ($\bar{x}$) of every parameter with 95%-confidence interval (CI), as well as the number of subjects (n) had a result outside the reference range (RR) are displayed. T_0 = before the start of the cycle, T_1 = in the last week of the cycle, T_2 = 3 months after the cycle, T_3 = 1 year after the start of the cycle. CI and P values were calculated with mixed models and compare visit T_1, T_2 and T_3 with T_0. †P=.01-.05, ‡P<.001.

	T_0 (n = 60)	**T_1 (n=58)**	**T_2 (n=54)**	**T_3 (n=49)**
Semen analysis (units, RR)	**$\bar{x}$ [CI]**	**$\bar{x}$ [CI]**	**$\bar{x}$ [CI]**	**$\bar{x}$ [CI]**
	n (%)	**n (%)**	**n (%)**	**n (%)**
Semen volume (ml, ≥2.0)	3.7 [3.3-4.2]	2.7‡ [2.1-2.8]	2.9† [2.4-3.3]	3.0† [2.6-3.4]
	10 (17%)	22 (38%)‡	19 (35%)†	13 (27%)
Concentration ($x10^6$/ml, ≥15)	65.2 [46.4-84.0]	13.9‡ [1.2-26.6]	43.8‡ [35.6-52.0]	52.3 [43.4-61.3]
	2 (3%)	36 (81%)‡	11 (21%)†	9 (16%)
Total sperm count (x106, ≥40	216 [183-250]	38.3‡ [4.42-72.2]	114‡ [80.1-149]	160† [124-196]
	0 (0%)	41 (71%)	16 (30%)	10 (20%)
Progressive motility (%, >32%)	50.4 [45.8-54.9]	34.5‡ [29.7-39.3]	45.4 [40.7-50.1]	49.2 [44.3-54.1]
	11 (18%)	24 (41%)‡	15 (28%)†	7 (14%)
Mean testicular volume (ml, ≥12)	18.3 [17.5-19.1]	14.1‡ [13.2-14.9]	17.8 [17.0-18.6]	18.4 [17.6-19.3]
	0 (0%)	13 (22%)	1 (2%)	0 (0%)

Determinants of recovery

Results of multivariable regression analysis are summarized in *Supplementary table 2*. Cycle duration, average weekly dose and number of androgen types used were not associated with total and free testosterone nor TSC at T_2 and T_3. This also held true if the analysis was done in subgroups comparing subjects with and without a history of androgen abuse and for the group of subjects with normal levels of total and free testosterone and TSC at baseline.

PCT was not associated with total or free testosterone, or TSC at T_2 or T_3. However, the use of PCT was associated with lower semen volume at T_3 if semen volume was viewed as a percentage of the baseline value (β = -65.2, CI -107 to -23.7, P<.01).

DISCUSSION

The HAARLEM study is a unique trial that prospectively analysed the gonadal consequences of androgen exposure. Blood and semen were analysed before, during and after an androgen cycle in a cohort of 100 male amateur athletes. The cycles performed were very heterogeneous but invariably contained very high supraphysiological doses of androgens.[15] Because the study was promoted through (social) media and websites on a nationwide level, evidenced by subjects participating from throughout the country, the cohort is believed to be representative of the general population of amateur athletes using androgens in the Netherlands. In addition, cycle characteristics were similar to those described in earlier reports.[6,10]

Changes during androgen abuse

As expected, serum total and calculated free testosterone were extremely high during androgen abuse. Obviously, this reflected the use of exogenous testosterone and the very significant suppression of SHBG levels. However, serum testosterone and oestrogen concentrations measured at the end of the androgen cycle did not provide a reliable estimate of the androgen and oestrogen exposure during the cycle. Other bioactive androgens and oestrogens were not measured and since testosterone and oestradiol concentrations were measured by an immunoassay, and not by mass spectrometry, cross-reactivity of other steroid hormones may have resulted in inadequate estimates of the actual concentration. Also, the serum testosterone and oestradiol concentrations may have varied considerably in the days after injection of a depot. Most importantly, the androgen cycles covered many months in most subjects with a wide variety of androgen types and doses during the cycle. Irrespective of androgen dose and duration of use, androgen exposure resulted in complete suppression of the hypothalamic-pituitary-gonadal axis as demonstrated by undetectable LH and FSH concentrations in nearly all subjects. Furthermore, testicular volume declined during androgen abuse and spermatogenesis decreased, with two-third of subjects having oligo- or azoospermia by the end of the cycle.

Cycle dose was calculated by adding up the androgen dose in milligram based on label information. This estimate does not accurately reflect the actual androgen exposure, due to the fact that it is based on the declared and not the actual concentration of the abused products. Moreover, it combines oral and injectable products and different types of androgens. However, acknowledging these important shortcomings, it gives some indication of the actual dose of the abused androgens. The estimated androgen cycle dose did not have a significant impact on the degree of suppression of spermatogenesis. This may not be a surprise, knowing that LH and FSH were undetectable in nearly all subjects, indicating that all subjects were exposed to supraphysiological doses of androgens, sufficient to arrest spermatogenesis.

More interesting is the observation that a longer duration of androgen exposure was not associated with the degree of suppression of spermatogenesis either. Hormonal contraceptive studies in males showed that spermatogenesis was suppressed between 2 and 4 months after the start of contraception.[16,17] In our study, cycle duration ranged between 2 and 52 weeks, therefore an inverse relation between duration of androgen exposure and TSC was expected. We are unable to explain the

absence of such a relation. Maybe this relates to the wide variety of androgen regimes as used by our subjects. Since this reflects the daily practice of androgen abuse, one can conclude that long term androgen abuse cannot be regarded as reliable contraception and that even relatively short term exposure may result in azoospermia in some individuals.

Recovery of endogenous testosterone production after androgen abuse

After 3 months of recovery, mean testosterone concentrations had returned to baseline levels for the entire cohort. When the pre-exposure gonadal function was normal, there was a 90% chance of having a normal total testosterone concentration after 3 months of recovery and a 100% chance at the end of follow-up. These data suggest that full recovery of endogenous testosterone production takes place for the vast majority of androgen abusers independent of cycle cumulative dose or duration, provided pre-exposure gonadal function is normal.

In contrast to this observation, 37 individuals had signs of abnormal gonadal function at baseline. To be included in the study, subjects were not allowed to use androgens up to 3 months prior to inclusion, which was verified by interview when subjects were screened for eligibility. Based on the evidence presented above, such a high rate of low testosterone and/or low TSC was not expected based on intraindividual and test variability alone. Of this group, 95% had used androgens in the past, indicating past androgens as a determinant of gonadal insufficiency. These subjects had a significantly higher cumulative past exposure to androgens, having abused androgens twice as long in their lifetime compared to the group with normal baseline gonadal function. Also, the pattern of gonadal dysfunction, i.e. hypogonadotropic hypogonadism, found in this subgroup was concordant with previous androgen use. Although we did not find spuriously elevated androgen levels in any of the subjects at baseline, we cannot exclude that some of them had used androgens within 3 months from inclusion.

In addition, a subgroup of 9 subjects still had a low total testosterone at the end of follow-up. It is uncertain whether endogenous testosterone production will eventually normalize in these men. Their high cumulative history of androgen exposure probably plays an important role in their prolonged hypogonadism. This association has been reported previously.[10,18] However, we were unable to determine other risk factors that predicted their slow recovery of gonadal dysfunction compared to other subjects that had a similar history of androgen abuse but did recover during follow-up.

It is not possible to exclude that subjects surreptitiously used androgens after clinic visit T_1, besides the 15 subjects that reported so beforehand (see *Figure 1*), which represents an important limitation. However, it is likely that surreptitious androgen use would have been detected with blood analysis because it would cause suppression of the hypothalamic-pituitary-gonadal axis. Only in 8 clinic visits after T_1 the gonadotrophins were below the detectable range and in all cases the testosterone concentration was low or normal but not above the reference range, whereas a cycle almost always contains a testosterone ester.

Recovery of spermatogenesis after androgen use

The recovery of spermatogenesis took longer than the recovery of endogenous testosterone production. Mean TSC was still significantly lower compared to baseline 3 months after the end of the cycle. This is not surprising as it takes approximately 90 days before spermatozoa appear in the ejaculate after spermatogenesis commences.[19] Also, from male hormonal contraception studies, it is known that recovery of spermatogenesis takes much longer than hormonal recovery.[4,17] This explains the rather high percentage of individuals in our study with subnormal TSC at baseline. Most likely, this is a carryover effect of recent androgen use, as is suggested by the very high percentage of past androgen users in this group. In our study, the estimated mean time of TSC to recover to baseline values was 48 to 69 weeks, depending on which group was taken as the reference (own control vs. non-user control group), which is comparable to the 14 months estimated by Shankara-Narayana et al. (2020).[7] Recovery was somewhat faster in the subgroup of subjects with normal gonadal function at baseline, with a calculated recovery time of 47 to 56 weeks.

Post-cycle therapy

In our study, 80 subjects performed PCT, which usually represented a combination of tamoxifen (20-40 qd), clomiphene citrate (25-50 qd) and/or hCG (1-3 times per week 500-1000 IU). The medication were generally started within 1 to 4 weeks after the last androgen dose and continued for about 2 to 4 weeks. However, PCT did not show any beneficial effect on the recovery of parameters 3 months after the cycle. The only association that reached statistical significance was a negative association between the use of PCT and semen volume. It cannot be fully excluded that the use of PCT shortened recovery time of endogenous hormone production. For most individuals, plasma testosterone concentrations had fully recovered 3 months after androgen exposure. If we had tested 1 or 2 months after androgen exposure, perhaps we might have found a difference between users of PCT and non-PCT users. Although the use of PCT is widely advocated among androgen users, there is hardly any evidence to support its use. Most PCT regimes consist of tamoxifen and/or clomiphene citrate used for four weeks after the use of androgens. Taking into consideration the very high supraphysiological doses of androgens and the long half-life of the intramuscular depots, it is unlikely that anti-oestrogens are able to counteract the strong suppressive effects of androgens on gonadotropin production.

Furthermore, the use of hCG had no detectable effect on testicular size or spermatogenesis, whereas this would be expected based on clinical experience and the medical literature.[20] Perhaps, the products acquired by subjects did not contain any, or less, active ingredients than declared on the label.[15,21] Dosage regimens used may also have been insufficient as many subjects injected less than 1500 IU per week.

Conclusion

Clinicians such as endocrinologists, urologists, fertility specialists and general practitioners may be confronted with (ex) users of androgen that present with gonadal dysfunction. Based on our data and provided normal pre-exposure gonadal function, hormonal recovery is expected within 3 months after stopping androgens for the vast majority of users, whereas recovery of spermatogenesis is expected after about 1 year. Cycle characteristics such as dose or duration of use cannot be reliably used to predict recovery in these subjects. Eleven percent of our cohort completed the follow up period with

a subnormal testosterone level, without signs of gonadal recovery. The characteristics of these subjects indicate that repetitive exposure to highly supraphysiological androgen doses may lead to chronic disruption of endogenous testosterone production. We were unable to demonstrate any beneficial effect of medications referred to as PCT, therefore the use of these agents should be discouraged.

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CHAPTER 7

A PROSPECTIVE STUDY ON BLOOD PRESSURE, LIPID METABOLISM, AND ERYTHROCYTOSIS DURING AND AFTER ANDROGEN ABUSE

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ABSTRACT

Background

Androgen abuse, i.e. use of androgens without prescription, is associated with unfavourable changes in blood pressure, lipid metabolism and erythrocytosis. Most of our knowledge is based on retrospective or cross-sectional studies which are sensitive to bias. Therefore we assessed the magnitude of these effects and degree of recovery in a prospective study.

Methods

The cohort included 100 men (≥18 years) who intended to start an androgen cycle on short notice. Clinic visits took place before and at the end of the cycle, as well as 3 months after the end of the cycle, and 1 year after start of the cycle, and included measurement of blood pressure, lipid parameters and haematocrit.

Results

During androgen use, systolic and diastolic blood pressure increased 6.87 (95%CI 4.34-9.40) and 3.17 mmHg (95%CI 1.29-5.04) compared to baseline, respectively. LDL cholesterol and ApoB increased 0.45 mmol/l (0.29 to 0.61) and 18.2 mg/dl (13.5-22.8), respectively, whereas HDL cholesterol, ApoA and Lp(a) decreased with 0.40 mmol/l (-0.45--0.35), 36.6 mg/dl (30.2-42.9) and 37.6% (13.9-61.3), respectively. ANGPTL3 increased with 20.3% (7.38-33.2). Mean haematocrit increased 0.03 l/l (0.02-0.03). Three months after the cycle, and 1 year after start of the cycle, the mentioned parameters returned to baseline levels.

Conclusions

Androgen abuse induces small but clinically relevant adverse changes of blood pressure, lipid metabolism and erythrocytosis. These changes are rapidly reversible after cessation of androgens. Because the study follow-up was limited to one year, the impact of androgen abuse on the incidence of cardiovascular disease remains uncertain.

INTRODUCTION

The abuse of androgens, or anabolic androgenic steroids, is not uncommon among amateur strength athletes. Androgens entail testosterone, synthetic derivatives such as nandrolone and trenbolone, and the nonsteroidal selective androgen receptor modulators (SARMs). The term anabolic androgenic steroids is oxymoronic as there is no genuine distinction between anabolic and androgenic effects. Androgen abuse is defined as the use of androgens without a prescription, usually for the enhancement of muscle mass. The prevalence for men visiting fitness centres to use androgens is estimated at 4-6%.[1,2] Production and trading of androgens without a license is prohibited in most countries. Nevertheless, androgens can be easily acquired illegally through local dealers or the internet. The quality of androgens on the black market is remarkably poor and only about 50% of products contain the androgens declared on the label and many contain undeclared ingredients.[3,4]

The use of androgens is harmful but exact data about negative health effects is lacking. There is evidence showing androgen abuse is associated with premature coronary atherosclerosis.[5,6] The mechanisms through which androgen abuse may lead to cardiovascular morbidity comprise high blood pressure[7,8] and unfavourable changes in lipoprotein metabolism.[9,10] This knowledge, however, is mostly based on low-level evidence, such as case reports and cross-sectional studies of which the majority is retrospective in nature. Small prospective studies investigating the effects of androgen abuse on blood pressure sometimes yield ambiguous data.[11] It is also not certain whether the change in lipid metabolism conveys an elevated risk for cardiovascular disease as studies mostly only assessed HDL and LDL cholesterol and no other parameters of lipid metabolism.

In addition, whether cardiovascular risk factors are influenced by androgen type, cycle dose and duration is not well-known. Small clinical trials show that oral androgens have a worse impact on lipid metabolism than injectables ones,[7] but it is unclear whether this holds true for androgen abuse in general practice where most athletes combine oral with injectable androgens. Furthermore, it is undetermined to what extent effects on blood pressure, lipid metabolism and erythrocytosis are reversible and if recovery depends on androgen cycle characteristics.

We initiated a prospective observational study with a systematic approach, the HAARLEM-study (acronym for Health risks of Anabolic Androgenic steRoid use by maLE aMateur athletes). The goal of the study was to obtain more reliable information about the health effects of androgen abuse. A cohort of 100 men was assembled and health analysis including measurement of blood pressure, lipid parameters and haematocrit was performed during a one year follow-up period: before, during and after a cycle of androgens.

METHODS

A detailed description of the methods of subject recruitment are described in a previous report.[3] In short, the 100 subjects included in the HAARLEM-study were men attending fitness centers of at least 18 years old intending to start a self-initiated androgen cycle on short notice (i.e. within two weeks).

The androgens had to be acquired through their usual illegal channels. Androgens and other medication used by subjects during the study were not prescribed by the investigators. The study was purely observational and subjects used androgens according to their own agenda and did not receive any advice. Subjects were required to not have used androgens for at least three months prior to inclusion. The study took place in the outpatient AAS clinic in Haarlem, the Netherlands, after the study was promoted on national television and in regional newspapers. The study was approved by the local institutional review board (IRB) and subjects provided written informed consent.

Clinic visits

Subjects visited the study clinic four times during a one year study period. A medical investigation was performed at baseline within two weeks before initiation of the androgen cycle (T_0), and comprised measurement of height, weight and body mass index (BMI) and blood pressure (manual, upper right arm, seated, appropriate cuff size, in threefold). Blood analysis was performed and included lipid parameters and haematocrit. Investigations were repeated in the last week of the androgen cycle (T_1), three months after the end of the cycle (T_2), and one year after the start of the cycle (T_3). The results of the study were not disclosed to the subjects before the end of the study.

The androgen cycle performed by subjects during follow-up was recorded in detail, with duration and dosage of each androgen type used, in addition to use of other performance and image-enhancing drugs (PIEDs), recreational soft and hard drugs, self-obtained medication, and post-cycle therapy (PCT). Mean weekly androgen dose during the cycle was based on label information and, because the exact content of androgens used was unknown, all androgen types were considered equivalent.

Blood analysis

Subjects visited the hospital laboratory for collection of blood after an overnight fast. Blood was drawn by venepuncture by a qualified laboratory assistant. Venous blood collected in K_3-ethylenediaminetetraacetic acid (EDTA) tubes was used for analysis of blood cell count using an Abbot CELL-DYN Sapphire Hematology Analyzer (Abbott Diagnostics, Santa Clara, CA, USA). A lithium heparin sample was analysed on the Abbott ARCHITECT system for total, HDL and LDL cholesterol, and triglycerides.

After completion of the HAARLEM study, stored serum samples were analysed for apolipoprotein A (ApoA1), apolipoprotein B (ApoB), lipoprotein (a) (Lp(a)), angiopoietin-like 3 (ANGPTL3) and proprotein convertase subtilisin/kexin type 9 (PCSK9). Serum ApoA1, ApoB, and Lp(a) were measured using commercially available assays (DiaSys Diagnostic Systems, Holzheim, Germany) on a Vitalab Selectra E analyzer (Vital Scientific, Dieren, the Netherlands). Serum ANGPTL3 and PCSK9 concentrations were determined using a human ANGPTL3 DuoSet ELISA (R&D Systems, Minneapolis, MI). These analyses were performed on samples of 86 subjects from clinic visit T_0, T_1 and T_2. The 14 subjects left out of this analysis had missed clinic visit T_2 (2), were using androgens at that time (7), or stored serum samples were not available (5).

Statistical analysis

Simple descriptive statistics were used to display quantitative data. As measurements were clustered within patients, linear and logistic mixed models were used for continuous and dichotomous variables, respectively, to calculate mean differences, 95%-confidence intervals (95%CI), odds ratios (OR) and P values. This analysis takes missing data into account. Outcomes of laboratory parameters were displayed as a mean with 95%CI and as the number of subjects that had a result outside the reference range.

Subgroup analysis was carried out to compare subjects with and without a history of androgen abuse. Multivariable regression analysis was performed with mixed models and assessed the confounding role of BMI, recent history of androgen abuse, androgen cycle dose and duration, number of different androgens used, use of oral androgens in the week leading up to T_1, as well as the use of PCT or other substances or medication in the period prior to the clinic visit, i.e. amphetamines, cocaine, selective estrogenic receptor modulators (SERMs) and aromatase inhibitors. To correct for multiple testing, a Bonferroni adjustment to P values for significance was employed. Stata software (for Windows, version 15, StataCorp, 2017) was used for analysis.

RESULTS

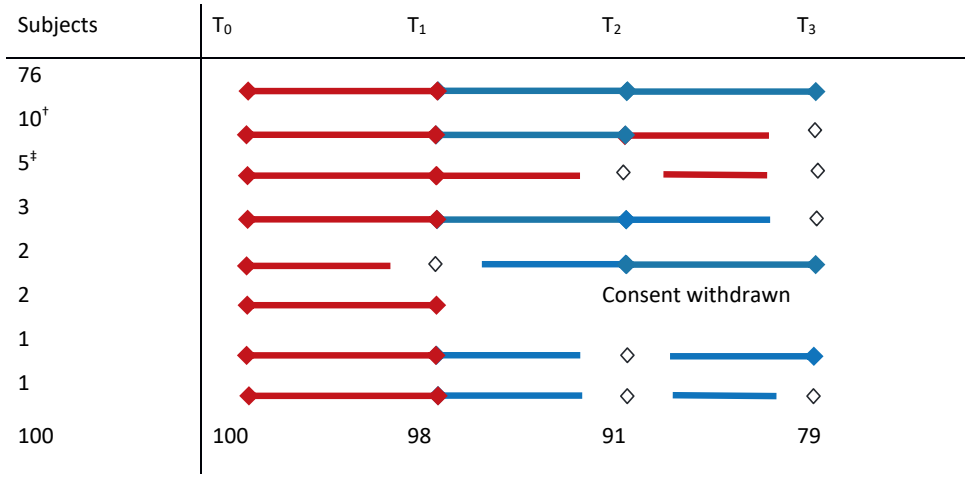
Follow-up of the 100 men included in the HAARLEM study took place between October 2015 and May 2018. The median age at inclusion was 31 years (range 19-67). Seventy-nine subjects had a history of androgen abuse but 20 of them had not used androgens in the year prior to inclusion. During follow-up subjects performed an androgen cycle with a median duration of 13 weeks (range 2-52) and 4 types of androgens (range 1-11). The mean dose of androgens, based on label information, was 898 mg per week (range 250-3382 mg/week). Twenty-nine subjects used oral androgens during clinic visit T_1. Eighty subjects carried out PCT. Demographic data and further details of androgen cycles and performance and image-enhancing drugs (PIEDs) used during follow-up period are described elsewhere.[3]

The course of follow-up with respect to androgen abuse and clinic visits of the 100 subjects is shown in *Table 1*. Two subjects withdrew consent after T_1, of whom one due to emigration, and therefore no data were available from these subjects on T_2 and T_3. Fifteen subjects used androgens continuously or started a second cycle of androgens during the follow-up of the study. In these subjects, T_2 and/or T_3 measurements were not useful for analysis of recovery. Another nine clinic visits were missed (twice T_1, thrice T_2, four times T_3) due to obligations for work or personal circumstances. One subject visited the clinic at T_2 but did not go to the laboratory for blood analysis.

The median total follow-up period (i.e., from clinic visit T_0 to T_3) was 11.9 months (range 8.0-17.0 months). T_1 took place in the last week of the androgen cycle after a median of 3.5 months after inclusion. Including the 15 subjects that continued or restarted androgen abuse after the first cycle, the 100 subjects used androgens for 593 months (49%) during 1201 months of follow-up. T_2 and T_3

took place after a median of 3.2 and 9.4 months after T_1, respectively, and this reflected recovery after the cycle. Details of blood analysis from the different clinic visit are displayed in *Table 2*. Box and whisker plots of systolic and diastolic blood pressure as well as haematocrit and platelet count are shown in *Figure 1A-D*.

Table 1. Overview of follow-up period of the 100 subjects included in the HAARLEM study. Red lines correspond to subjects using androgens, whereas blue lines correspond to subjects not using androgens. Coloured diamonds are performed clinic visits, whereas white diamonds are missed clinic visits or clinic visits without useful data due to androgen abuse not according to study protocol. *Subjects who started a new cycle after T_2. **Subjects who did not discontinue androgens after T_1.



Baseline values

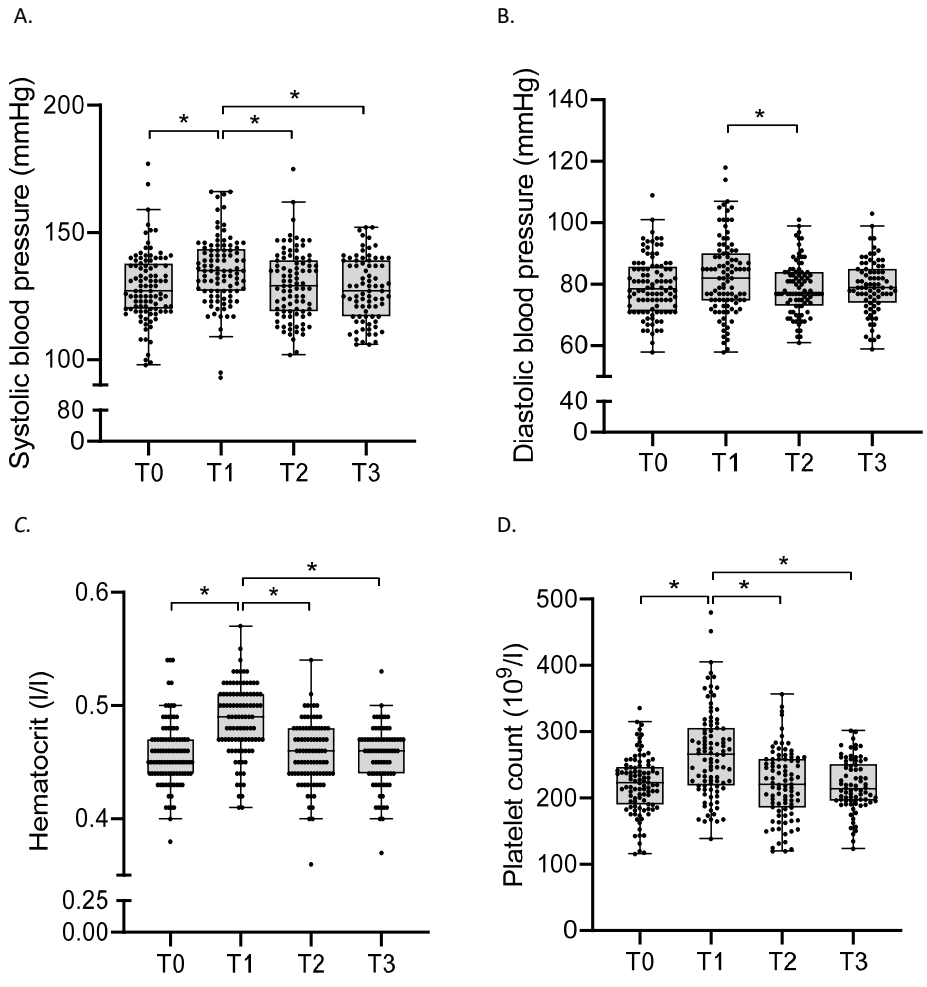
Mean systolic and diastolic blood pressure were 129 (SD±14) and 79 (SD±9) mmHg at baseline (T_0; see *Figure 1A* and *1B*), respectively. Sixteen and 13 subjects had a systolic and diastolic blood pressure above 140 or 90 mmHg, respectively. There was no statistically significant difference between subjects with or without a history of androgen abuse with respect to these data. BMI was positively correlated with systolic (β=0.95, 95%CI 0.41 to 1.48, P<.01) and diastolic blood pressure (β=1.03, 95%CI 0.48 to 1.58, P<.01).

Subjects with a history of androgen abuse had a higher total and LDL cholesterol by 0.54 mmol/l (95%CI 0.03 to 1.05, P=.04) and 0.67 mmol/l (95%CI 0.15 to 1.19, P=.01), respectively, and a lower HDL cholesterol by 0.15 mmol/l (95%CI -0.28 to -0.02, P=.03), at baseline. There was a positive association between BMI and total (β=0.08, 95%CI 0.03 to 0.13, P<.01) and LDL cholesterol (β=0.09, 95%CI 0.04 to 0.15, P<.01), and ApoB (β=3.22, 95%CI 1.57 to 4.89, P<.01). The numbers of subjects outside the reference range for these parameters were not significantly different between subjects with and without previous androgen abuse. All five subjects with a haematocrit above 0.50 had a history of androgen abuse but mean haematocrit was not significantly different (0.01, 95%CI -0.01 to 0.02, P=.46) between previous users and new users.

Changes during androgen abuse

At the end of the cycle (T_1), mean systolic and diastolic blood pressure were 6.87 (95%CI 4.34 to 9.40, P<.01) and 3.17 mmHg (95%CI 1.29 to 5.04, P<.01) higher, respectively, compared to baseline (T_0; see *Figure 1A* and *1B*). The number of subjects with a systolic and diastolic blood pressure above 140 or 90 mmHg, respectively, were 35 (OR 5.65, 95%CI 2.28 to 14.0, P<.01) and 23 (OR 3.42, 95%CI 1.24 to 9.44, P=.02).

Figure 1. Box and whisker plots of systolic (A) and diastolic (B) blood pressure, (C) haematocrit and (D) platelet count. T_0 = before the start of the cycle, T_1 = in the last week of the cycle, T_2 = 3 months after the cycle, T_3 = 1 year after the start of the cycle. *P<.01.



Between baseline (T_0) and the end of the cycle (T_1), mean LDL cholesterol and ApoB increased with 0.45 mmol/l (95%CI 0.29 to 0.61, P<.01) and 18.2 mg/dl (95%CI 13.5 to 22.8, P<.01), respectively, whereas mean HDL cholesterol and ApoA1 decreased with 0.40 mmol/l (95%CI -0.45 to -0.35, P<.01) and 36.6 mg/dl (95%CI 30.2 to 42.9, P<.01). Waterfall plots of relative change of HDL cholesterol and ApoA1 as well as LDL cholesterol and ApoB are shown in *Figure 2A* and *2B*, respectively. Mean total cholesterol did not change significantly in this period. The use of oral androgens during clinic visit T_1 was associated with a greater increase of LDL cholesterol (β=0.53, 95%CI 0.15 to 0.91, P<.01) and ApoB (β=19.9, 95%CI 9.65 to 30.2, P<.01), and a greater decrease of ApoA1 (β=-19.8, 95%CI -33.9 to -5.66, P<.01), compared to subjects not using oral androgens during T_1. A small but significant increase of mean triglycerides with 0.18 mmol/l (95%CI 0.06 to 0.31, P<.01) was observed at the end of the cycle (T_1) compared to baseline (T_0).

***Table 2*.** Results of blood analysis comprising blood cell count and lipid parameters during clinic visits. Of every parameter the mean (x̄) parameter with 95%-confidence interval (95%CI) is shown as well as the number of times (n) a result was outside the reference range (RR). T_0 = before the start of the cycle, T_1 = in the last week of the cycle, T_2 = three months after the cycle, T_3 = one year after the start of the cycle. 95%CI, odds ratios and P values were calculated with mixed models and compare visit T_1, T_2 and T_3 with T_0. For the number of times subjects deviated from the RR only statistical significance is indicated without odds ratios or P values. PCSK9 = proprotein convertase subtilisin/kexin type 9. †P=.01-.05, ‡P<.01.

	T0 (n = 100)		**T1 (n=98)**		**T2 (n=90)**		**T3 (n=79)**	
Blood analysis (unit, RR)	**x̄**	**[95%CI]**	**x̄**	**[95%CI]**	**x̄**	**[95%CI]**	**x̄**	**[95%CI]**
	n	**(%)**	**n**	**(%)**	**n**	**(%)**	**n**	**(%)**
Haemoglobin (mmol/l, 8.5-11.0)	9.8	[9.7-10.0]	10.2‡	[10.0-10.3]	9.8	[9.7-9.9]	9.7	[9.7-9.9]
	3	(3%)	11‡	(11%)	2	(2%)	3	(4%)
Haematocrit (l/l, 0.40-0.50)	0.46	[0.45-0.46]	0.49‡	[0.48-0.49]	0.46	[0.45-0.47]	0.45	[0.45-0.46]
	5	(5%)	32‡	(33%)	2	(2%)	2	(3%)
Platelet count (x 10^9/l, 150-400)	223	[212-234]	270‡	[259-280]	221	[210-232]	219	[213-236]
	0	(0%)	5	(5%)	7	(8%)	3	(4%)
White blood cells (x 10^9/l, 4.0-10.0)	6.0	[5.6-6.4]	7.8	[7.4-8.2]	6.0	[5.7-6.5]	5.8	[5.5-6.3]
	2	(2%)	16	(16%)	5	(6%)	1	(1%)
Cholesterol. total (mmol/l, 1.5-6.5)	4.4	[4.2-4.6]	4.3	[4.1-4.5]	4.2	[4.0-4.4]	4.4	[4.1-4.6]
	2	(2%)	5	(5%)	0	(0%)	1	(1%)
LDL-cholesterol (mmol/l, 1.5-4.5)	2.9	[2.7-3.1]	3.3‡	[3.1-3.6]	2.8	[2.5-3.0]	2.8	[2.6-3.1]
	12	(12%)	23†	(23%)	10	(11%)	9	(11%)
HDL-cholesterol (mmol/l, 0.9-1.7)	1.2	[1.1-1.2]	0.8‡	[0.7-0.8]	1.1	[1.1-1.2]	1.2	[1.1-1.3]
	14	(14%)	58‡	(59%)	10	(11%)	8	(10%)
Triglycerides (mmol/l, 0.6-2.2)	1.0	[0.8-1.1]	1.2‡	[1.0-1.3]	1.1	[0.9-1.2]	1.1	[1.0-1.3]
	22	(22%)	21	(22%)	18	(20%)	19	(24%)

	T0 (n = 84)		**T1 (n=82)**		**T2 (n=83)**	
Lipoprotein (a) (mg/dl, <30)	19.7	[15.0-24.4]	10.1‡	[5.40-14.8]	19.3	[14.6-24.0]
	19	(23%)	8†	(10%)	21	(25%)
Apolipoprotein A1 (mg/dl, 110-180)	140	[135-146]	104‡	[98.2-109]	136	[130-141]
	8	(10%)	47‡	(57%)	9	(11%)
Apolipoprotein B (mg/dl, 40-125)	72.1	[66.1-78.1]	90.3‡	[84.3-96.3]	72.5	[66.6-78.5]
	8	(10%)	17†	(21%)	8	(10%)
Angiopoietin-like 3 (ng/ml)	903	[795-1011]	967	[858-1075]	906	[799-1014]
	n/a	n/a	n/a	n/a	n/a	n/a
PCSK9 (ng/ml)	316	[285-347]	280†	[249-311]	332	[301-362]
	n/a	n/a	n/a	n/a	n/a	n/a

Mean Lp(a) decreased 37.6% (95%CI 13.9 to 61.3, P<.01) between clinic visit T_0 and T_1 (see *Figure 2C*). Lp(a) was negatively associated with cycle duration (β=-0.51, 95%CI -0.89 to -0.14 P<.01) and weekly cycle dose (β=-9.4e^{-3}, 95%CI -1.6e^{-2} to 3.1e^{-3}, P<.01) during the clinic visit at the end of the cycle (T_1). ANGPTL3 increased 20.3% (95%CI 7.38 to 33.2, P<.01) between baseline (T_0) and the end of the cycle (T_1; see *Figure 2D*). There was a positive association between ANGPTL3 and total (R^2=108.0, P<.01) and LDL cholesterol (R^2=108.7, P<.01). Mean PCSK9 decreased 35.6 ng/ml (95%CI 5.27 to 66.0, P=.02) in this time period.

Mean haemoglobin and haematocrit increased with 0.33 mmol/l (or 0.53 g/l; 95%CI 0.22 to 0.44 mmol/l, or 0.35 to 0.71 g/l, P<.01) and 0.03 l/l (95%CI 0.02 to 0.03, P<.01; see *Figure 1C*), respectively, between baseline (T_0) and the end of the cycle (T_1). The number of subjects with a haematocrit above 0.50 l/l increased from 5 to 32 (OR 27.3, 95%CI 5.53 to 134, P<.01). The increase of haematocrit was not associated with longer cycles or higher androgen dose. The mean platelet count increased by 46.3 x10^9 (95%CI 37.5 to 55.0, P<.01; see *Figure 1D*) and was positively correlated with the use of oral androgens (β=50.7, 95%CI 30.5 to 70.8, P<.01).

Recovery after androgen abuse

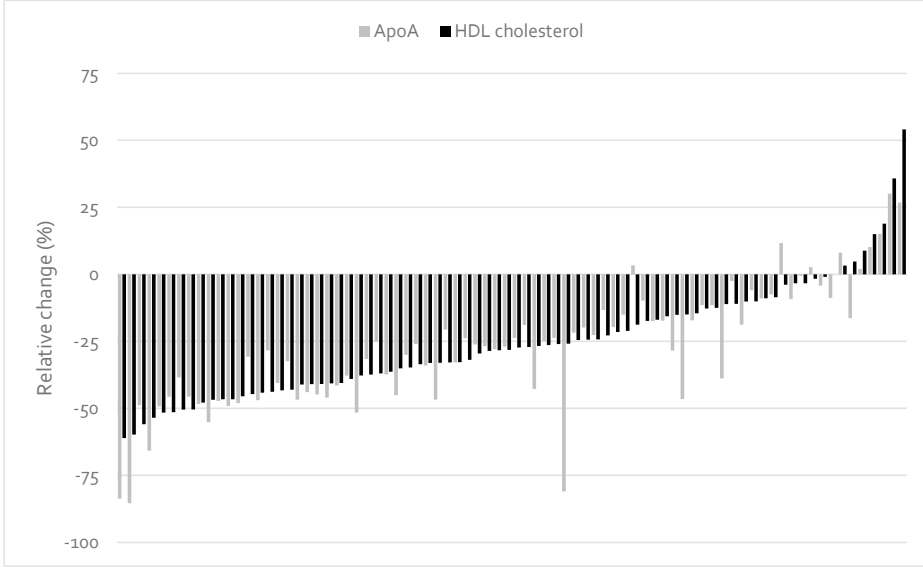
Approximately three months after the cycle (T_2), mean systolic and diastolic blood pressure were not significantly different from baseline (T_0). Likewise, lipid parameters, haematocrit and platelet count showed complete recovery. History of androgen abuse, cycle duration, weekly cycle dose, number of androgen types and the use of oral androgens did not have a significant association with the recovery of any of the measured parameters, nor did the use of PCT or other illegal substances. The only exception was the use of aromatase inhibitors, which was positively associated with PCSK9 at the clinic visit three months after the cycle (T_2).

DISCUSSION

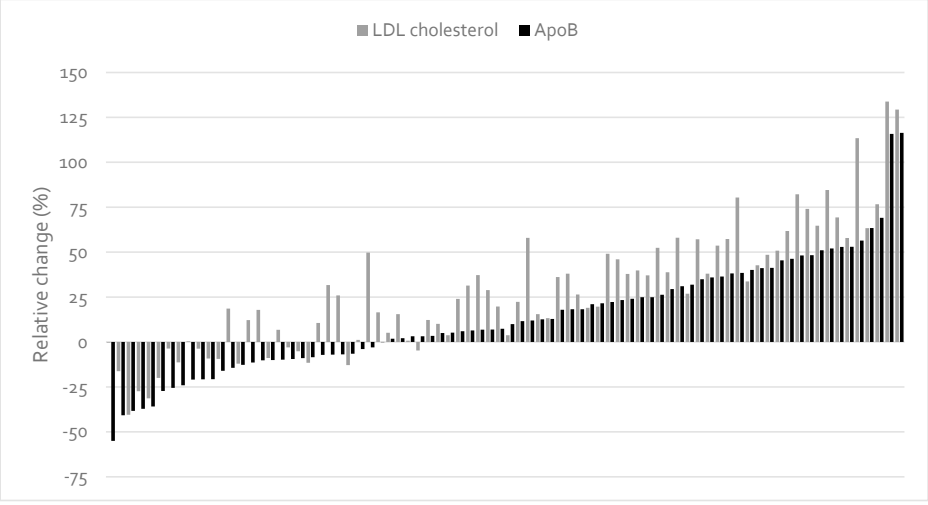
The HAARLEM study is a unique trial that prospectively analysed the short to medium-term cardiovascular effects of androgen abuse. Blood pressure, lipid parameters and haematocrit were measured before, during and twice after an androgen cycle in a cohort of 100 male amateur athletes. Because the study was promoted through (social) media and websites on a nationwide level, the cohort is believed to be representative of the general population of amateur athletes abusing androgens in the Netherlands. The cycles performed contained very high supraphysiological doses of androgens. Adherence of subjects to follow-up was nearly 100%. It is not possible to exclude that subjects surreptitiously used androgens after their initial cycle, besides the 15 subjects that reported so beforehand and whose data were excluded for analysis, and this is an important limitation. However, it is likely that undisclosed androgen use would have been detected with blood analysis because the use of exogenous testosterone – which is almost always included in an androgen cycle – would cause suppressed gonadotrophin and high or high-normal testosterone levels and this has not been observed.

***Figure 2*.** Waterfall plots of (A) HDL cholesterol and ApoA, (B) LDL cholesterol and ApoB, and (C) Lp(a) and (D) ANGPTL3, showing the relative change of these parameters during androgen abuse (T_1) in comparison to baseline (T_0) values. The bars display paired data of subjects from whom data were available from both clinic visits.

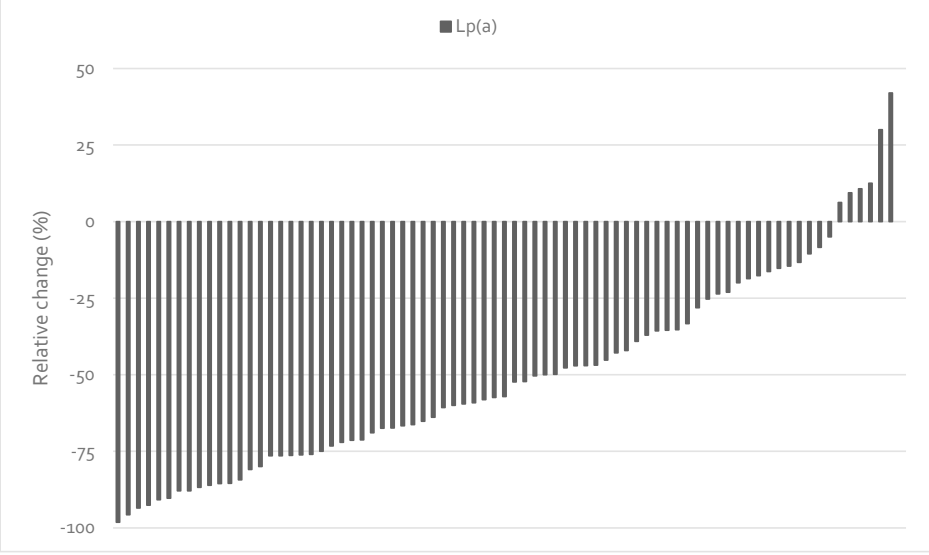
A.



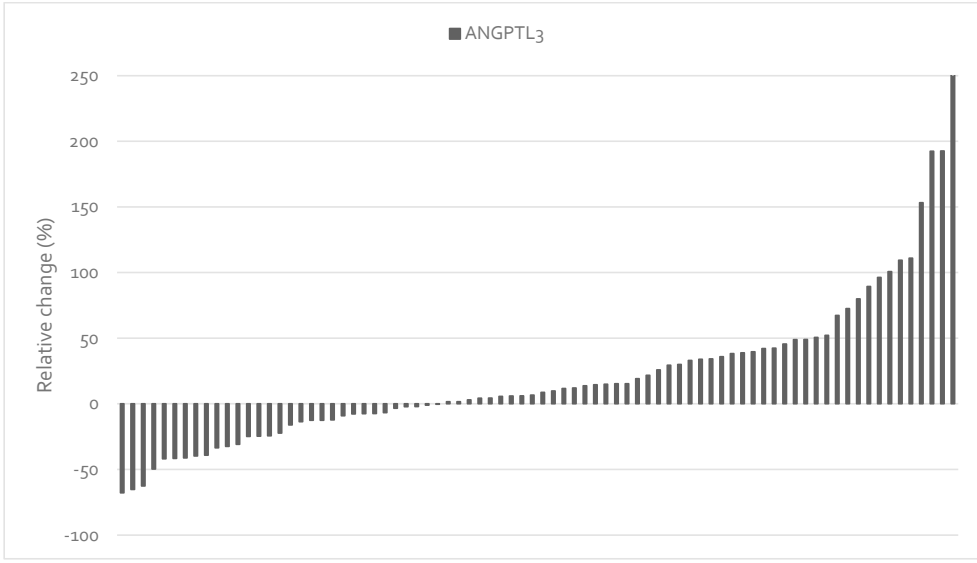
B.


LDL cholesterol
ApoB
Relative change (%)
150
125
100
75
50
25
0
-25
-50
-75

C.


Lp(a)
Relative change (%)
50
25
0
-25
-50
-75
-100

D.



The study was promoted through (social) media and websites on a nationwide level in order to recruit a representative group of amateur athletes abusing androgens in the Netherlands. Our cohort indeed much resembles the group of patients seen in the AAS clinic (12) and participants of earlier survey studies (13,14) in terms of age of first androgen abuse, sport, educational level, cycle characteristics and hard drug use. Nonetheless, the study provided the opportunity to monitor health – for free – and may have led to selection of more wary and careful strength athletes. The design of the study possibly led to similar type of selection as a subgroup of users (~5%) uses androgens more or less continuously (12) and they were not included because health measurements after a cycle were supposed to assess recovery. These strength athletes may abuse androgens more hazardously, and the cumulative dose is usually larger.

In our study, the use of androgens had several adverse cardiovascular effects: a relatively small but significant increase in systolic and diastolic blood pressure, a rise in haematocrit and platelet count, an increase in LDL cholesterol, ApoB and ANGPTL3, and a decrease in HDL cholesterol and ApoA1. All effects were reversible within three months after stopping androgens. The use of oral androgens was associated with higher platelet counts and a more adverse lipid profile. Weekly dose of androgens, although highly variable between study subjects, was not associated with any of the variables.

A recently published cross-sectional study by Shankara-Narayana et al. compared 41 current and 31 past androgen abusers with 21 healthy non-users.[10] They reported similar findings with respect to blood pressure, lipid metabolism and erythrocytosis. In their analysis, however, the higher platelet count and LDL cholesterol in current users compared to past users and non-users did not reach statistical significance. The relatively large size of our cohort, the prospective nature of our study and

the meticulous follow-up over a period of one year make our findings very robust. In addition, we are the first to document changes of ANGPTL3 and PCSK9 during an androgen cycle, thereby expanding current knowledge of the effect of androgen abuse on lipid metabolism. Also, reversibility of the cardiovascular effects and the (absent) association with cycle characteristics has not been described in such detail before.

Effects of androgen abuse on blood pressure

The observed effects of androgen abuse on blood pressure are in accordance with the results from previous studies.[7,8,10] Forty-one percent of our subjects was hypertensive during the cycle according to current guidelines. Blood pressure was measured with an appropriate cuff size, as a relatively small cuff on muscular arms may overestimate blood pressure.[12] One of the mechanism by which androgen abuse may lead to elevated blood pressure is water and sodium retention,[13] which is often reported by androgen users.[14,15]

Effects of androgen abuse on lipid metabolism

Unfavourable changes in serum LDL and HDL cholesterol due to androgen abuse have been well documented before.[8,10,16,17] It is not entirely certain whether these changes convey cardiovascular disease risk, but this becomes more likely considering an equal rise in ApoB and decline in ApoA1. These parameters correlate more strongly with cardiovascular disease than do LDL and HDL cholesterol alone.[18] However, the rise in LDL and ApoB as well as the reduction in HDL and ApoA1 disappear shortly after cessation of androgen use, as is the case with all androgen-induced changes in lipid parameters. Consequently, any adverse cardiovascular effect of androgen abuse will not only be determined by the magnitude of the effect on lipid profile, but also, and perhaps predominantly, by the duration of androgen abuse.

Contrarily, the effect of androgen abuse on Lp(a) appeared to be beneficial with a ~50% reduction at the end of the cycle. The reduction was greater in cycles with a higher weekly dose or longer duration. A similar effect on Lp(a) has been observed in healthy men and weight lifters, and is primarily an androgenic, not estrogenic, effect.[19,20,21] Why serum Lp(a) concentrations are reduced by androgens is currently not known. It has been suggested that effects of androgens on Lp(a) are mainly due to an effect on the liver, which produces and secretes apolipoprotein(a).[22] It remains to be elucidated whether the reduction of Lp(a) could counterbalance the change of parameters which promote the development of atherosclerosis and cardiovascular disease.

ANGPTL3 is a member of the angiopoietin-like family and an important regulator of lipid metabolism, among others by inhibiting lipoprotein lipase and endothelial lipase. Its reduced expression has been linked to lower LDL cholesterol and triglyceride levels and a lower risk of atherosclerosis.[23] The increase of ANGPTL3 seen in our subjects could therefore be considered disadvantageous and may, to some extent, explain the increase of LDL cholesterol. Indeed, in our cohort we found a strong correlation between ANGPTL3 and LDL cholesterol. The mechanism by which this occurs is uncertain but may be an enhanced hepatic VLDL synthesis and/or a reduced endothelial lipase-mediated VLDL clearance.[23,24] The effect of androgen abuse on ANGPTL3 may rather be related to elevated oestrogens, and not so

much androgens, but this requires more investigation. There may also be a role for insulin resistance as this has been demonstrated to elevate ANGPTL3.[25] However, in our subjects we observed a small but statically significant decline in fasting glucose, probably due to an increase in exercise activity during the cycle, so this mechanism is not likely.

PCSK9 is a central regulator of cholesterol metabolism. It acts as a chaperone for the internalized LDL receptor and guides it to the lysosome for premature termination. Decreased (circulating) PCSK9 has been strongly associated with a reduced LDL cholesterol and a lower prevalence of atherosclerotic cardiovascular disease.[26] In our subjects, PCSK9 was reduced by ~11% during the cycle which may be brought about by elevated oestradiol concentrations[27] due to aromatization of the administered androgens. Although this change may be regarded as protective, it did not prevent the significant rise in LDL cholesterol.

Effects of androgen abuse on erythrocytosis

Haematocrit increased during androgen abuse in such a way that one third of subjects had a level above 0.50 l/l. Obstructive sleep apnoea may have occurred in some subjects and contributed to this result. The association of an elevated haematocrit with cardiovascular disease risk is well established.[28] We did not observe a dose-dependent effect on erythropoiesis, contrary to physiological testosterone replacement therapy in hypogonadal patients.[29] It is possible this effect is curvilinear and plateaus above a supraphysiological dose of androgens. Of note, unreliability of black market androgens may have clouded the estimation of weekly cycle dose and therefore hindered our analysis.

The demonstrated ~20% rise in platelet count has been reported in androgen users before.[30] In concert with a presumed enhanced platelet aggregability,[31] higher haematocrit and elevated platelet count may confer a prothrombotic risk to users of androgens. Little evidence exists, however, about the humoral coagulation cascade in androgen users but data point towards procoagulant and fibrinolytic pathways of activation.[32] An adjoining coagulation study will provide data into these mechanisms in order to create a better understanding of thrombosis risk in androgens users.

Recovery after androgen abuse

As soon as androgens were discontinued, blood pressure, lipid parameters and haematocrit fully recovered within 3 months. Our data were not able to demonstrate androgen cycle characteristics such as dose, duration and the use of oral androgens, nor PCT, to have a relevant impact on degree of recovery. It is imaginable that these factors play a role but were not detected because the exact type and dose of androgens used by our subjects was not known. We assume that, at least for non-endocrine biochemical markers, normalization commences rapidly as soon as androgen concentration drop to a near-physiological level, which primarily depends upon the dose and half-life of the androgen esters last used in the cycle. The rate of recovery then depends on factors such as intermediate enzymes, hormones and cells, e.g. erythropoietin and red blood cells for haematocrit.[33]

Androgen abuse as a cardiovascular risk factor

The demonstrated elevated blood pressure, unfavourable change of lipid metabolism, and rise in haematocrit, position androgen abuse as a cardiovascular risk factor. This is aside from other substances used by many subjects that may also predispose to cardiovascular morbidity, such as growth hormone (21%), amphetamines (46%) and cocaine (32%). We performed only one clinic visit during the cycle, but results were probably representative for the entire cycle as most outcomes were not associated with weekly cycle dose and duration. The absence of this association also makes it unreasonable to believe that there would be a 'safe' or 'responsible' way of performing androgen cycles. The cardiovascular risk of androgen abuse is proportionate to the duration of androgen abuse as recovery of parameters takes place within several weeks after the end of the cycle. In other words, the longer an athlete abuses androgens in his sporting career, the higher the risk of cardiovascular disease becomes.

Our current cohort used androgens for 49% of the follow-up time of 1 year. The 79 subjects with a history of androgen abuse had spent an average of 17 months using androgens before inclusion.[3] This puts the average cumulative exposure to androgens for the entire cohort around 20 months, albeit with a range extending from 1 month to 30 years. Since subjects were on average 32 years old at the end of follow-up and 77% planned to start a new cycle afterwards, of whom half within three months, this exposure time is likely to increase appreciably. It is possible that cardiovascular disease eventually will be more prevalent in this group compared to non-users. To establish the true contribution of androgen abuse to cardiovascular disease, however, larger and long-term prospective studies or well-designed large case-control studies are needed.

PERSPECTIVE

The HAARLEM study is the largest systematic and prospective study investigating blood pressure, lipid parameters and haematocrit associated with androgen abuse to date and provides reliable one year follow-up data during and after an androgen cycle. During androgen abuse blood pressure increased together with haematocrit and platelet count, while HDL and LDL cholesterol, ApoA1 and ApoB, and ANGPTL3 changed adversely. Androgen cycle dose did not play an important role but the use of oral androgens had a worse impact on lipid parameters and platelet count than the use of only injectable androgens. The derangements reversed almost completely within three months after the end of the cycle. Our results thus indicate that androgens have reversible atherogenic effects and convey cardiovascular risk for as long as they are used. It is not uncommon for amateur athletes to be exposed to androgens many years in their sporting career, although with tremendous variation. The cumulative duration of androgen abuse should therefore be considered a risk factor for cardiovascular disease.

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CHAPTER 8

COAGULATION FACTOR LEVELS DURING AND AFTER ANABOLIC ANDROGENIC STEROID USE: DATA FROM THE HAARLEM STUDY

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ABSTRACT

Background

Anabolic androgenic steroids (AAS) are thought to increase venous thromboembolism (VTE) risk through an unknown mechanism. We investigated whether AAS use influences coagulation parameters associated with VTE by assessing their changes during and after AAS use.

Methods

The HAARLEM study enrolled 100 men intending to start an AAS cycle between 2015-2018. Coagulation parameters (factor[F]II, FVIII, FIX, von Willebrand factor [vWF], protein S [PS], D-Dimer [DD]), and endogenous thrombin potential (ETP) were measured at baseline and during two years follow-up. Changes in coagulation parameters during AAS exposure (T_1) and 3 months after discontinuation (T_2) compared with before (T_0) were estimated using linear mixed models. Furthermore, we studied the associations between AAS dose and cycle duration with these outcomes by means of multivariable linear regression, adjusted for potential confounders.

Results

Participants performed an AAS cycle with a median duration of 13 weeks (interquartile range [IQR] 10-23) and a median weekly dose of 901 mg (IQR 634–1345). Mean levels of procoagulant markers increased during use (T_1-T_0) for factors FII, FIX and DD, whereas FVIII remained unchanged and vWF decreased. The anticoagulant PS showed the biggest increase (22%, 95%CI 15-29). ETP was -165nm*min (95%CI -205 to -124) lower during AAS exposure. A high weekly AAS dose and short cycle duration were associated with increased PS during use. Coagulation parameters returned to baseline at T_2 and did not change further; neither weekly dose or cycle length were associated with their recovery.

Conclusions

AAS use was associated with a reversible increase of pro- and anticoagulant parameters. The overall balance, as suggested by the lower ETP, did not shift towards an apparent procoagulant change.

INTRODUCTION

Anabolic androgenic steroids (AAS) are synthetic derivates of testosterone, the male sex hormone. These substances belong to the class of performance and image-enhancing drugs (PIEDs), commonly used with the aim of gaining muscle mass, strength and increasing the oxygen carrying capacity of blood.[1,2] Although the word "doping" is traditionally associated with professional athletes, the majority of AAS users are amateur bodybuilders or frequenters of gyms. It is estimated that approximately 1-6% of regular visitors of gyms use AAS.[3,4] However, in 2009, the Netherlands organisation for applied scientific research (TNO) found a prevalence of over 8% among visitors to fitness centres aged 15 and older,[5] indicating that AAS use may even be higher. As in most countries, the production and trading of AAS without a licence is illegal in the Netherlands, however, AAS can be easily acquired through local dealers or the internet.[6]

AAS use is generally regarded as harmful. There is an important association with a higher risk of cardiovascular events.[7,8] Venous thrombosis is also linked to AAS use.[9,10] However, the level of evidence is low, primary based on case reports or few small epidemiological studies.[11] Data are lacking on the mechanism behind a possible association between AAS use and thromboembolic disease. AAS are thought to be able to potentiate both activation and regulation of coagulation during use, which may translate into an increased risk of venous thrombosis when using a longer AAS cycle. The effect of AAS on coagulation proteins has been studied by employing a single type of androgen, in intervention studies as therapy for coagulation factor deficiencies, or in small cross-sectional studies of AAS abusers.[11] Several studies showed that AAS use is associated with increased levels of factor (F)II, FIX, FVIII,[12,13] yet not all studies confirmed these findings.[12,14,15] Levels of coagulation inhibitors, such as free protein S (PS), were also found to be increased.[12,14] Limited data are available on thrombin generation parameters, as only one study showed an augmented coagulation potential in current and former AAS users.[12] Nevertheless, some data are case reports and most studies lack power, due to a modest number of subjects included (ranging from 10 to 40). Moreover, only short term intervention studies have addressed the effect of AAS on haemostasis, while long term effects or the extent of recovery after AAS withdrawal have not been studied prospectively.

Our aim was to investigate the effect of an AAS cycle on the coagulation system in a population of amateur strength athletes. Moreover, we aimed to assess whether these changes are influenced by androgen dose and route of administration, and to investigate the extent of eventual recovery after AAS withdrawal.

METHODS

Study design and subjects

This study used data from the HAARLEM study (Health risks of Anabolic Androgenic steRoid use by maLE aMateur athletes), which study design details have been described previously.[16] Briefly, the HAARLEM study is a purely observational study that enrolled 100 men of 18 years or older intending to start a self-initiated cycle of AAS on short notice between October 2015 and May 2018. The study was launched through the media on a national level and promoted on several popular websites where many AAS users are known to gather. Subjects were included if they intended to start a self-initiated cycle of AAS within two weeks after signing informed consent. The scheduled AAS cycles were to be of at least 6 weeks duration, comprise at least two different types of AAS (or one type of AAS that contained different AAS esters) and with a weekly dose of at least 200 mg. This is the lower-bound supraphysiologic dose which is customary for AAS users. The AAS had to be acquired through participants' usual channels and were not provided by the investigators. The subjects were enrolled within 14 days before the beginning of a new cycle (T_0) and then followed-up at fixed time points. The study design required the subjects to completely discontinue AAS after the cycle within the first 12 months of follow-up, in order to assess adverse events after androgen exposure and potential recovery. Between 12 and 24 months of follow-up, subjects were allowed to use AAS in the way they chose. In the end, 35% of subjects did not use AAS again, whereas 48% performed one or multiple new cycles, and 16% used AAS throughout the second year. Exclusion criteria for the HAARLEM study were the use of AAS within 3 months prior to inclusion and the diagnosis of a new chronic somatic or psychiatric illness within 6 months prior to inclusion. Men were also refused to participate if, within 6 months prior inclusion, treatment for a chronic illness had been started or altered significantly, or a somatic illness (other than trauma) or a psychiatric illness had led to admission to a general hospital or a psychiatric institution, respectively. For this substudy, we excluded one subject who was treated with acenocoumarol during T_1 because of arrythmia.

The AAS cycle performed by the participant during follow-up was recorded meticulously, including the duration and dosage of each type of AAS. In addition, the use of drugs of abuse, other PIEDs (e.g. growth hormone, thryroid hormone, clenbuterol), medications (selective estrogenic receptor modulators, aromatase inhibitors), and post-cycle therapy (PCT), commonly used to prevent testosterone withdrawal symptoms after the cycle, was documented. As the use of other PIEDs,[17] PCT and other recreational drugs, such as amphetamines[18] and cocaine,[19] during and after AAS cycle may influence coagulation, their use was accounted for in the statistical analysis.

Outcome: measures of coagulation

After enrollment, subjects visited the laboratory for baseline blood analysis (T_0). Clinic visits with repeat blood analysis followed at fixed time points: in the last week of the AAS cycle (T_1), 3 months after the end of the cycle (T_2), 12 months after inclusion (T_3) and 24 months after inclusion (T_4). Clotting factors (FII, FVIII, FIX, FXI, von Willebrand factor antigen (vWF), D-Dimer (DD), free PS) were measured at each time point. All coagulation-related laboratory measurements were analyzed on the ACL-Top 700 analyzer (Instrumentation Laboratory). FVIII, FIX and FXI levels were measured using modified activated partial thromboplastins time assays and FII using modified prothrombin time assay, using

immunodepleted plasmas. VWF, DD, free PS levels were measured using an automated latex enhanced immunoassay using the HemosIL vWF:Ag, HemosIL D-dimer HS 500 and HemosIL Free Protein S reagent kit, respectively. To assess fibrinolysis, Clot Lysis Time (CLT, min) was measured. Thrombin generation potential was analyzed by means of a thrombomodulin-modified thrombin generation assay (TGA), using the Calibrated Automated Thrombogram (Diagnostica Stago, Asineres, France) according to the manufacturer's specifications. The thrombin generation parameters determined were the lag time of the thrombin formation process (lag time, minutes), the peak thrombin concentration (peak, nm), the effective rate of thrombin generation between lag time and time to peak (velocity index, nm/min) and the endogenous thrombin potential (ETP, nmol*min) recording the total amount of thrombin formed. CLT and thrombin generation parameters were measured only up until T_3.

Statistical analysis

Mean levels and standard deviations of all coagulation parameters were calculated at baseline (T_0) and at each follow up visit (T_1, T_2, T_3 and T_4). Coagulation parameters were checked for their normal distribution and DD was log-transformed to achieve normal distribution. A linear mixed model was used to assess changes of coagulation measurements during follow-up up until T_3. Estimates and 95% confidence intervals (95%CIs) on the log scale were back-transformed to the original scale.

Between 12 and 24 months of follow-up, patients were allowed to use AAS if they were willing to. Therefore, to assess the measurement at 24 months of follow-up (T_4), we stratified the subjects in three groups according to their AAS use between 12 and 24 months of follow-up: subjects who did not perform another AAS cycle (non-users), subjects who performed one or more cycles, but were not taking AAS at the time of sampling (repeat cycle users) and subjects who were using AAS at the time of blood draw (current users). We compared coagulation parameters of non-users and cycle users with their baseline measurement (T_0) to assess the extent of their recovery. Measurements of current users were instead compared to their measurement at the end of AAS cycle (T_1), to assess possible changes due to prolonged use.

We used multivariable linear regression to assess whether a higher weekly cycle dose was associated with more prominent changes in coagulation parameters. In a first crude model, we included as outcome the difference between coagulation measurements at baseline and during the last week of the cycle ($\Delta T_1 = T_1 - T_0$) and as exposure the average weekly cycle dose or the cycle duration. In a second model, we further adjusted for possible confounders (the number of different AAS used, the use of AAS at time of T_1, the use of other PIEDs, recreational drugs use, previous AAS use).

To assess whether a higher weekly cycle dose was associated with incomplete coagulant recovery, we included as outcome variable the difference between the measurements of coagulation at baseline and at three months after the end of the cycle ($\Delta T_2 = T_2 - T_0$) with cycle dose or duration as the exposure. In the fully adjusted model, the use of post-cycle therapy was also included, together with the previously mentioned confounding variables. As a sensitivity analysis, to ensure that recreational drugs did not influence our results, we performed the aforementioned analysis in a subgroup of non-drug users.

To investigate changes by route of AAS administration, we further stratified the analysis between subjects who were using oral AAS at time of blood draw at T_1 and subjects who were using injectable steroids only.

RESULTS

Study population

A total of 100 men were enrolled in the HAARLEM study (Table 1), of whom 99 were eligible for analysis in this substudy. Their median age was 30 years (interquartile range [IQR] 26 to 37). The median androgen dose of the AAS cycle performed during follow up was 901 mg per week (IQR 634 to 1345), with a median of 4 different types of AAS per cycle (IQR 3 to 5). The median cycle duration was 13 weeks (IQR 10 to 23).

Table 1. Characteristics of the HAARLEM population at baseline.

General	
Age, median (IQR)	30 (26 - 37)
Men, (%)	99 (100 %)
Height, cm, mean (SD)	182 (7)
Weight, kg, mean (SD)	89 (13)
BMI, kg/m^2, mean (SD)	26 (3)
Previous AAS use, (%)	79 (79 %)
Current sport	
Fitness/bodybuilding, (%)	92 (93 %)
Competitive bodybuilding, (%)	19 (19 %)
Weight lifting, (%)	3 (3 %)
Strongman athlete, (%)	3 (3 %)
Combat sports (e.g. kickboxing, karate, judo), (%)	44 (44 %)
Characteristics of AAS cycle	
Cycle duration, week, median (IQR)	13 (10 - 23)
Types of AAS used, median (IQR)	4 (3 - 5)
Cumulative cycle dose (mg), median (IQR)	13100 (770 - 22825)
Average weekly dose (mg), median (IQR)	901 (634 – 1345)

Use of other PIEDs during the cycle	
Growth hormone	22 (22 %)
Clenbuterol	18 (18 %)
Thyroid hormone	18 (18 %)
Insulin	6 (6 %)
ECA-stack (ephedrine, caffeine, aspirin)	7 (7 %)
Insulin-like growth factor 1	8 (8 %)
Ephedrine	3 (3 %)
Growth hormone releasing hormone	4 (4 %)
Amphetamine	8 (8 %)
Sibutramine	1 (1 %)
Selective androgen receptor modulator	1 (1 %)
Dinitrophenol (DNP)	1 (1 %)
Medication during the cycle	
Human chorionic gonadotropin	25 (25 %)
Tamoxifen	23 (23 %)
Anastrozole	21 (21 %)
Exemestane	9 (9 %)
Isotretinoin	6 (6 %)
Diuretics	4 (4 %)
Clomiphene citrate	5 (5 %)
Bromocriptine	3 (3 %)
Finasteride	3 (3 %)
Cabergoline	3 (3 %)
Letrozole	2 (2 %)
Placental growth factor peptide	1 (1 %)

Differences in coagulation parameters between baseline and follow-up

Mean levels of procoagulant factors FII, FIX, FXI increased at the end of the cycle (T_1) compared to T_0 (Table 2, Figure 1 and Supplementary figure 1), whereas FVIII levels remained unchanged and vWF levels decreased. Levels of the natural anticoagulant PS increased the most, with a mean difference of 22% (95%CI 15 to 29) between T_1 and T_0. DD levels were 1.3 times higher at the end of the cycle compared to baseline (95%CI 1.2 to 1.5). CLT was increased, whereas all parameters of thrombin generation were decreased, except for the lag time. ETP was -165 nm*min (95%CI -205 to -124) lower at T_1 compared to baseline. Three months after the end of the cycle (T_2), all coagulation parameters

were restored to baseline value, and did not change in the remaining 12 months after enrollment (T_3, Supplementary figure 1). Twenty-four months after enrolment, mean levels of coagulation parameters in non-users and cycle users were similar to their baseline level (Table 3). Subjects using AAS at the end of year 2 of the study (T_4) had coagulation factor levels similar to their measurement at the end of the first cycle.

Differences in administration route

Subjects using oral AAS at the end of their cycle had higher mean levels of FII, FIX, FXI and longer CLT at T_1 (Figure 1) compared with users of injectable AAS. FVIII and vWF mean levels were lower at all time points in users of oral AAS compared with users of parenteral AAS. As for thrombin generation parameters, ETP, peak and velocity index were lower whereas lag time was increased compared with subjects using only injectable AAS.

Association between cycle characteristics and differences in coagulation parameters

In the crude model, higher weekly dose and shorter cycle duration were positively associated with the increase of FII, FIX and PS between T_1 and T_0, whereas in the adjusted model only the increase in PS was associated with a higher dose and shorter duration (Supplementary table 2). Neither weekly dose nor cycle length were associated with the recovery of coagulation parameters, both in the unadjusted and adjusted model (Supplementary table 3).

Sensitivity analysis

In the 37 subjects who reported the use of recreational drugs, coagulation parameters were overall higher compared with subjects not using recreational drugs. The results of the linear mixed model were similar to the main analysis, with similar mean differences. Due to relatively small size of this sample, we did not have enough power to investigate the association between cycle dose and duration and differences in coagulation parameters.

Table 2. Mean levels of coagulation factors at baseline and at each follow-up visit up until T_3

	T_0 n=98	T_1 n=97		T_2 n=91		T_3 n=73	
	Mean (SD)	Mean (SD)	Mean difference vs T_0* (95%CI)	Mean (SD)	Mean difference vs T_0* (95%CI)	Mean (SD)	Mean difference vs T_0* (95%CI)
Coagulation measures							
FII [%]	98 (11)	112 (19)	14 (10 to 18)	97 (12)	-1 (-3 to 1)	99 (12)	1 (-1 to 3)
FVIII [%]	121 (26)	120 (24)	0 (-5 to 4)	123 (28)	3 (-2 to 7)	118 (26)	-3 (-7 to 1)
FIX [%]	108 (20)	128 (27)	20 (15 to 26)	110 (18)	3 (0 to 7)	108 (19)	1 (-2 to 5)
FXI [%]	118 (20)	122 (24)	4 (0 to 8)	113 (18)	-4 (-6 to -2)	113 (20)	-3 (-6 to -1)
vWF [%]	136 (42)	130 (40)	-7 (-14 to 0)	138 (42)	1 (-5 to 6)	133 (37)	-5 (-10 to 0)
PS [%]	114 (31)	135 (36)	22 (15 to 29)	112 (28)	-1 (-6 to 4)	111 (24)	-2 (-6 to 3)
DD [ng/mL]	285 (213)	405 (583)	1.3 (1.2 to 1.5)ǂ	351 (647)	1 (0.9 to 1.2)ǂ	292 (229)	1 (0.9 to 1.2)ǂ
Fibrinolysis							
CLT [min]	71 (14)	79 (13)	8 (5 to 10)	73 (11)	2 (0 to 4)	73 (12)	2 (-1 to 4)
Thrombin generation							
ETP [nm*min]	510 (170)	343 (213)	-165 (-205 to -124)	519 (176)	13 (-19 to 45)	526 (146)	-8 (-36 to 20)
Lag time [min]	2 (0.3)	2 (0.5)	0.2 (0.1 to 0.3)	2 (0.3)	0.03 (-0.02 to 0.1)	2 (0.3)	0.04 (-0.03 to 0.1)
Peak [nm]	143 (50)	89 (58)	-53 (-64 to -42)	142 (50)	0 (-9 to 8)	146 (43)	-5 (-12 to 3)
V.index [nm/min]	73 (29)	43 (31)	-29 (-35 to -24)	71 (28)	-2 (-6 to 3)	74 (27)	-3 (-8 to 2)

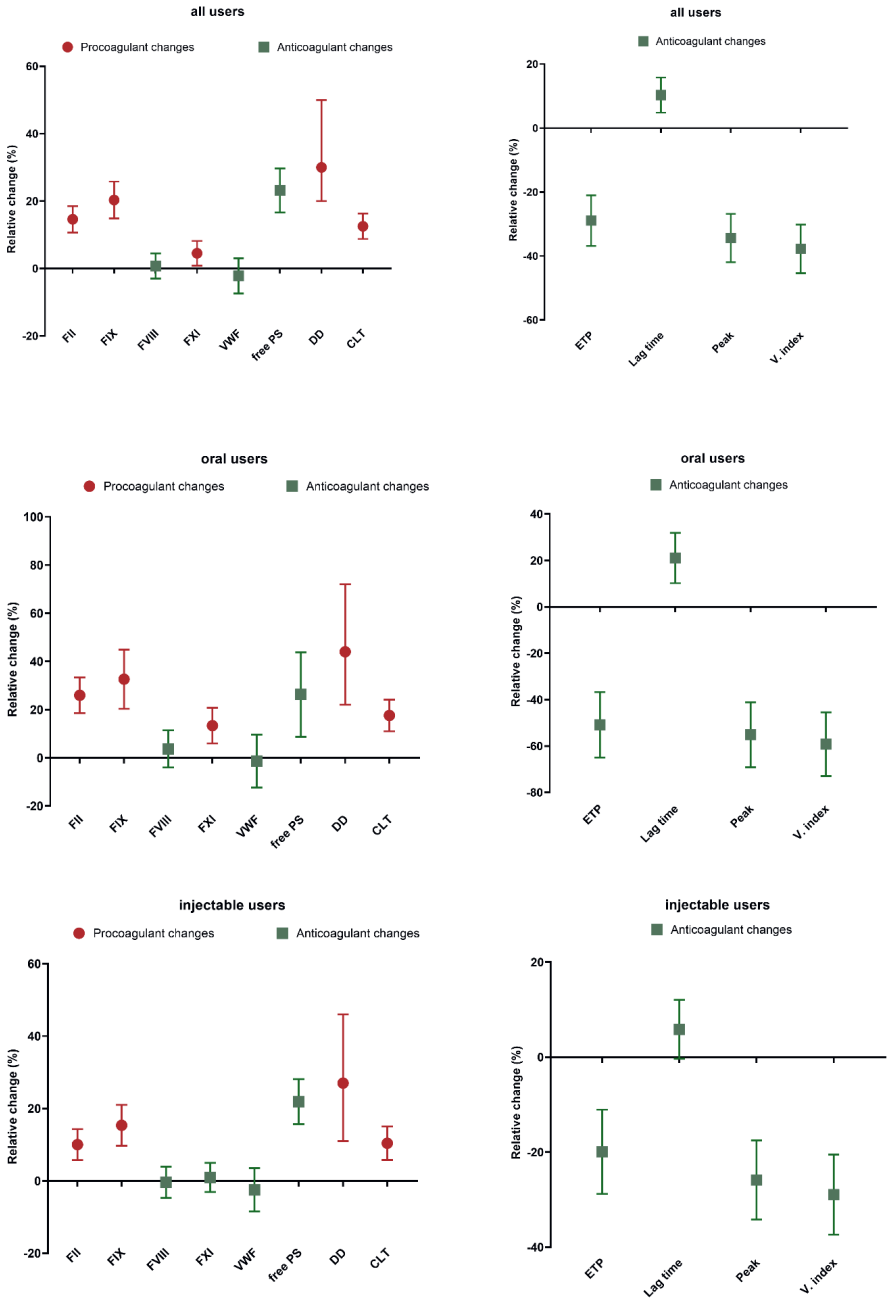
At T_0 the available measurements were 96 for CLT and 97 for thrombin generation, at T_2 90 measurements were available for free protein S, CLT and 97 for thrombin generation. T_0 = before the start of the cycle, T_1 = in the last week of the cycle, T_2 = 3 months after the cycle, T_3 = 12 months after the start of the cycle. * Mean difference calculated with linear mixed model using T_0 as reference. ǂ Mean ratio back-transformed after logarithmic transformation.

Table 3. Mean levels of coagulation factors 24 months after enrollment (T_4) on 77 participants.

	Non users n=23		Repeat cycle users n=32		Current users n=22	
	Mean (SD)	Mean difference vs T0 (95%CI)	Mean (SD)	Mean difference vs T0 (95%CI)	Mean (SD)	Mean difference vs T1 (95%CI)
FII [%]	101 (17)	4 (-1 to 8)	98 (13)	-1 (-4 to 3)	107 (16)	-1 (-9 to 5)
FVIII [%]	122 (31)	-4 (-13 to 5)	113 (25)	-7 (-16 to 1)	119 (23)	-11 (-22 to -1)
FIX [%]	113 (16)	5 (0 to 9)	105 (15)	-2 (-8 to 4)	129 (25)	-2 (-17 to 14)
FXI [%]	118 (18)	2 (-3 to 6)	113 (17)	-4 (-8 to 0)	121 (23)	0 (-12 to 11)
vWF [%]	136 (40)	-9 (-21 to 2)	130 (41)	-7 (-20 to 6)	126 (39)	-7 (-21 to 7)
PS [%]	108 (34)	-6 (-14 to 2)	113 (28)	3 (-6 to 12)	141 (25)	4 (-17 to 24)
DD [ng/mL]	298 (167)	0.9 (0.7 to 1.2)Ŧ	271 (147)	1.1 (0.9 to 1.3)Ŧ	335 (240)	1.0 (0.8 to 1.2)Ŧ

Not users = subjects that did not start another AAS cycle; repeat cycle users = subjects that performed one or more cycle between 12 and 24 months after enrolment; current users = subjects that were using AAS at the time of blood drawn 24 months after inclusion. Ŧ Mean ratio back-transformed after logarithmic transformation.

Figure 1. Percentage change in the coagulation parameters between baseline (T_0) and during AAS use (T_1), for all users and stratified by route of administration of AAS (oral vs. injectable only) at the end of the cycle. The red circle indicates a procoagulant effect of the measured change, whereas a green square indicates an anticoagulant effect. Anchored lines indicate 95% confidence intervals.



DISCUSSION

In this study, we prospectively investigated changes in coagulation parameters during and after AAS use in a cohort of amateur strength athletes. We observed that AAS use affected clotting factors involved in different stages of the activation and inhibition of the coagulation cascade, as reflected by an increase of both pro- and anticoagulant parameters during AAS exposure. Of interest, we observed major differences in thrombin generation curves before and during AAS use, reflecting the overall coagulation potential. The time to initiation of coagulation was prolonged during androgen exposure and all other TGA parameters, in particular ETP, were decreased. In addition, AAS use resulted in an impairment of the fibrinolytic process, as we observed a prolongation of CLT. All changes appeared to be completely reversible, as three months after AAS withdrawal all coagulation parameters restored to their baseline values, and did not change further until 12 months after inclusion. These results provide some reassurance on a possible long term effect on the coagulation system after AAS exposure.

Only few previous studies investigated the effect of AAS on clotting factors in AAS users and used only a cross-sectional design,[12,14,20] but some inferences can be extrapolated from studies in healthy volunteers or patients with coagulation deficiencies. A study in patients with haemophilia showed an increase in concentration of FVIII during administration of danazol.[21] Other studies,[15,22] including one in AAS abusers,[14] did not confirm this finding, similarly to our results. Another study on AAS abusers[12] reported an increase in FII in current and former AAS users when compared with age-matched controls, similar to what we observed. In regard to coagulation inhibitors, three cross-sectional studies on AAS abusers all reported an increase of free PS during AAS use,[12,14,20] in accordance with our results. Increased levels of free PS, albeit lower in magnitude, were also observed in a recently published study on the effect of testosterone therapy in transmen,[23] in whom a concomitant decrease in FII was also reported. To the best of our knowledge, vWF has not been previously investigated in AAS abusers, but a study on the effect of testosterone esters on a small group of transmen showed a decrease of vWF levels.[2] Likewise, the effect of AAS on fibrinolysis was investigated in only one earlier study, in which fibrin clot lysis was impaired in AAS abusers compared with controls.[24]

Equally limited evidence is available on the effect of AAS abuse on thrombin generation, as only one previous study evaluated TGA in AAS users.[12] While we observed a reduced thrombin potential, in this study ETP was found to be increased in AAS users in comparison with age-matched healthy controls. It must be noted, however, that two different thrombin generation assays were used which might explain the opposite results. While Chang *et al.* used a standard TGA assay, we performed a thrombomodulin-modified thrombin generation test. In this latter assay the capacity of the protein C system to downregulate coagulation is more taken into account. An increase of protein C during AAS use was reported in previous studies,[12,14,20] which might explain our observed overall reduced thrombin potential.

An additional difference with the study of, Chang *et al.* is the observed differences in coagulation parameters in former AAS abusers, who had stopped AAS for a median of 2.5 years.[12] In contrast, all clotting parameters were completely restored to baseline levels in our population, up to one year after AAS withdrawal. Our findings are strengthened by the analysis on coagulation measurements at two-

years follow-up, when participants were free to perform a second cycle if they were willing to. None of our analyses, stratified by AAS use, showed any difference between clotting parameters measured at two-years follow-up and baseline. There was also no difference between measures at two-years follow-up and during the first cycle in those subjects that were using AAS at the two-years visit. Furthermore, other laboratory parameters, such as markers of liver and kidney function, were fully restored to baseline value after AAS withdrawal.[25] Taken all together, our finding points towards the complete reversibility of the changes in coagulation system after AAS withdrawal and against a possible cumulative effect after multiple AAS exposure. However, it cannot be entirely excluded that multiple years of consecutive AAS use may affect coagulation in such a way that the clotting cascade may remain modified even when AAS are withdrawn.

Interestingly, as shown in Supplementary Figure 2 and by the increased residual covariance of the linear mixed model during androgen exposure (Supplementary Table 1), the observed changes in coagulation parameters were not uniform in each subject. This may be due to individual, i.e. genetic, differences. Alternatively, different AAS types might have a different effect on the clotting system, as suggested by the diverging effect of stanozolol and danazol on FVIII levels observed in haemophiliac patients.[15,21] Our study population used diverse combinations of AAS compounds in different durations and dosages It is possible that these differences also had a diverse effect on the coagulation parameters. We hypothesized that a higher cycle dose was associated with increased differences in coagulation parameters. However, in the model adjusted for possible confounders, cycle dose and duration were associated only with an increased difference in PS. Furthermore, cycle dose and duration were not associated with the recovery of coagulation parameters three months after AAS withdrawn. Nevertheless, it is important to acknowledge that the documented AAS types and cycle dose were based on the information from the label of the AAS compounds. In a subset of 55 subjects, qualitative analysis of the AAS products delivered by the subjects was performed.[16] The results indicated a low quality of the illegal AAS products used in this study, as only about one half of the analysed AAS samples contained the specific AAS subtype as declared on the label. Consequently, the reported label information dose only serves as a rough estimate of the actual androgen exposure and may have clouded the effect in the multivariable model. Furthermore, as specific AAS types could not be reliably derived from label information, an analysis assessing the relationship between specific compounds and effects on coagulation could not be performed.

The different routes of AAS administration could also partly explain between-subject differences. We observed increased differences in coagulation parameters in subjects using oral AAS at the time of blood collection, compared with subjects using injectable AAS only. This might be explained by oral compounds causing a higher peak plasma androgen concentration, a shorter time to maximum plasma concentration and shorter half-life, and by an immediate effect on the liver due to first-pass metabolism.[26,27] It must be noted however, that the first group also performed a cycle with a higher median dose compared to the injectable steroids' users (median dose of 1120 mg [IQR 884 to 1504] and 804 mg [IQR 591 to 1130] respectively). Hence, the observed difference might also be explained by the overall higher cycle dose performed by oral AAS users.

The mechanisms by which AAS induce changes in the coagulation system are currently not known. AAS might increase the overall liver protein synthesis through their action on the androgen receptor in human hepatocytes.[28] A targeted action on the synthesis of liver-produced clotting factors is also a possible explanation. Indeed, we observed changes in the liver-produced pro- and anticoagulant proteins, such as FII, FIX, FXI and PS,[29] whereas we observed no changes in the endothelial-derived components (e.g. vWF). FVIII, albeit synthetized both in endothelial cells and in sinusoidal cells of the liver, is rapidly cleared if not bound to vWF and its levels are therefore strictly dependent on vWF concentrations.[30] Hence, the overall lack of changes in FVIII might be explained by the absence of an effect on vWF concentrations. Nonetheless, it is worth noting that AAS have been associated with increased levels of TFPI,[12] which is mostly produced by vascular endothelial cells,[31] and on other makers of endothelial cell function.[2] Therefore, androgens might also act via different pathways. In addition, liver toxicity been described in association with AAS and especially oral compounds,[32] which could lead to release of the hepatocytes' intracellular contents. Liver biochemistry was investigated during the follow-up of subjects in the HAARLEM study, and although a modest rise in concentration of liver-related enzymes was observed during androgen exposure,[25] this was primarily due to muscle breakdown and there have not been signs of acute liver damage in any of the subjects.

As we observed changes in both pro- and anti-coagulant parameters, it is difficult to estimate whether an overall pro- or anti-coagulant shift in the balance takes place and the corresponding increase or decrease in VTE risk. The reduced TG potential would suggest that the anticoagulant changes prevail over the procoagulant ones. Moreover, FVIII and vWF, the clotting factors which are most strongly associated with VTE risk,[33,34] did not increase during AAS use. Nevertheless, studies that assess the absolute incidence of VTE during AAS use remain the benchmark to assess the real risk. Unfortunately, no such study has been performed to date. In the HAARLEM study participants, no episode of VTE was recorded during 203 person years of follow-up and 68 person years of androgen exposure. Some inference can be obtained through studies of patients using testosterone replacement therapy, albeit there are major differences between androgen dosage and therapy duration compared with AAS abusers as well as in clinical characteristics. A recent meta-analysis suggested that androgen therapy was not associated with an increased risk of VTE.[35] However, the authors observed an overall low quality of evidence and a high risk of bias in most studies, as well as wide confidence intervals, so the existence of an increased risk cannot be completely ruled out.

Our study has several important strengths. We were able to prospectively investigate a relatively large group of AAS users, the largest to date, and to follow them through their cycle and withdrawal period for a total of two years. Previous studies on AAS users were only cross-sectional, which weakens any possible causal interpretation of the findings, and only included a limited number of subjects.[11,14,20] The use of thrombomodulin modified TGA allowed us to include the anticoagulant protein C pathway and to obtain a complete picture of the overall haemostatic balance. Moreover, the use of the single-subject design, in which each subject acts as their own control, reduced problems with incomparability of groups (minimizing confounding from fixed factors such as age and genetics) and with sampling bias that can be introduced by selection of controls.

Few aspects of the study should be mentioned as possible limitations. Since the study design urged subjects to completely discontinue AAS after their cycle, it excluded users who intended to use AAS continuously (i.e., not in a cycle). This notwithstanding, in total 5 subjects did use AAS the entire follow-up period. Whether changes in coagulation factors were reversible in these men is not known. Moreover, the study provided subjects the opportunity to monitor health for free, even though the results were disclosed only after completing the study. This may have led to, on the one hand, a cohort composed of more health-conscious subjects who use AAS more moderately, or, on the other hand, users who did not feel the need to hold back as their health was checked anyway. This possible selection bias is not likely to impact the results by much, as cycle characteristics such as dose and duration played only a minor role in the observed effects on the coagulation system As previously mentioned, the use of label information to calculate the cycle dose in the analysis probably did not reflect true AAS dose, due to unreliability of AAS obtained through the illegal market. However, our results possibly better reflect the effect of stacking of AAS compounds in the uncontrolled real life setting, where the formulation and dose is variable and mostly not known by the subjects. Finally, as the subjects were using other PIEDs and medication during the cycle, as well as drugs of abuse, confounding by the use of other substances cannot be excluded. Nevertheless, the similar results of our sensitivity analysis excluding users of drugs of abuse strengthen our results.

In conclusion, we observed changes of pro- as well as anti-coagulant parameters in a population of amateur strength athletes during androgen exposure. The overall reduced thrombin potential and the absence of changes in FVIII and vWF do not point towards an increased VTE risk during androgen exposure. Nevertheless, further prospective studies on AAS amateur users, powered to investigate an increased incidence of VTE, are necessary.

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CHAPTER 9

ANABOLIC ANDROGENIC STEROIDS INDUCE REVERSIBLE LEFT VENTRICULAR HYPERTROPHY AND CARDIAC DYSFUNCTION. ECHOCARDIOGRAPHY RESULTS OF THE HAARLEM STUDY

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ABSTRACT

Background

The use of anabolic androgenic steroids (AAS) is not uncommon among strength athletes. Several cross-sectional studies have linked AAS use to heart disease, but a causal role for AAS is not certain and it is unknown whether cardiac changes are reversible.

Methods

Men of at least 18 years old intending to start an AAS cycle on short notice were included for comprehensive 3D echocardiographic examination before (T_0), at the end of the cycle (T_1), and 1 year after inclusion (T_2) after a recovery period. Details of the AAS cycle performed and the use of other performance and image-enhancing drugs (PIEDs) as well as illicit drug use were recorded. Trend analysis and multivariable regression analysis were performed with mixed effects linear models.

Results

Thirty-one subjects were included. Between start (T_0) and end of the cycle (T_1), after a median AAS cycle duration of 16 weeks, 3D left ventricular ejection fraction declined with 4.9% (CI -7.2 to -2.5, P<.001), E/A-ratio declined with -0.45 (CI -0.69 to -0.21, P<.001), and 3D left atrial volume increased with 9.2 ml (CI 2.9 to 15.4, P=.004). Left ventricular mass increased with 28.3 g (CI 14.2 to 42.4, P<.001) and was positively correlated with AAS average weekly dose. After a median recovery time of 8 months (T_2), all parameters returned to baseline.

Conclusion

AAS induce left ventricular hypertrophy and impaired systolic and diastolic function in amateur strength athletes. The structural cardiac changes are positively associated with AAS dose and complete recovery occurred after AAS were discontinued.

INTRODUCTION

The use of anabolic androgenic steroids (AAS) is not uncommon among strength athletes and regular visitors of fitness centres. The lifetime prevalence for men is estimated at 3%.[1] Production and trading of AAS without a license is prohibited in most countries, but AAS can be illegally acquired through local dealers or the internet.

It is beyond doubt that AAS are harmful. Current knowledge however is based on rather low levels of evidence including expert opinion, case reports and case control studies. Prospective studies describing adverse effects of AAS are scarce. The use of AAS is associated with agitation, mood and anxiety disorders,[2] liver toxicity and hepatocellular neoplasia,[3,4] and disruption of gonadal function after cessation of use.[5,6] AAS use could also pose a risk factor for cardiovascular disease due to unfavourable effects on blood pressure, haematocrit and lipid metabolism.[7] A cross-sectional case control study using computed tomography angiography showed higher coronary artery plaque volumes in weightlifters using AAS compared to non-users.[8]

Several reports have also linked AAS use to heart disease, such as left atrial dysfunction,[9] ventricular diastolic[10] and systolic dysfunction,[11,12,13] impaired ventricular strain[14] and left ventricular hypertrophy.[15,16] These studies are all cross-sectional in nature and a causal role for AAS is thus not certain. It is also not known whether cardiac changes are reversible. An additional limitation is the heterogeneous and often poorly documented records on AAS use of the strength athletes in these study groups.

The HAARLEM study is a prospective observational cohort study that started in 2015 and investigated the effects of AAS use by amateur strength athletes.[4] We recruited male athletes who intended to start an AAS cycle on short notice. Baseline measurements were performed prior to the start of the AAS cycle. Repeat analyses were performed in the last week of the cycle and 1 year after the start of the cycle. The study posed a unique opportunity to obtain prospective data about both structural and functional cardiac changes during AAS use and therefore we performed comprehensive echocardiographic examinations at these three points in time.

All androgens used by subjects in our study were illegal and not registered for use in humans. It would therefore be unethical to conduct a controlled intervention study in which subjects would use androgens according to a study protocol. Prospective, observational studies like the HAARLEM study may thus be the only feasible approach to collect valid data.[17]

METHODS

Details of subject recruitment of the HAARLEM study are described in a previous report [4]. In short, men of at least 18 years old intending to start an AAS cycle on short notice (i.e. within 2 weeks) were invited to sign up after the study was promoted on national television, regional newspapers and social

media. We did not interfere with the chosen dose, duration or contents of the planned AAS cycle. However, to guarantee significant exposure, the cycle had to be at least 6 weeks in duration, average a weekly dose of a minimum of 200 mg of androgens, and comprise at least two different types of AAS. Subjects were excluded if they had used AAS in the 3 months before inclusion or had suffered any major somatic or psychological health issues in the previous 6 months.

The HAARLEM study started in October 2015 and 100 men were included for health analysis. Echocardiography was added to the analysis in May 2017 after approval by the local Medical Ethics Committee. From this point until April 2018, the last 31 subjects included in the HAARLEM study were offered analysis with echocardiography, all of whom provided informed consent.

Clinic visits

Subjects underwent 3 echocardiographic examinations during a 1 year study period. The baseline visit took place immediately after inclusion and before the initiation of the AAS cycle (T_0). Subjects returned to the clinic in the last week of the AAS cycle (T_1). The cycle performed was recorded in detail, with duration and dosage of each type of AAS used, in addition to use of other performance and image enhancing drugs (PIEDs), medication and post-cycle therapy (PCT). Symptoms or side effects experienced by subjects were recorded. After clinic visit T_1 subjects discontinued AAS use for the remaining part of the study period. The last visit followed 1 year after the start of the cycle (T_2). As a result, the interval between T_1 and T_2 was variable, i.e. the recovery phase, dependent on the duration of the AAS cycle.

Echocardiography

Cardiac 3D echocardiography was performed on a state-of-the-art Philips Epiq 7 device. The HeartModel application was used for cardiac chamber 3D quantification. Two EACVI certified analysts carried out the echocardiography and echocardiograms of the same subject were made by the same analyst as much as possible to reduce interobserver variability. Interpretation of the echocardiographic data was performed by the study cardiologist after full data collection and after blinding for the type of clinic visit.

Resting heart rate (/min), blood pressure (mmHg), height (cm),weight (kg) and body surface area (BSA, m^2) were obtained during each clinic visit. The following variables were extracted from the obtained echocardiogram (with corresponding units):

- Left ventricle:
    - Left ventricular end-diastolic dimension (LVEDd, mm), left ventricular end-systolic dimension (LVEDs, mm), left ventricular end-diastolic volume 3D (LVEDV 3D, ml), left ventricular end-systolic volume 3D (LVESV 3D, ml), intraventricular end-diastolic septal thickness (IVSd, mm), left ventricular end-diastolic posterior wall thickness (LVPWD, mm), left ventricular mass (LV mass, g), left ventricular ejection fraction 3D (3D LVEF, %), global longitudinal strain (LV strain global, %), myocardial performance index (MPI, Tei-index, no dimension).

- Diastolic function:
    - Mitral valve E wave (E, cm/s), mitral valve E duration time (E-DT, ms), mitral valve A wave (A, cm/s), mitral valve A wave duration time (A-DT, ms), E/A-ratio, lateral e' wave (e' lat, cm/s), septal e' wave (e' sept, cm/s), lateral E/e'-ratio (E/e' lat), septal E/e'-ratio (E/e' sept), pulmonary vein Arev (PV Arev, cm/s), pulmonary vein Arev duration time (PV Arev DT, ms), pulmonary vein D (PVD, cm/s), pulmonary vein S (PVS, cm/s), left atrial volume index (LAVI, ml/m^2), left atrial volume 3D (LAvol3D, ml), LAD parasternal long-axis view (LAD PSLAX).
- Right ventricle:
    - Right ventricular tricuspid annular plane systolic excursion (RV TAPSE, mm), right ventricular tissue Doppler imaging (RV TDI, cm/s), right ventricular annulus diastolic (RV annulus, mm), right ventricular fractional area change (RVFAC, %), pulmonary artery acceleration slope (PA acc slope, cm/s^2), pulmonary artery acceleration time (PAAT, ms), tricuspid valve insufficiency severity (TV severity, 0-4), systolic pulmonary artery pressure (SPAP), right atrium pressure (RA pressure, mmHg), right ventricular outflow tract (RVOT, mm).

Mitral inflow velocities were recorded in the apical 4-chamber view with the pulsed-wave Doppler sample volume placed at the level of the mitral valve tips. The peak velocity of early (E) and late (A) diastolic waves and the E-DT were measured. The peak myocardial systolic (s'), early diastolic (e'), and late diastolic (a') velocities were measured in the apical 4- and 2-chamber views using Doppler tissue imaging (DTI). LV mass was calculated using the Devereux cube formula incorporated in the IntelliSpace workstation.

Analysis

Simple descriptive statistics were used to display quantitative data. If the variables were normally distributed, mean and standard deviation were calculated. If the distribution of a variable was skewed, a median is presented with range. For the comparison of the results of clinic visits, mixed effects linear models were used to calculate 95%-confidence intervals (CI) and P-values. In this model missing data are accounted for with maximum likelihood estimation.

Multivariable regression analysis was also performed with mixed models, assessing the effects of AAS cycle dose and length, number of AAS used, whether or not oral AAS were used, previous AAS use, mean arterial blood pressure (MAP), cumulative history of AAS use, training time, concurrent use of growth hormone (GH), ecstasy (XTC), cocaine, and the use of post-cycle therapy (PCT). In the multivariable regression analysis, to correct for multiple testing, only a P-value <.01 was considered statistically significant.

RESULTS

Thirty-one subjects of the HAARLEM study were included for echocardiography. Relevant baseline characteristics and AAS cycle characteristics are shown in *Table 1*. Subjects were on average 33 years old. All of them were active in strength sports, averaging 307 minutes per week at the gym at baseline, which increased to 390 minutes per week during the cycle (P=.002) and decreased to 243 minutes after the cycle (P=.010). Twenty-seven subjects had performed an AAS cycle before. The cycle performed during the study period had a median duration of 16 weeks and median weekly dose of 904 mg testosterone equivalents. This was similar to the cycle characteristics of the subjects in the HAARLEM study cohort who were not included for echocardiography. In this group the median cycle duration was 15 weeks and the median weekly dose was 898 mg (unpaired t-test, P=.31 and P=.81, respectively).

***Table 1*.** Baseline characteristics, AAS cycle characteristics and details of drug abuse of the 31 subjects of the HAARLEM study included for echocardiography.

General	**n (%), mean (SD, ranges)**
Male	31 (100%)
Age	33 (±8.4; 20-67)
Height (cm)	182 (±6.8; 171-193)
Weight (kg)	88,2 (±10.5; 71-118)
Body mass index (BMI, kg/m^2)	26,6 (±2.2; 23.1-32.7)
Previous AAS use	27 (87%)

Current sport	**n (%)**
Fitness/bodybuilding	31 (100%)
Competitive bodybuilding	3 (10%)
Weight lifting	1 (3%)
Combat sports (e.g. kickboxing, karate, judo)	12 (39%)

Fitness schedule at baseline	**Mean (SD, ranges)**
Number of training sessions (/week)	4 (±1.1; 1-7)
Duration of training sessions (minutes)	73 (±15; 60-105)
Time weekly spent at gym (minutes)	307 (±110; 80-630)

Cycle characteristics	**n (%), median (ranges)**
Cycle length (weeks)	16 (±8.0, 7-42)
Number of AAS	4 (1-11)
Average weekly dose (mg)*	904 (250-3382)
Cumulative dose (mg)*	13.200 (3.000-74410)
Post-cycle therapy	26 (84%)

Use of other PIEDs during cycle	**n (%)**
Creatine	9 (29%)
Growth hormone	8 (26%)
Levothyroxine	4 (13%)
hCG	3 (10%)
Insulin	2 (6%)
Tamoxifen	6 (19%)
Aromatase inhibitors (anastrozole, exemestane)	9 (29%)

Drug use during study period	
Nicotine	13 (42%)
Alcohol	21 (68%)
Ecstasy/amphetamines	13 (42%)
Cocaine	11 (35%)
Gamma-hydroxybutyric acid (GHB)	11 (35%)
Cannabis	10 (32%)
Other (3-FMP, ketamine, 2-CB)	7 (23%)
≥3 drugs (except nicotine and alcohol)	8 (26%)

***** Cumulative dose is the sum of all different AAS compounds used in a cycle in mg. For practical purposes, all types of AAS were regarded as having a 1 to 1 equivalence with testosterone (e.g. 1 mg testosterone = 1 mg nandrolone = 1 mg stanozolol). The average weekly dose is calculated by dividing the cumulative dose by cycle length in weeks.

Analysis with echocardiography was performed in all 31 subjects at the start (T_0) and the end of the cycle (T_1). The median time between T_1 and T_2, corresponding to recovery time after the AAS cycle, was 8 months (range 5-12). Four subjects started a new cycle before T_2, hence echocardiography was not carried out in them as it could not assess recovery. Another 2 subjects missed T_2 due to obligations for work and emigration, respectively. Consequently, 25 subjects were analysed with echocardiography after the recovery period (T_2). As intended, T_2 took place after a median of 12 months (range 8-16) after inclusion (T_0), i.e. total follow-up period.

Details on the side effects reported by subjects of the HAARLEM study are published separately [18] but none reported dyspnoea or peripheral oedema at the end of the cycle (T_1). Two of 31 subjects complained of fatigue. Fluid retention was reported by 15 of 31 subjects. Between the start (T_0) and the end (T_1) of the cycle, there was an increase in heart rate of 10.0 beats/min (CI 5.8 to 14.2, P<.001) and BSA of 0,05 m^2 (CI 0.01 to 0.10, P=.020). BSA at the end of the cycle (T_1) was positively associated with AAS cycle dose and GH use, but negatively associated with training time. Mean systolic and diastolic blood pressure increased with 6 mmHg (CI 1.2 to 11.0, P=.015) and 5 mmHg (CI 0.9 to 8.2, P=.013), respectively. Heart rate, blood pressure and BSA returned to baseline after the recovery period (T_2).

Detailed results of the trend analysis and multivariable regression analysis of all measured echocardiographic parameters are shown in *Supplementary Table 1* and *Supplementary Table 2*, respectively. Important findings are elaborated in the subsequent paragraphs.

Left ventricle

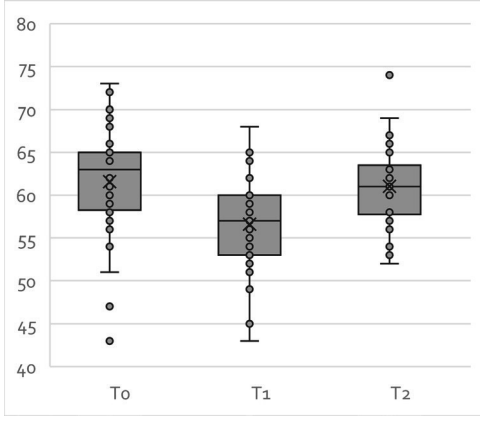
The 3D LVEF trend is shown in *Figure 1A*. 3D LVEF declined with 4.9% (CI -7.2 to -2.5, P<.001). 3D LVEF was negatively associated with training time at the end of the cycle (T_1).

Between the start (T_0) and end of the cycle (T_1), there was an increase in 3D LVEDV and 3D LVESV of 10.3 ml (CI 1.0 to 19.7, P=.030) and 11.5 ml (CI 6.8 to 16.2, P<.001), respectively. After the recovery period (T_2), these volumes declined with -16.9 ml (CI -26.5 to -7.2, P=.001) and -8.0 ml (CI -12.9 to -3.0, P=.002) compared to T_0, respectively. GH use was associated with a higher 3D LVEDV and 3D LVESV at the end of the cycle (T_1), whereas the number of AAS used in the cycle was negatively associated with 3D LVEDV and 3D LVESV both measured at the end of the cycle (T_1) as after the recovery period (T_2).

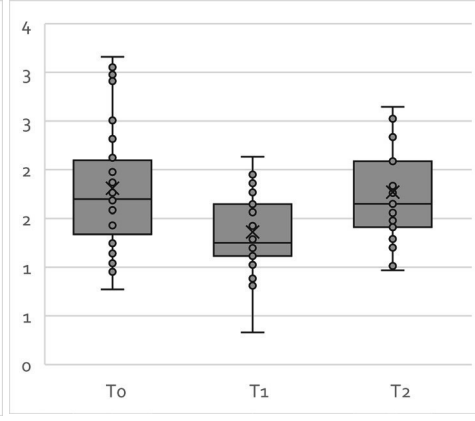
LV mass increased with 28.3 g (CI 14.2 to 42.4, P<.001) between the start (T_0) and the end of the cycle (T_1). This trend was not different when LV mass was adjusted for BSA, i.e. g/m^2. IVSd and LVPWD both increased during the cycle (T_1) with 0.87 mm (CI 0.44 to 1.30, P<.001) and 1.18 mm (CI 0.76 to 1.61, P<.001), respectively. There was a positive correlation between AAS average weekly dose and LV mass, LVPWD and IVSd at the end of the cycle (T_1). Contrarily, IVSd was negatively associated with training time at the end of the cycle (T_1) and after the recovery period (T_2). XTC use also had a negative impact on LV mass measured at the end of the cycle (T_1). LV mass, IVSd and LVPWD all returned to baseline after the recovery period (T_2).

Figure 1. Box and whisker plots of the relevant parameters measured with echocardiography before (T_0) and at the end of the cycle (T_1) with AAS, and after the recovery period (T_2).

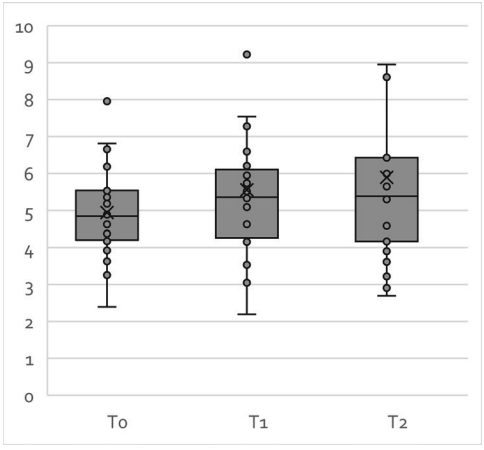
A. 3D LVEF (%)



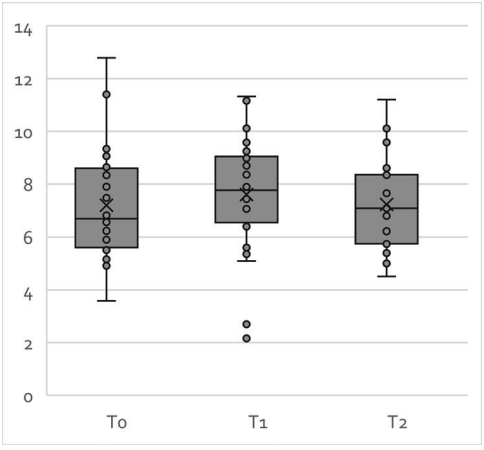
B. E/A-ratio



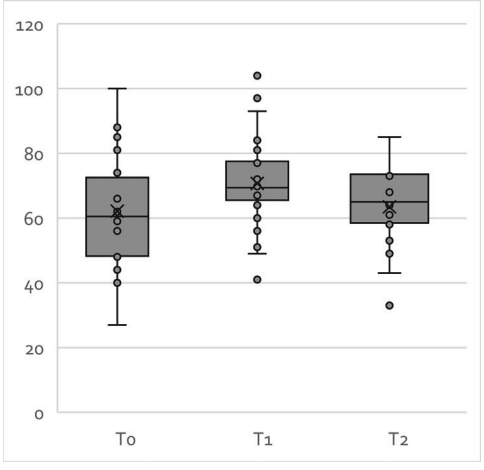
C. E/e'-ratio lat



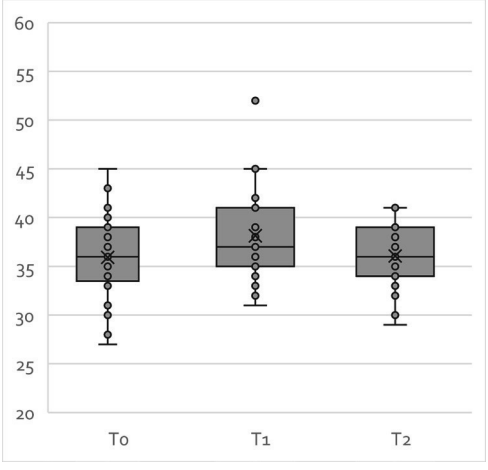
D. E/e'-ratio sept



E. LAvol 3D (ml)



F. LAD PSLAX (mm)



The parameters LVEDd, LVEDs, LV strain global and MPI did not show a significant change during the course of clinic visits.

Diastolic function

The trend for E/A-ratio during clinic visits is shown in *Figure 1B*. A increased with 9.1 cm/s (CI 2.6 to 15.7, P=.006) during the cycle (T_1). No significant changes occurred in A-DT, E and E-DT. The resulting decline of the E/A-ratio between the start (T_0) and the end of the cycle (T_1) was -0.45 (CI -0.69 to -0.21, P<.001) with normalization to baseline after the recovery period (T_2).

The changes of the E/e' lat and sept through the course of clinic visits are shown in *Figure 1B*. None of these changes were statistically significant. There was a decline of e' lat during the cycle (T_1) with -1.8 cm/s (CI -3.5 to -0.1, P=.038). Age, cocaine use and training time were positively associated with E/e' sept at the end of the cycle (T_1), whereas AAS average weekly dose was negatively associated with E/e' sept.

The change of LAvol3D and LAD PSLAX during clinic visits is shown in *Figure 1C*. During the cycle (T_1), LADvol3D increased with 9.2 ml (CI 2.9 to 15.4, P=.004) and LAD PSLAX with 1.9 mm (CI 0.7 to 3.2, P=.002). The increase in LAD PSLAX was greater for subjects using oral AAS (β = 3.7, CI, 1.2 to 6.2, P=.004). The parameters normalized after the recovery period (T_2).

The parameters PV Arev, PV Arev DT, PVD and PVS did not show any significant change during the study period.

Right ventricle

During the course of clinic visits, there were no significant changes in RV TAPSE, TV TDI, RV annulus, RVFAC, PA acc slope, PAAT, RVOT and RVAC. Tricuspid valve insufficiency was grade 1 in only 4, 2 and 4 subjects at the start (T_0) and end of the cycle (T_1), and after the recovery period (T_2), respectively, so SPAP and RA pressure could not be analysed reliably.

DISCUSSION

The main findings of this study were that an AAS cycle with supraphysiological doses of androgens induced an increase in left ventricular mass, a reduction of left ventricular ejection fraction by ~5%, and a higher left ventricular stiffness reflected by the reduction of E/A-ratio, although changes in filling pressures cannot be excluded. There was a positive relationship between average weekly dose of AAS and left ventricular mass. When AAS were discontinued, after a median recovery time of 8 months, all parameters returned to their baseline values.

The increase in left ventricular mass was due to an increase in interventricular septum as well as posterior wall thickness. This is in compliance with the results of a recent large cross-sectional study by Baggish and colleagues.[8] This group also showed a reduction of left ventricular ejection fraction in AAS users. A lower E/A-ratio has also been documented in previous studies comparing bodybuilders to non-users and sedentary controls.[10,19]

The data from our cohort study are novel because the prospective single-subject design allowed to assess not only the effect of AAS on cardiac function but also whether recovery occurred after the cycle. This showed complete normalization of all parameters after discontinuation of AAS. In addition, we meticulously characterized the AAS cycles performed by the subjects and as such we could demonstrate that androgen dose was associated with increased left ventricular mass and diameters.

Limitations

Several shortcomings of the HAARLEM study are mentioned in the baseline data report.[4] The main limitation was the fact that the study design precluded the possibility to establish causality between androgen abuse and the observed cardiac effects. The course of the data in time, however, as well as the association of left ventricular parameters and androgen dose, leave little doubt that androgen abuse was the main determinant of the observed cardiac changes. Moreover, androgens were shown to independently cause cardiac hypertrophy and impaired systolic and diastolic function in rat studies.[20]

Furthermore, several other variables play a confounding role in cardiac function, such as blood pressure and exercise. Although mean blood pressure increased mildly during AAS use, we did not find it to interact significantly with cardiac function. This was probably because rise in blood pressure was fairly small and existed for a relatively short amount of time. Long-term intensive exercise may also induce myocardial changes of the left ventricle, i.e. the Morganroth hypothesis. Even though our subjects spent an average 7.5 hours in the gym per week during the cycle, it is unlikely that the observed cardiac changes were a result of vigorous strength training. First, there is evidence indicating that the effect of exercise on the heart particularly applies to endurance athletes and not strength athletes.[21] Secondly, included subjects usually had a long history of strength training and the actual increase in training time during the cycle was only modest in comparison to their training time before the cycle. Cardiac changes reversed after discontinuation of AAS even though subjects kept an intensive training regimen. Thirdly, our multivariable analysis did not show an association between training time and observed myocardial changes.

Clinical relevance

Our findings strongly support the cardiotoxic nature of AAS. The changes in cardiac structure and function did not lead to symptoms, however, such as dyspnoea or peripheral oedema, as these were not reported by the subjects. The occurrence of heart failure in users of AAS nevertheless has often been reported.[22] We hypothesize that cumulative cardiac damage may follow long-standing AAS use when recovery time in between cycles is too short or when AAS are used continuously. Our data could not substantiate the presence of such cumulative damage as we did not observe a relationship between the extent of prior AAS use and cardiac abnormalities at baseline. Of note, our cohort displayed a wide variety of historic AAS use, ranging from no prior use to 8 years of cumulative AAS use, but no subjects had abused androgens continuously for longer than 1 year. It is therefore likely that progression to clinical heart failure only occurs in those athletes with an excessive history of AAS use, or when an athlete has a prior medical condition affecting the heart, e.g. cardiomyopathy.

CONCLUSION

We have shown that AAS induce left ventricular hypertrophy and impaired systolic and diastolic function in amateur strength athletes. This effect was independent from changes in blood pressure and training time. The structural cardiac changes were greater if larger androgen doses were used.

Impaired cardiac function did not lead to clinical signs of heart failure. All parameters normalized within the one year follow-up period after the AAS were discontinued.

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CHAPTER 10

PREDICTORS OF ONGOING ANABOLIC ANDROGENIC STEROID USE. A TWO YEAR PROSECPTIVE OBSERVATIONAL COHORT STUDY

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(Submitted for publication)

ABSTRACT

Background

The use of anabolic androgenic steroids (AAS) is common among visitors of fitness centers. Prospective data regarding patterns of AAS use and predictors of future use.

Methods

Participants performed a single AAS cycle in the first year and remained in follow-up for a second year in which they used AAS in the way of their choice. Details of AAS use were recorded. The analysis aimed to identify baseline sociodemographic factors and cycle characteristics that would predict future AAS use.

Results

Ninety-seven (97%) men completed the second year of follow-up. Sixty-three subjects (65%) used AAS again and 16 (16%) for the entire duration of the second year. Training time at baseline and taking part in bodybuilding competitions were associated with a longer duration of AAS use during follow-up. Cycle duration in the first year predicted repeat AAS use in the second year.

Conclusion

The majority of androgen abusers have a tendency to use AAS repeatedly. The factors that predicted future AAS use may assist in harm reduction strategies that aim to minimize long-term health problems in strength athletes.

INTRODUCTION

The use of anabolic androgenic steroids (AAS) is not uncommon among amateur strength athletes. The prevalence for men visiting fitness centers is estimated to be 4-6%.[1-2] AAS are commonly used in cycles where multiple androgens are combined in a supraphysiological dose for a certain amount of time, usually 6 to 20 weeks.[3-5] After the cycle, serum androgen concentrations wane to physiological levels, and below, during a phase of variable duration called 'post AAS hypogonadism'. It is during this time that muscle mass and strength rapidly decline. Athletes therefore often quickly reengage in a new cycle to maintain muscle mass.[6]

In case cycles are recurring, the alternating periods of supraphysiological androgen levels and periods of androgen deficiency, lead to fluctuating sexual dysfunction – reduced libido and sexual activity, somatic symptoms – gynecomastia and acne, and poor general well-being – depressed mood and emotional lability.[7] It is a loop that predisposes to dependency and repeated use, and eventually a longer suppression of endogenous testicular function, which delays ultimate recovery. About 6-20% of strength athletes escapes androgen withdrawal symptoms by using a maintenance dose of androgens in between their cycles, the so-called blast-and-cruise strategy,[3,6] thereby increasing their exposure time to underground AAS.

There is little known about which factors determine whether a strength athlete will keep on using after an initial cycle. A single previous study has looked into this and reported an association between longer AAS use and higher age as well as marital status.[8] However, it is unknown whether taking part in bodybuilding competitions, side effects, or post-cycle testosterone concentrations levels play a role. Recognition of these factors is important as it could help focusing harm reduction strategies on athletes who run the most risk of suffering health hazards from androgen abuse. It is likely that repeated AAS use increases the risk of long-term health issues, such as the chronic disruption of testicular function[9,10] and cardiovascular disease.[11,12]

The HAARLEM study (acronym for Health risks of Anabolic Androgenic steRoid use by maLE aMateur athletes) is a prospective initiative to study health effects of AAS use. One hundred volunteer strength athletes were included and requested to adhere to a study protocol of performing a self-composed cycle of anabolic steroids during a one year follow-up period. The first year of the study was purely observational and there were no significant limitations for subjects as to how they performed their cycle. After the first year, subjects remained in follow-up for another year. Subjects decided themselves if, and how, they would use androgens again, so the natural course of AAS use would become apparent. Data from the second year could therefore be used to address the research question which factors are associated with longer AAS use and/or predict repeated AAS use.

METHODS

A detailed description of the methods of subject recruitment are described in a previous report.[5] To summarize, subjects included in the HAARLEM-study were men of at least 18 years old intending to

start an AAS cycle on short notice (i.e. within two weeks). Subjects were required to not have used androgens for at least three months prior to enrollment. Inclusion took place between October 2015 and April 2018, after the study was promoted on national television, regional newspapers and popular bodybuilding websites. The study was approved by the local Medical Ethical Committee and subjects provided written informed consent.

Clinic visit

At inclusion, subjects were requested to adhere to a protocol of one androgen cycle and to not use androgens for the remainder of the first year in order to assess recovery. There was no upper limit for cycle duration or dose. Subjects visited the study clinic four times in this year: before the start of the cycle (T_0), in the last week of the cycle (T_1), 3 months after the end of the cycle (T_2), and 1 year after inclusion (T_3). Fifteen subjects decided to continue using androgens after the presumed end of the cycle (T_1) or started a new cycle before the end of the year. During the last clinic visit (T_3), subjects were requested to return to the clinic one year later (T_4). In the second year, subjects could use androgens in the way they chose, which meant they could now decide not to use androgens at all, to perform multiple cycles, or use AAS continuously. Follow-up data were collected by telephone interview if the subject did not return to the clinic.

The clinic visit or telephone interview approximately two years after inclusion (T_4) included the documentation of characteristics of AAS use in the past year, i.e. duration and types of AAS used. Androgen dose used by subjects was not recorded in detail because subjects could not recall dose details precisely and, more importantly, label information of AAS products do not accurately reflect true androgen content.[5] The use of other performance and image-enhancing drugs (PIEDs) and recreational drugs was recorded.

Analysis

Simple descriptive statistics were used to display quantitative data. Outcomes of variables in the first follow-up year, e.g. results of psychological questionnaires and reported side effects, were compared between subjects that used AAS in the second year, i.e., repeat users, and those who did not. Differences between continuous variables were analyzed with the unpaired student's t-test and between dichotomous variables with the Chi-square test (χ^2) or Fisher's exact test.

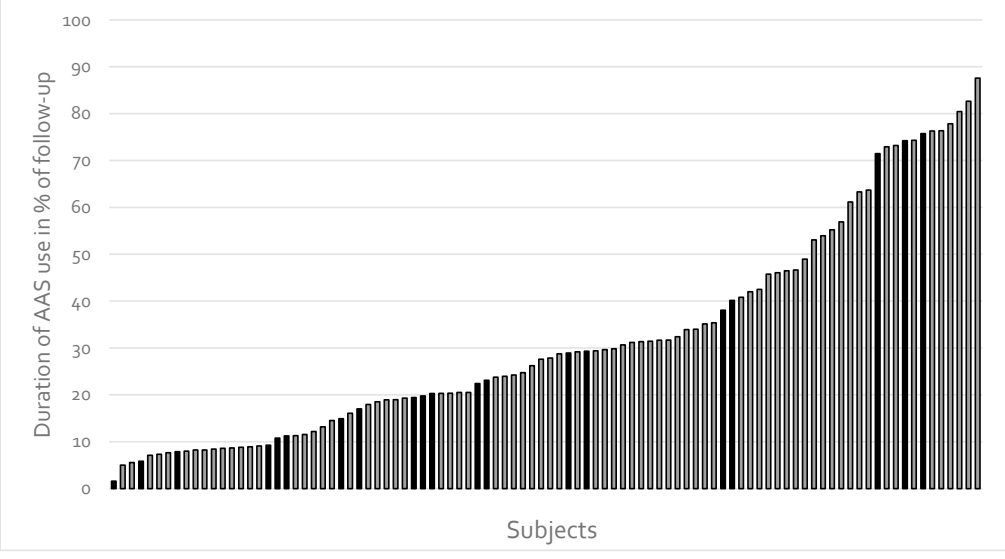
In addition, the research question was analyzed by using standard and stepwise multivariable regression models. In one model, the dependent variable was the total number of months of AAS use during total follow-up time (between the intake visit, T_0, and clinic visit after two year, T_4). The independent variables were sociodemographic factors (i.e., age, children, level of education, marital status, smoking, recent hard drug use, competitive bodybuilding, weekly training time, history of AAS use) and psychological factors (i.e. body dysmorphia, depression, wellbeing, quality of life, level of aggression, self-reported dependence of AAS) which were investigated with questionnaires during the intake visit, T_0. The details of these questionnaires and their results are described in another report.[13]

In two other models, the independent variables were characteristics of the cycle in the first year (i.e., weekly androgen dose, cycle duration) as well as outcomes of variables collected during the first year (i.e., reported side effects, laboratory results). The dependent variables of these two models were, respectively, whether a subject started a new cycle in the second year or not, and the number of months of AAS use in the second year of follow-up. The analysis was performed with Stata/SE 14.1 for Windows.

RESULTS

The disposition of subjects for the HAARLEM study is discussed in detail elsewhere [Smit, 2020]. Of the 100 men enrolled in the study, two were lost to follow-up in the first year. Another subject withdrew consent in the second year due to personal circumstances. Follow-up data were available from all other 97 subjects. Of these 97 subjects, 80 visited the clinic after the second year (T_4); the other 17 were interviewed via telephone. The mean follow-up time between the end of the first year (T_3) and the end of the second (T_4) was 12.7 months (range 9-19). Including the men who were lost to follow-up, the 100 subjects in the HAARLEM study cohort used AAS for a cumulative 68 years during 203 person years, of which 35 in the first year and 33 in the second year. *Figure 1* shows the percentage of follow-up time (both first and second year) that each subject used AAS.

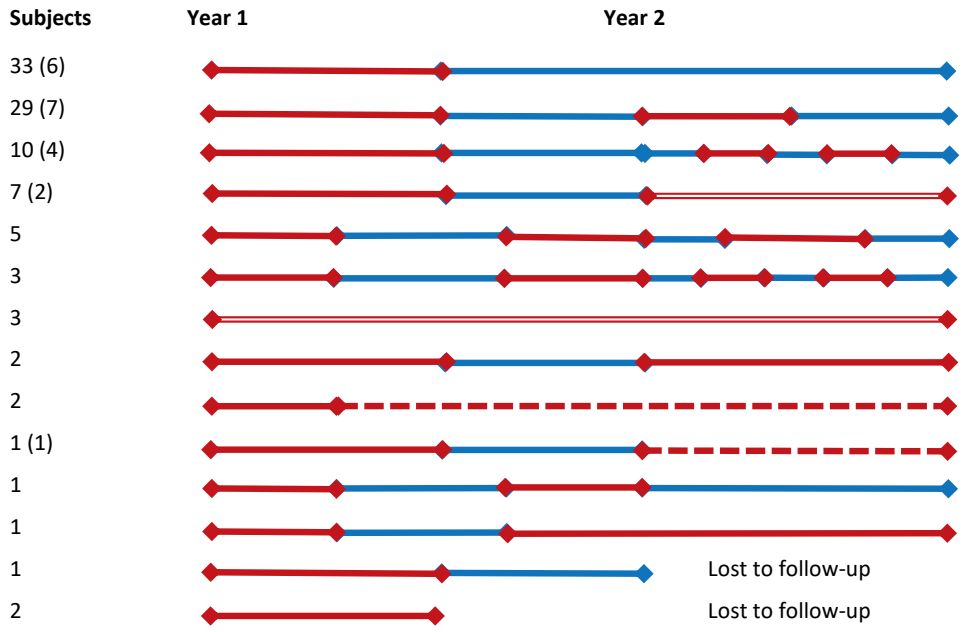
Figure 1. Waterfall plot showing the percentage of follow-up time (both first and second year of follow-up) each subject was using AAS. Black bars indicate subjects without a history of AAS use before inclusion in the cohort study.



Repeated AAS use

The course of AAS use by subjects during the entire two years of follow-up (from clinic visit T_0 to T_4) is shown in *Table 1*. Thirty-four subjects (35%) did not use AAS in the second year of follow-up. Of the 63 (65%) subjects who did use AAS again, 37 (38%) performed one cycle, of which 3 lasted the entire second year, and 13 (13%) performed two cycles. Ten (10%) followed a blast and cruise protocol, and the remaining three (3%) subjects self-initiated testosterone replacement therapy (TRT).

Table 1. Overview of androgen abuse by study subjects in the two year follow-up period. The number of subjects without a history of AAS use before inclusion in the cohort study is indicated between brackets.



Characteristics of AAS and PIED use by the 63 subjects who used AAS in the second year of follow-up are displayed in *Table 2*. Mean cycle duration was 16 weeks (range 4-65) and the mean number of different androgen types used was 3 (range 1-11). Mean cycle duration was longer in subjects performing one cycle compared to subjects performing multiple cycles (20 vs. 12 weeks). Forty-two (67%) subjects spent more time in a cycle in the second year compared to the first year. Post-cycle therapy was performed after 41 of the 60 cycles (68%) in the second year.

Predictors of AAS use

Key sociodemographic variables and characteristics of the cycle performed in the first year of both repeat users in the second year and subjects who would not use AAS again and are displayed in *Table*

3. At baseline, T_0, repeat users would significantly more often self-report that they are addicted to AAS than subjects who would not use AAS again (37% vs. 9%, P<.01). These users reported a lower well-being score (65 vs. 72, P=.03) than subjects who did not use AAS again. During the cycle, they reported an increase in well-being score, compared to a decrease in subjects who would not use AAS again (+1.1 vs. -6.6, P=.04). This group also reported a slightly higher level of physical (23 vs. 21, P=.02) and verbal (14 vs.13, P=.03) aggression during the cycle in the first year.

Table 2. Characteristics of AAS cycles in year 2 (n = 63). Data are derived from 37 subjects who performed one cycle, 13 subjects who performed 2 cycles, and 3 subjects who performed a non-stop cycle. For the AAS used, the 10 subjects who performed the blast and cruise strategy were also considered.

Mean cycle duration	16 weeks (range 4-65)
Mean number of AAS types	3 (range 1-11)
Duration cycle year 2 > year 1	42 (67%)
On cycle >50% of follow-up	32 (51%)
Post-cycle therapy	41/60 (68%)
AAS used	
- Testosterone	69/73 (95%)
- Trenbolone	30/73 (41%)
- Boldenone	19/73 (26%)
- Nandrolone	19/73 (26%)
- Drostanolone	17/73 (23%)
- Oxandrolone	16/73 (22%)
- Stanozolol	15/73 (21%)
- Methandienone	8/73 (11%)
Other PIEDs	
- Creatin	19/63 (30%)
- Growth hormone*	10/63 (16%)
- hCG	20/63 (of which 8 as PCT; 32%)
- Tamoxifen	20/63 (of which 12 as PCT; 32%)
- Clomiphene	12/63 (of which 8 as PCT; 19%)
- Anastrozol	8/63 (13%)
- Exemestane	7/63 (11%)
- Thyroid hormone	7/63 (11%)

- IGF-1	5/63 (8%)
- Insulin	5/63 (8%)
- Letrozol	5/63 (8%)
- Cabergoline	3/63 (5%)
- GHRP-2/-6	3/63 (5%)
- Ostarine	1/63 (2%)
- CJ-1295	1/63 (2%)
- PGF-2-alpha	1/63 (2%)
- Ipomorelin	1/63 (2%)
- Fulvestrant	1/63 (2%)

* Another 2 subjects used growth hormone in year 2 but did not use AAS.

Multivariable regression analysis showed a correlation between training time (β=0.03, P=.04) and taking part in bodybuilding competitions (β=3.0, P=.04), and the number of months of AAS use during the entire period of follow-up. Cycle duration in year one (β=$1.3e^{-2}$, P<.01) and training time at baseline (β=$7.5e^{-5}$, P<.01) were associated with repeated use in year two. Cycle duration in the first year was also positively associated with the number of months that AAS were used in the second year (β=0.19, P<.01). For all the other investigated baseline and cycle characteristics, no associations with future use were found.

DISCUSSION

The HAARLEM study is a prospective initiative investigating methods of AAS use and, to our knowledge, is the cohort study in this field with the longest follow-up. Because the study was promoted through (social) media and websites on a nationwide level, the cohort is believed to be representative of the general population of amateur athletes using androgens in the Netherlands. With only three men lost to follow-up in the course of two years, the study has an excellent adherence rate which increases its internal validity. Because subjects were allowed to use androgens in the way of their choice in the second year, the extended follow-up provided valuable insight into patterns and predictors of androgen abuse by athletes after a first cycle and serious adverse events.

Repeated AAS use

Sixty-five percent of subjects used androgens again in the second year. Of the repeat users, 25% used continuously throughout the extended follow-up period, mainly in a blast and cruise fashion, but also as TRT or a non-stop cycle. Interestingly, subjects without a past history of androgen abuse prior to enrollment used androgens in the same rate and patterns in the second year as their more experienced counterparts. The mean duration of AAS use increased from 18 weeks in the first year to 27 weeks in the second year, after excluding the subjects who did not use AAS again. The observed increase in

duration of AAS use was mainly due to the subset (16%) of continuous users, as mean cycle duration in subjects who only performed cycles was similar in the second year compared to the first year, i.e. 18 vs. 16 weeks, respectively.[5]

Table 3. Baseline characteristics of subjects arranged by patterns of AAS use.

Pattern of AAS use	**Repeat users (n = 63)**	**No repeat use (n=34)**
Age	31	34
Children	16 (25%)	9 (26%)
Married	9 (14%)	3 (9%)
Bachelor degree	21 (33%)	18 (53%)
Competitions	15 (24%)	4 (12%)
Recent hard drug use	36 (57%)	16 (47%)
History of AAS use (yes/no)	49 (78%)	28 (82%)
History of AAS use (months)	16	9
Weekly training time (min)	299	308
Cycle duration year 1 (weeks)	20	13
Self-reported addiction to AAS	23 (37%)	3 (9%)
Body dysmorphia*	11 (17%)	2 (6%)

* Score on the BDD-YBOCS (Yale-Brown Obsessive-Compulsive Scale Modified for Body Dysmorphic Disorder) questionnaire of ≥15 points

The difference in patterns of AAS use may indicate that a considerable group of athletes have the tendency to increase their use and perform longer cycles. However, it is hard to draw firm conclusions as the observed difference in AAS use between the first and second year may be explained by the request to adhere to a protocol of one cycle in the first year. Even though 15% of subjects had continued or restarted androgen abuse in the first year after their initial cycle despite this request, the second year probably better reflected true patterns of androgen abuse by amateur strength athletes.

Predictors of AAS use

It is important to define what characterizes the subset of athletes that uses AAS the most. These users do not necessarily have a much higher risk of short-term health problems but a longer duration of AAS use poses a larger burden on health and they probably accumulate the most risk for long-term health problems.

The most important predictor of future AAS use was cycle duration in the first year. This may portray the hesitancy experienced by subjects to terminate their cycle, for example due to the perceived

benefits of AAS use or fear for withdrawal symptoms. The strong tendency to continue AAS use experienced by some subjects was illustrated by the fact that two of the four subjects who suffered a serious adverse event in the first year decided to use androgens again in the second year. In other words, athletes continue AAS use even in the face of critical health effects. They often reason that specific androgen types, e.g. oral AAS or trenbolone, or other circumstances, e.g. a certain diet, had led to the side effect, and not the androgens per se.

Another factor associated with future AAS use was a high amount of weekly training time, probably because it signifies the devotion to strength sports and the willingness of an athlete to invest in the building up of muscle mass. Taking part in bodybuilding competitions was associated with longer AAS use (52 weeks vs. 31 weeks), presumably because losing muscle mass after quitting AAS would put these users in an unfavorable position towards their competitors.

Perhaps even more curiously, we did not find an association between positive and negative side effects reported during or after the cycle in the first year, so these did not appear to significantly affect the decision whether to continue AAS use in the future or not. Similarly, other factors such as education level, history of previous AAS use, hard drug use, and post-cycle testosterone concentrations were not associated with longer AAS use. The one exception was wellbeing, which was lower at baseline in subjects who would use again in the second year of follow-up, and also increased more significantly during AAS use. Perhaps it is a sign that athletes who experience a beneficial effect of AAS on their state of mind are more inclined to further their use.

Primary and secondary prevention

Obviously the best way to avoid harm caused by androgen abuse is to refrain amateur athletes from starting a cycle in the first place. As can be deduced from our data, once an athlete ventures into a cycle for the first time, there is little in the way of ongoing androgen abuse. Not even serious health hazards hold back users from continuing their quest for enhanced strength and muscle mass. Primary prevention focuses on, among others, making anabolic steroids less accessible and less exposure of young men to unnaturally muscular men in (social) media. It is unthinkable, however, that primary prevention measures would be as effective as to prevent strength athletes to engage in androgen abuse entirely.

This is why there should be more attention for secondary prevention or harm reduction strategies. The amount of evidence in this field is currently very limited.[14] With our data we can draw a distinction between two groups of users, each possibly benefiting from a different approach. On the one hand, there is a group of more recreative AAS users who do not take part in bodybuilding competitions, do not spend a great deal of time in the gym, and may have started AAS use in a more impulsive way. In a previous report outlining different typologies of AAS users,[15] these men would correspond to the well-being or YOLO type. They are probably receptive to expert advice and could be persuaded to use less AAS and focus more on a proper diet and better training methods.

On the other hand, there is a fanatic group of (near) professional bodybuilders that spend an excessive amount of time in the gym preparing for competitions. They often agree with the athlete or expert typology,[15] use AAS the most and the longest, and have the highest risk to run into health issues later in life. They are mostly indifferent about advice to use less, are already at the top of their game in terms of diet and training, and would rather have a doctor monitor their health and prescribe maintenance therapy or TRT instead of criticizing their AAS use. Upon confronting these users with the health risks, they reason it is not a short-term issue and that their passion is worth it. It is questionable whether any type of harm reduction is effective in this group of users. It may be more effective to change the bodybuilding culture, which has become strongly interrelated with AAS use, by appealing bodybuilding federations to improve their anti-doping code and uphold its compliance.

CONCLUSION

The HAARLEM study prospectively followed a cohort of 97 amateur strength athletes for two years. All subjects performed a cycle of AAS in the first year and two-thirds used AAS again in the second year. About 1 in 4 of the repeat users abused androgens for the entire second year. A longer first cycle duration, a higher amount of training time, and taking part in bodybuilding competitions were associated with longer and repeated AAS use. Symptoms or health events experienced during or after a cycle did not play a significant role. The exposure time to AAS in the sporting career of a strength athletes is likely to be related to long-term endocrine and cardiovascular morbidity, so their androgen abuse should be curtailed. As it is very difficult to intercept AAS use in abusers entirely, harm reduction strategies should be sought, counseling those athletes who are amenable to expert advice, and impelling the bodybuilding federations to ban the use of AAS.

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CHAPTER 11

ANABOLIC ANDROGENIC STEROIDS ABUSE IN YOUNG MALES

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Endocrine Connections
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ABSTRACT

This review summarizes 10 years experience with male abusers of anabolic androgenic steroids (AAS). The typical user of AAS is male, aged between 20 and 40 and lifting weights. Illegal AAS are cheap and easily obtained via internet or local suppliers. AAS are mostly used in cycles with a duration between 6 and 18 weeks. Most AAS cycles contain multiple agents, used simultaneously in a dose vastly exceeding a substitution dose. A variety of other performance and image-enhancing drugs are commonly used, including human growth hormone, thyroid hormone, tamoxifen, clomiphene citrate and human chorionic gonadotrophin. Short-term clinical and biochemical side effects are well established. Long-term side effects are uncertain, but may include heart failure, mood-and anxiety disorders, hypogonadism and subfertility. We share our views on the management of common health problems associated with AAS abuse.

Introduction

Every now and then a clinical endocrinologist will be visited by a patient that uses anabolic androgenic steroids (AAS) or has been using them in the past. The interaction between doctor and patient may be hampered for a number of reasons. First, some doctors may feel reluctant to help a patient who has self-inflicted health issues due to the use of banned substances. They do not understand why someone would jeopardize his health in order to gain muscle mass or strength and simply advise the patient to stop using steroids at once. Secondly, most doctors, including endocrinologists, do not have much experience with AAS abusers and do not have detailed knowledge of the different compounds and the adverse health effects they may inflict. This is partly due to the fact that there is limited scientific evidence about the health effects of AAS and hardly any evidence to guide treatment of side effects. Thirdly, most AAS abusers have low expectations concerning a doctors knowledge of AAS and may be reluctant to disclose details about their AAS abuse.[1] They frequently have strong opinions about laboratory results and treatment strategies, based on biased and incomplete knowledge.

These are the reasons why we started our AAS outpatient clinic almost 10 years ago in Haarlem, the Netherlands. Goals were to gain more insight into the characteristics of AAS users, the methods of AAS use and the health risks associated with AAS use. Users of AAS can be referred to us by their general practitioner or medical specialist if they want advice or treatment for health problems associated with current or past use of AAS. In the past years we have seen almost 400 patients. A summary of our findings in these patients can be found elsewhere.[2] The population seen in our outpatient clinic is selected on the basis of health problems and may not be representative for all steroid users in the Netherlands. Therefore, we started an observational cohort study in 100 AAS abusers without health problems, the HAARLEM study (acronym for Health risks of Anabolic Androgenic steRoid use by maLE aMateur athletes), of which the design and baseline characteristics have been published recently.[3] The results of 12 months follow-up are expected soon. Based on our experience, we discuss the management of steroid abuse and give treatment recommendations for the clinical endocrinologist.

What are AAS?

AAS comprise a group of compounds that are structurally similar to testosterone and have similar actions when administered in an appropriate dose. The term 'anabolic androgenic steroids' refers to the anabolic (muscle building) and androgenic (virilizing) effects of these compounds. The term has been criticized and deemed obsolete since all compounds included in this group bind and activate the androgen receptor, making them basically androgens, which, by definition, have muscle building and virilizing effects.[4] Injectable testosterone esters are among the AAS most used, but there is a wide variety of synthetic derivatives available. Testosterone, as most other AAS, undergoes extensive metabolism when administered orally. Therefore, some AAS have been alkylated to increase bioavailability after oral administration. However, decades ago, it became evident that 17-alkylated androgens are hepatotoxic and clinical application was largely abandoned.[5] Although most AAS abusers are well aware of this, oral AAS, such as methandienone (Dianabol), chlorodehydromethyltestosterone (Turinabol), oxandrolone (Anavar) and stanazolol (Winstrol), are still widely abused. To bypass first pass metabolism in the liver, AAS are injected. Without modification, steroids rapidly enter the blood, resulting in high peak levels and a very short plasma half-life. To

improve pharmacokinetics, a fatty acid chain is attached to the steroid. The longer the fatty acid, the slower the release from the injected depot.[6]

Testosterone can be converted to estradiol via the aromatase enzyme and converted to dihydrotestosterone (DHT) via the 5α-reductase enzymes. Administration of supraphysiological doses of testosterone thus results in increased levels of estradiol and DHT. Elevated estradiol levels are responsible for a number of side effects: they may stimulate breast glandular tissue and suppress endogenous LH and FSH production. Dihydrotestosterone, primarily produced in the skin, liver and prostate, due to the high local 5α-reductase activity, may lead to male pattern baldness and increased body hair. Although intraprostatic DHT levels are primarily derived from conversion of plasma precursors such as testosterone, local DHT concentrations appear largely unrelated to plasma levels of either DHT or its precursors, when present in physiological concentrations. As a result, there is no evidence that plasma androgen concentrations have any relevance with respect to development of prostate pathology.[7] If this also holds true when plasma androgen levels are highly supraphysiological, as can be encountered in AAS abusers, is unknown.

By modifying the testosterone molecule, aromatization or 5α-reduction can be prevented, attempting to improve the benefit-to-harm ratio of the compound. Although the majority of these steroids have not been extensively studied in humans, users and sellers claim relevant differences and suggest synergism between compounds. There should be no misconception that using anabolic steroids, in combination with an adequate diet and strength training, is very effective. Bhasin *et al*.[8] showed that administration of testosterone increases muscle mass and strength in healthy male volunteers and that the effects of testosterone are dose-dependent. Strength training enhances the effects of steroids significantly.

Who is using anabolic steroids and why are they using it?

The typical user of AAS is male, aged between 20 and 40 and engaged in weight lifting, bodybuilding, strongman competitions or martial arts, primarily kickboxing and mixed martial arts. A minority participates in competitions. Although most body building organizations have a drug free policy, drug tests are mostly not executed. As a result, using anabolic steroids among competitors is widespread and is a necessity to be competitive, especially at the elite levels. In our clinic, only 1% of AAS abusers was female.[2] More than 50% of users reported recent (<3 months) recreational drug use, such as ecstasy, amphetamines, cocaine and cannabis.[3]

It is not surprising that gaining muscle mass and strength are the most important motives to start AAS. Since the majority of AAS abusers is non-competitive, their AAS abuse appears to be internally motivated, such as the ambition to achieve a more ideal body, to reach a new level of performance or to improve self-esteem. Only 3% of our HAARLEM cohort reported that becoming more attractive was a reason to start using AAS. Although the internet is filled with drug-build physiques, only 5% of users admitted that this inspired them to use AAS.[3]

One-third of users have friends or relatives who are also using AAS. This indicates that potential users frequently seek the company and advice of other users. Thirty-two percent of subjects took the initiative to start using AAS entirely by themselves and usually had carried out extensive research beforehand. Almost half of AAS users consider themselves to be addicted to AAS, mainly due to the perceived positive effects on mind and body. Almost all users report positive effects when using AAS – more muscle mass, more strength, less fat mass, more energy and enhanced concentration. Although almost all users also report negative effects, these are mostly expected, mild and transient and do not outweigh the beneficial effects.[3]

What are the origin and quality of anabolic steroids?

In the Netherlands, as in most other European countries, anabolic steroids, such as testosterone, can only be obtained via a pharmacy with a doctor's prescription. Since most doctors are not willing to prescribe steroids for performance and image-enhancing purposes, most steroid abusers are dependent on illegal suppliers. It appears to be easy to obtain illegal anabolic steroids. On the internet, many websites market anabolic steroids and offer shipment all over the world. In our experience, most users of AAS use the internet as a source of information, but retrieve products via local dealers. Mostly, products are obtained via personal contacts in the gym. With some experience, abusers of anabolic steroids are easily spotted based on physical appearance. A short conversation with such a person in the gym usually suffices to retrieve information about the local supplier. Although selling and buying of AAS is prohibited in most countries, the chances of being caught are negligible.

Table 1. Percentage of participants of the HAARLEM study that used one of the below mentioned androgens during one cycle of anabolic steroids (based on label information).

Testosterone	96%
Trenbolone	52%
Drostanolon	39%
Boldenone	38%
Nandrolone	33%
Stanazolol	29%
Methandienone	25%
Oxandrolone	23%
Mesterolone	19%
Methenolone	17%
Oxymetholone	15%
Dehydrochlorotestosterone	2%

A cycle of anabolic steroids is not expensive. In our HAARLEM cohort, the mean cost per week was €30, adding up to €400 per cycle.[3] This means that cost is rarely a barrier to start using AAS. Due to their illegal nature, it is hard to find exact figures about the origin and quality of illegal steroids. Moreover, these figures may be different between countries, dependent on local legislation, infrastructure and activity of regional suppliers. Nowadays, steroid production and trafficking is a multimillion-euro international business, mostly in the hands of organized crime. Due to the unregulated production, the

quality of the illegal products may be poor. Therefore, users are at risk of overdosing and being exposed to other drugs than anticipated. Although exact figures are lacking, one may question the microbial safety of injectable products produced in illegal 'underground' labs.

For the HAARLEM study, we made a qualitative inventory of the products obtained by our 100 study participants (Table 1). Only about one half of analyzed AAS samples contained the AAS type as indicated on the label and more often than not the samples contained AAS types not indicated on the label. In a minority of products, no active ingredient was found.[3] We suspected that roughly 40% originated from Eastern Europe and Asia, whereas 60% appeared to be produced locally in illegal, so called underground laboratories. Underground labs are improvised labs hidden in cellars or warehouses where raw materials, mostly originating from Asia, are processed into tablets and injectable depots.

How are anabolic steroids used?

Although there is hardly any scientific evidence supporting the common practice of AAS abuse, most users have strong opinions about which type, dose and combination of AAS best suits their purpose. AAS cycles are rarely identical, not even for a single individual. Abusers tend to experiment, frequently escalating AAS dose and duration of use during their career. However, some common principles can be deduced when examining AAS cycles and questioning users.

Anabolic steroids are mostly used in cycles with a duration between 6 and 18 weeks. The unproven rationale behind this strategy is to gain muscle mass and strength during a cycle, allowing the body to recover between cycles. The contents, dose and duration of the cycles are mostly directed by advice from self-proclaimed experts and are based on unproven beliefs and personal experience. Since muscle mass and strength decline after discontinuation of AAS, multiple cycles or continuous use are deemed necessary to maintain or further increase gained muscle mass. In our cohort study, the mean weekly estimated androgen dose was almost 1000 mg, ranging between 250 and 3300 mg (3). This estimate does not accurately reflect the actual androgen exposure, due to the fact that it is based on the declared and not the actual concentration of the abused products. Moreover, it combines oral and injectable products and different types of androgens. However, it gives an indication that the weekly dose varies enormously between users and that the mean dose is highly supraphysiologic. For comparison, a normal substitution dose of an injectable testosterone-ester to treat male hypogonadism should not exceed 100 mg per week.[6]

Most AAS cycles contain multiple agents, used simultaneously, referred to as 'the stack'. A stack usually contains an injectable testosterone ester, mostly combined with nandrolone, trenbolone, drostanolone and/or boldenone esters. In first users or prudent individuals, cycles sometimes comprise only oral anabolic steroids, mostly a single agent, in a low to moderate daily dose (20–50 mg). More frequent, oral anabolic steroids are added to injectable ones, for instance, in the first few weeks of the cycle, referred to as a 'kick start'. Some have adopted the so-called 'blast and cruise' strategy, in which cycles with multiple high dose AAS are alternated with a lower maintenance dose, to prevent muscle loss in between cycles.

Among users, cycles can be characterized as 'bulking' or 'cutting' cycles. Bulking refers to the period in which an individual maintains a caloric surplus in combination with heavy weight training in order to maximize muscle growth. Testosterone, boldenone, nandrolone and methandienone are typically, but not exclusively, seen as bulk agents. Bulking invariably results in an increase of s.c. fat tissue, whereas for body builders, a very low body fat percentage is required to obtain a lean or 'shredded' look. A cutting phase aims to minimize body fat and maintain muscle mass as much as possible. Therefore, a relatively low caloric diet combined with weight and cardio training mostly follows the bulking phase. To prevent loss of muscle, the cutting phase is accompanied by a cutting cycle of anabolic steroids. Since estrogens are assumed to promote s.c. fat apposition, androgens in the cutting cycle should not be prone to aromatization. Therefore, trenbolone, stanozolole and drostanolone are typically regarded as cutting agents.

What is post cycle therapy?

Post cycle therapy, or PCT, is an unproven strategy that aims to restore endogenous testosterone production as soon as possible after a cycle of AAS. An inevitable side effect of AAS abuse is suppression of gonadotropin production, mostly to undetectable levels, and subsequent shutdown of testicular testosterone production. This will become clinically evident once exogenous androgen levels start to decay after the last pill or injection of the cycle. In the recovery phase, there may be a variable period of low plasma androgen levels. This may result in clinical signs of hypogonadism such as fatigue, loss of libido, erectile dysfunction and depressed mood. This period is particularly feared because it may result in loss of strength and muscle mass due to a lower anabolic state and less frequent and intense training.

The speed of recovery of endogenous testosterone production depends primarily on the type and dose of the anabolic steroids used in the last phase of the cycle. As stated before, anabolic steroids are mostly injected as an i.m. depot. Depending on the type of fatty acid chain attached to the steroid, the plasma half-life after injection may be weeks or months.[6] Taking into consideration that the administered doses are many times the natural endogenous production, it may take many weeks before exogenous androgen levels are low enough to allow endogenous testosterone production to ignite (9). Estrogens, although present in much smaller concentrations compared to androgens, have strong suppressive effects on gonadotrophin production.[10] Anti-estrogens, such as tamoxifen and clomiphene citrate, have been shown to stimulate gonadotrophin and testosterone production moderately in eugonadal men.[11] Consequently, these substances are frequently used as PCT, based on the unproven assumption that they will speed up recovery of the male hypothalamic-pituitary-gonadal axis.

Human chorionic gonadotropin (hCG) is frequently used to start or maintain spermatogenesis and endogenous testosterone production. Although it is effective to stimulate gonadal function, it does not stimulate gonadotrophin production. It may actually delay recovery of gonadotrophin production by artificially increasing plasma testosterone levels and thereby prolonging the underlying hypothalamic suppression of reproductive function.

What else do they use?

Although anabolic steroids are by far the most abused drugs, a variety of other performance and image-enhancing drugs are commonly used.[2,3] These can be categorized as muscle builders, fat burners, pre-workout agents and agents to prevent or treat side effects. Depending on the nature and dose of these agents, additional adverse health effects can be expected. Similar to AAS, most of these products are illegally obtained and their quality should be questioned. Moreover, scientific evidence supporting the claimed effects is mostly absent.

Human growth hormone is typically used as a muscle builder in addition to anabolic steroids. Insulin is used during a bulking phase to facilitate weight gain. Selective Androgen Receptor Modulators (SARMs) are viewed by illicit sellers and users as 'boutique' agents, claiming a better ratio of muscle growth vs side effects. Although this claim is far from being supported by clinical trials, SARMs are promoted as a safer, albeit more expensive, alternative to steroidal androgens. Thyroid hormone, clenbuterol and dinitrophenol (DNP) may be used in the cutting phase to reduce s.c. fat. DNP raises the basal metabolic rate and may result in life threatening hyperthermia.[12] Pre-workout agents are basically stimulants such as caffeine, clenbuterol and ephedrine to improve training intensity. As stated previously, tamoxifen, clomiphene and hCG are frequently used to speed up recovery of gonadal function after a cycle of anabolic steroids. Tamoxifen or aromatase inhibitors may also be used to prevent or treat gynaecomastia. Isotretinoine is sometimes used to treat acne. Sildenafil or other phosphodiesterase inhibitors are used to improve erectile function. Diuretics are typically used by competitive bodybuilders days before a contest to reduce body water and improve muscle definition.

What are side effects of AAS and when should I suspect AAS abuse?

All users of anabolic steroids, assuming a significant exposure, have side effects, although the majority of these side effects is mild and transient and some go unnoticed by the abuser. Some of these effects are sensitive indicators of androgen abuse and can be used to confront the patient if he is unwilling to disclose.

Suppression of gonadotrophins is a very sensitive finding, even with relatively low doses and short exposure. Plasma testosterone and estradiol levels may be high or low, depending on the type of androgen abused. Spermatogenesis is usually reduced following suppression of gonadotrophins and may take months to recover. Testicular volume decreases during abuse. Sex Hormone Binding Globulin and High Density Lipoprotein-cholesterol are liver derived-markers of androgen use and both are markedly suppressed during and weeks after exposure. Erythropoiesis is stimulated by androgens resulting in a mild rise in hematocrit, which may be exaggerated by smoking, sleep apnea or the use of diuretics. Androgens stimulate sebum production and therefore androgen abuse is frequently associated with oily skin and acne. Androgens affect mood and fluctuating androgen levels may result in mood swings and agitation, particularly in vulnerable individuals. Due to disruption of the estrogen-androgen balance, breast tenderness or gynecomastia is frequently reported. The use of oral anabolic steroids is associated with liver toxicity; however, the mild elevation of ALT and AST frequently encountered in steroid abusers mostly reflects muscle damage that results from intensive weight training. Improper injection hygiene or contaminated steroids may give rise to local inflammation or

infection. The increased muscle mass and strength that results from AAS abuse may result in injuries such as tendon rupture, lumbar hernia and overloaded joints.[13]

The long-term side effects are less well defined. Randomized controlled trials are ethically unrealizable, large prospective studies are unavailable and case-control studies and case series have methodological shortcomings. Several reports indicate that anabolic steroid abuse is associated with cardiac disease, ranging from diastolic dysfunction, overt heart failure to sudden cardiac death.[14] Also, AAS abuse is associated with mood and anxiety disorders.[15] In a retrospective study, men who tested positive for AAS in fitness centers had a higher mortality risk compared to matched controls.[16] It is unclear whether this can be causally attributed to anabolic steroids abuse. It has been shown that users of anabolic steroids are more prone to a hazardous lifestyle. For instance, in a Swedish study approximately 40% of deaths among those who had tested positive for AAS were homicide or suicide, compared with 14% among those who tested negative.[14]

Some studies indicate that AAS abuse may have detrimental effects that persist long after AAS abuse has ended or may even be permanent. A case-control study, comparing past abusers with healthy controls, showed significantly lower testosterone and gonadotrophin levels in past users up to 3.7 years after stopping AAS.[17] We and others[2,18] have reported on individuals with persistently symptomatic low testosterone levels more than 6 months after stopping anabolic steroids. We observed that individuals that had used a high cumulative dose of anabolic steroids in the past appeared to be more prone to post-exposure hypogonadism; however, such an association was not found in the aforementioned case-control study. Unfortunately, pre-AAS testosterone levels in these individuals are mostly unavailable, therefore a causal relationship between steroid abuse and persistent gonadal dysfunction cannot be established in most cases. A recent case-control study suggested complete restoration of gonadal function in all 31 past users, albeit this may take years after stopping anabolic steroids.[19] To date, it cannot be excluded that irreversible damage to gonadal function can be a result of AAS abuse. Additional, larger, prospective studies are necessary to clarify this topic.

What to do?

Treating (past) steroid abusers can be complicated for several reasons. As outlined previously, there is a lack of knowledge about adverse health effects of anabolic steroid abuse, especially concerning the long-term effects. There is uncertainty about the actual contents of the abused products and there is large variability in dose, duration and type of abused substances.

It is the policy of our clinic not to offer routine health and blood checks to active users *without* health problems. Although these checks may be part of a harm-reduction strategy, we have concerns that it may invite potential users to start using AAS and convince current users to keep on using or even start experimenting as long as the health checks indicate no (serious) harm. For similar reasons, we do not prescribe anabolic steroids for performance or image enhancing purposes.

Managing health problems in active users asks for a strategy of harm reduction. The problem is that the harms for the individual patient are hard to predict and there are no evidence-based harm-reduction strategies. Doctors may experience moral, ethical, legal or practical barriers, making them feel uncomfortable to check and treat patients who are continuously jeopardizing their health for a cause they cannot relate to. Whenever healthcare is provided to active AAS abusers, we advise to contemplate on these issues and to devise an individually tailored protocol, describing very clearly the type and extent of the care we are willing to offer.

Treating health problems in *past* users is less complicated. In our clinic, the reasons to visit were mostly related to symptoms indicating disrupted gonadal function, such as loss of libido, erectile dysfunction, low energy, depressed mood, subfertility and gynecomastia. As long as the patient refrains from anabolic steroids, we treat him according to applicable guidelines. The advice we will give subsequently is based on our experience with AAS abusers over the past 10 years who presented with health problems. Please note that it is expert-based and hardly evidence-based.

A careful history should be taken addressing prior use of AAS, including number of cycles, cycle length and weekly AAS dose. We also advise to routinely check for clinical signs that may indicate pre-AAS gonadal (dys)function, such as cryptorchidism, gynecomastia and infertility. We also check for recent use of recreational drugs, smoking and alcohol intake. Abuse of other drugs may contribute to health problems but may also indicate an addictive personality. We inquire about training frequency, type of training and diet. If these are suboptimal, potential users of anabolic steroids should be encouraged to consult a certified trainer or sports nutritionist before considering further use of anabolic steroids.

We also ask for side effects during or after previous cycles of anabolic steroids. We believe it is important to address the patients goals and the motives for anabolic steroid abuse. As stated previously, only a minority of users take part in competitions and thus their goals are self-constructed and their motives largely internal. In our experience, a lot of AAS users do not have clear goals for their use, apart from being ‘as big as possible’.

A considerable number of (former) AAS abusers seeking help have mental problems. Havnes *et al*. showed that mental problems such as depression, anxiety, behavioral change and AAS dependence are reported even more frequently than physical problems.[20] By the patient, these symptoms are mostly attributed to hormonal disturbances such as testosterone deficiency, but this may not always be the case. It may be quite revealing to ask why a muscular physique is so important for the patient and why he is even willing to use drugs for it. It may be of help to explore whether there is a low self-esteem or a distorted self-image that needs to be addressed. Lastly, we ask the patient if they have ever reflected on the possible health consequences of their abuse, now and in the future.

Based on this information, we try to discriminate between high-risk and low-risk abusers. Characteristics of high-risk or problematic abuse are escalating steroid use (longer cycles, experimenting with different steroids and higher doses over time), continuous use (’blast and cruise’), addictive behavior, impulsive behavior (e.g. starting a cycle without proper consideration), unrealistic,

ill-defined or absent goals, being overly concerned with body appearance (body dysmorphic traits) and having the tendency to treat AAS associated health problems with drugs instead of stopping the causative agent. On the basis of this assessment, we reflect with the patient on the potential risks of his current behavior and try to find out if and to what extent the patient is willing to change. If the patient is cooperative, we make a diagnostic and treatment plan. Mostly, blood or semen analysis is indicated and a psychologist or addiction specialist is consulted.

The wish to stop using anabolic steroids

If an AAS user wants to stop using steroids permanently, it may be helpful to withdraw from the 'steroid environment', such as the hard-core gym or the steroid using training partners.

We indicate that successful stopping is only possible if the user can accept a loss in muscle mass and strength. Furthermore, the user should be able to endure a period of weeks or several months with symptoms of testosterone deficiency. If the patient has not experienced severe withdrawal symptoms after discontinuation of AAS in the past and does not appear to have social, psychological or somatic issues that impede the patients capacity to cope with symptoms of withdrawal, we advise to stop steroids abruptly. Abusers mostly use injectable depots of anabolic steroids with a half-life up to 2 weeks in highly supraphysiological doses which will cause a gradual decay of the plasma testosterone concentration to subnormal levels. In other cases gradual tapering of steroids may be warranted. In committed individuals, we consider prescribing testosterone, aiming to maintain plasma testosterone levels in the high-normal range for a limited amount of time – needed to address issues that interfere with steroid tapering, such as substance abuse, mood disorders or signs of body dysmorphic disorder. Before prescribing testosterone, we make detailed agreements about testosterone dose, goals of therapy and adherence to the treatment plan. We stop testosterone prescription if the patient violates these agreements or starts using steroids again. Further tapering of testosterone dose should be individualized based on evaluation of treatment goals.

Symptoms of testosterone deficiency after stopping anabolic steroids

If the last administration of AAS has been within 3 months of presentation, provided that the symptoms are well tolerated, we advise to wait for spontaneous restoration of gonadal function. Checking blood is mostly not very helpful at this stage, knowing that levels are probably disturbed by the recent use of AAS. As explained previously, it may take weeks or months until exogenous androgen levels have decayed low enough for the Hypothalamo-Pituitary Gonad (HPG) axis to restart. Oral anabolic steroids have a much shorter half-life and recovery of the HPG axis is expected much faster.

If the patient plans a steroid cycle in the near future, checking blood may not be useful since the levels will be disrupted shortly afterwards. If symptoms persist for more than 3 months after the last injection, testing for plasma testosterone and gonadotrophins is warranted. Typically, the recovery phase after recent AAS use is characterized by low gonadotrophins, low testosterone and low SHBG levels. Three months after the last injection, at least partial recovery of the HPG axis is expected. If levels are still very low, the patient should be questioned about undisclosed steroid use in the weeks prior to blood testing. If this is denied, other, steroid unrelated causes of hypogonadotropic

hypogonadism should be explored. In the unlikely event of finding elevated gonadotropins and low testosterone levels, the patient should be questioned about current use of aromatase inhibitors or selective oestrogen receptor modulators. If this is denied, other, steroid unrelated causes of gonadal dysfunction should be explored.

If testosterone levels are not very low and symptoms are well tolerated, waiting for spontaneous recovery of the HPG axis is advised. If symptoms are not well tolerated, endogenous testosterone production may be stimulated by prescribing tamoxifen 20 mg once daily or clomiphene 50 mg once daily for several weeks. Both drugs mildly stimulate gonadotropin and testosterone production and do not suppress spermatogenesis. Testosterone substitution should be withheld as long as possible, since it interferes with HPG axis recovery, and prescribed only if no further recovery of the HPG is expected.

Although selective estrogen receptor modulators (SERMs), such as tamoxifen and clomiphene, have a potential advantage over testosterone substitution, some caveats need to be considered. SERMs act as an estrogen or an anti-estrogen, depending on the exposed tissue. The effects of SERMs have not been studied extensively in men and long-term effects are unknown. In men, aromatization of testosterone to estradiol is vital to reach and maintain bone mass, and the long-term effects of SERM administration on bone health in hypo- or eugonadal men have not been established. Also, there is evidence that sexual function in men depends on the combined effects of androgens and oestrogens.[21] Although detrimental effects of SERMs on sexual function in men have not been reported in small and short-term studies,[22] these effects cannot be excluded.

Finally, in postmenopausal women, SERMs have been shown to be mildly thrombogenic and similar, albeit, milder effects on coagulation parameters have been reported in men.[23] Eventually, if other causes of hypogonadism have been explored, testosterone levels remain unequivocally low and there is no desire to have children, testosterone substitution can be started under the same agreements as stated previously. If necessary, we prefer testosterone gel in the lowest effective dose. Testosterone gel results in fairly stable testosterone levels,[24] does not suppress gonadotropin levels as much as most injectables, is unpopular for misuse among steroid users and can be easily tapered in weeks or months.

Fertility

Anabolic steroid abuse inherently results in suppression of spermatogenesis. In our experience, normalization of sperm count lags behind normalization of plasma testosterone concentrations. Therefore, a wait-and-see policy is justified as a first step, that is, semen analysis should not be done within the first 6 months after stopping anabolic steroids. If the sperm count is severely compromised 6 months after the last injection and the patient denies AAS use in the last months, blood needs to be tested to check gonadotrophin and testosterone levels. As stated previously, the recovery phase after recent AAS use is characterized by low gonadotrophins, low testosterone and low SHBG levels. If gonadotrophin levels are elevated, especially if FSH is disproportionally elevated compared to LH, primary gonadal dysfunction, unrelated to steroid abuse, should be suspected. If gonadotrophins, testosterone levels and testicular volume are normal, obstructive azoo- or oligospermia should be suspected. If gonadotrophins and testosterone levels are low, and other causes of hypogonadotropic hypogonadism are explored and rejected, the wait-and-see policy should be continued for 6 more

months. If hypogonadotropic hypogonadism and oligospermia persist, spermatogenesis and endogenous testosterone production can be stimulated by administration of hCG 1500 IU two to three times weekly. Assuming that spermatogenesis has been normal in the pre-AAS period, hCG is effective in restoring spermatogenesis and endogenous testosterone levels to normal levels.[25]

Gynecomastia

Gynecomastia or breast tenderness is a common side effect of AAS abuse. It results from distortion of the androgen-estrogen balance during or after administration of AAS. Several AAS, including testosterone, can be aromatized to estrogens. As a result, finding supraphysiological estradiol levels in AAS abusers is not unusual. Aromatase inhibitors and anti-estrogens such as tamoxifen and clomiphene are frequently used by AAS abusers, either as PCT or to treat or prevent gynecomastia. Although symptoms are frequently transient, gynecomastia may persist after stopping anabolic steroids. After ruling out other causes, persistent breast tenderness and gynecomastia can effectively be treated with a trial of tamoxifen 20 mg once daily for several weeks.[25] In our experience there is a high chance of recurrence after stopping tamoxifen, especially if a new cycle of anabolic steroids is started. For recurrent or persistent gynecomastia, surgical treatment should be considered.

Concluding remarks

AAS abuse has been called 'a hidden epidemic'.[26] Over the past 40 years, the use of AAS has spread from use for performance enhancement by a relatively small group of elite athletes to widespread use among young men to obtain a more muscular physique. As described previously, AAS are easily obtained, cheap, of bad quality and used in huge quantities. All users experience side effects, a considerable percentage of users suffer long-term health problems after stopping and some long-term effects may not even be recognized. Among medical professionals there is a lack of knowledge to recognize and treat the problems associated with AAS abuse.

Due to its history in competitive sports, AAS abuse has been associated with cheating and foul play. Over the years, sport organizations and anti-doping institutions have emphasized, and perhaps exaggerated, the adverse effects of AAS to prevent athletes from taking them. This may explain why AAS abuse is frowned upon by the general public. As a result, most AAS users are reluctant to disclose the misuse of androgens. Knowing the numbers of users and the potential hazards associated with abuse, it is surprising that this topic has been largely neglected by the scientific community.

It is a challenge to manage the patient who uses AAS. More clinical studies are needed to fill the major gaps in knowledge regarding long-term side effects. Also, studies should be undertaken focusing on primary and secondary prevention and effective harm-reduction strategies. The results of these studies should be used to educate doctors on how to prevent and recognize these side effects, to treat patients without prejudice and to convince politicians that adequate measures should be taken to confine androgen abuse.

We believe that the endocrine community has a pivotal role in both research and treatment. Due to the controversial nature of AAS abuse and its medical management, it is the responsibility of national

and international endocrine societies to give some guidance. Therefore, we strongly recommend management guidelines to support the individual endocrinologist.

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GENERAL DISCUSSION

The use of AAS for the purpose of increasing strength and enhancing performance has transitioned from a small group of elite sportsmen in the previous century to a large group of amateur athletes visiting gyms nowadays. In 2009 there was an estimated 20.000 users of AAS in the Netherlands.[1] To put things in perspective, this number is similar to the amount of patients suffering from HIV or schizophrenia, each separately.[2] Despite this, and unlike for these illnesses, there is no broad understanding of the reasons, adverse effects or treatment of AAS use. This is mainly due to unfamiliarity of the topic among health professionals and ethical barriers for conducting scientific research. Current data in literature is therefore mainly derived from expert opinion, case series, retrospective and cross-sectional studies.

This thesis adds to the understanding of harm caused by AAS use by pooling data obtained from the outpatient AAS clinic in the Spaarne Gasthuis in Haarlem and a prospective cohort study carried out in this clinic, the HAARLEM study. Together they provide clinical and empirical data on different aspects of harm caused by AAS use. The study takes precedence to much of the existing literature in this field of research due to its prospective design and relatively large size. As such, the thesis lays an important foundation for the appropriate information of athletes who consider, or already use, AAS and paves the way for the development of preventative measures. This discussion section will elaborate on, summarize, and discuss the most important findings of the chapters in this thesis, and answer the research questions raised in the general introduction.

Outpatient AAS clinic

To recapitulate, the outpatient AAS clinic is an ambulatory care facility to which (former) users of AAS can be referred by their general practitioner or a medical specialist when experiencing health complaints that are – presumably – related to previous AAS use. After a medical assessment they receive an opinion from an internist with an expertise in AAS and, if necessary, treatment is initiated. This way the clinic has tapped into the world of, and medical problems experienced by, AAS users and also provided a means of diagnosing and treating them. In **CHAPTER 1** all patients evaluated in the clinic between 2011 and 2016 are summarized.

Patients were most often referred to the clinic because of symptoms occurring during or after an AAS cycle, such as a decreased libido or erectile dysfunction. Most patients were male, amateur strength athletes, who started using AAS in the third decade of life. Cycles were commonly between 6 and 18 weeks duration and contained two or more different AAS types. In addition, patients had used other PIEDs, such as growth hormone or clenbuterol, as well as medications to self-treat side effects, such as aromatase inhibitors. A typical user performed a mean number of 3 cycles before visiting the clinic and 1 in 5 users at some point had used AAS uninterruptedly for more than 1 year. There was a high number of patients reporting hard drug use, such as XTC, cocaine of GHB. Seventy-one percent of patients reported to always perform PCT after a cycle, with compounds such as tamoxifen and hCG.

All patients in the report documented one or more side effects related to the use of AAS, among others acne, gynecomastia and agitation. Remarkably, patients deemed side effects as mild and temporary, and acceptable with respect to the perceived increase in muscle size and strength. It is when these complaints persisted after discontinuation of the AAS that they became a reason for referral. In some cases this was due to – a newly established – post-AAS-hypogonadism, which was associated with a longer history of AAS use.

The summary of the first 5 years of the outpatient AAS clinic was insightful but had a few caveats. The report was retrospective in nature and the data were incomplete on many items and subject to recall bias and reporting bias. The group of patients represented a minority of the total group of AAS users and was a selection of people who were (self-)referred because of side effects or problematic AAS use. It did not include patients without health issues or, contrarily, life-threatening health problems due to AAS use. This is illustrated in **CHAPTER 2** and **CHAPTER 3** where patients ended up in a hospital with rare but critical medical issues that had resulted from escalating AAS and PIED use. They would normally not be patients of the AAS clinic but came to its attention by chance. The HAARLEM study with its prospective design and systematic approach did not have many of these biases and generated scientifically more reliable data regarding adverse events caused by AAS use.

Baseline characteristics of the HAARLEM study

As is outlined in **CHAPTER 4**, study subjects for the HAARLEM study were recruited by presenting the study concept on national television, in regional newspapers, and online discussion forums. Inclusion criteria entailed the male gender, a minimum age of 18 years, and the intention to start a cycle on short notice, i.e. within 2 weeks. The cycle had to have an average weekly androgen dose of 200 mg and a minimum duration of 6 weeks. The promotion measures combined with the strength of the AAS clinic reputation and word of mouth brought in 220 men in the course of two years of whom 111 were eligible for inclusion. Non-eligibility was mainly based on men having started a cycle already or not planning to initiate one any time soon. A probability sampling method, such as multistage cluster sampling, would have eliminated most of the selection bias. The chosen method, however, which could be considered a combination of convenience and snowball sampling, was preferred as it was the most practical, efficient, and effective in reaching a population of AAS users that was hard to identify.

Characteristics of the included subjects, all being strength athletes, most of them educated and employed, and frequently using hard drugs, showed great similarities with the group of patients interviewed in the outpatient AAS clinic and surveys from previous literature.[3,4] It must be clear that users of AAS are fanatic sportsmen that dedicate much of their time to hearty training, averaging over 6 hours per week in the gym. Eighty-six of the 111 initially included subjects of the HAARLEM study had past experience with AAS, meaning 25 of the subjects were new users of AAS. Reasons for subjects to use AAS were mainly intrinsically driven, albeit diverse and more than a straightforward desire to increase muscle mass an strength. For a subset of users, using AAS appeared to be a sine qua non for being a worthy contender in bodybuilding competitions. Contrary to popular belief, subjects rarely indicated that they used AAS specifically to be found more attractive.

After 11 subjects did not return to the clinic after their baseline visit because they had not started a cycle or had withdrawn consent – not for medical reasons, 100 remained in follow-up. The typical cycle performed by these subjects was 13 weeks in duration, contained 4 different AAS types, and the median dose of AAS equivalents was 901 mg per week. Subjects with a prior experience in AAS or taking part in bodybuilding competitions had a tendency to perform longer, heavier cycles. A subject had spent around €400 to retrieve the products from his dealer, in most cases, or the internet. The backbone of almost every cycle was an injectable testosterone ester. Apart from this, each subject followed a different regimen, where – presumed to be – complementary AAS types, mostly trenbolone, drostanolone and boldenone, were combined with other PIEDs, such as growth hormone, clenbuterol or thyroid hormone. PCT was employed by 80 subjects and constituted of a combination of 2 agents, mostly tamoxifen, clomiphene citrate and/or hCG, for period of 4 weeks.

As expected, the quality of the illicitly obtained AAS products was very low. This was shown by running a qualitative analysis with UPLC-QTOF-MS/MS on the samples provided by the first 55 enrolled subjects. In concordance with earlier reports,[7,8] the found constituents poorly concurred with the AAS types declared on the label information. In a mere 13% of the products the sample contained the declared AAS only. In all other cases the sample contained only undeclared AAS (47%), a mixture of declared and undeclared AAS (35%), or no active ingredients at all (6%). In particular, none of the 46 different AAS brands showed consistent results. Contamination with other harmful compounds appeared negligible, although in 14% of samples estrogens or progestogens were also detected. The results underline the vacuity of online information regarding AAS products, as well as the pointlessness of carefully assembling a cycle with different AAS types based on the presumed properties of specific AAS types, as true constituents of the acquired AAS can never be accurately predicted.

Of the 100 initially included subjects, 98 completed follow-up with a median duration of 12 months. Two subjects were lost to follow-up because they withdrew consent and emigrated, respectively. The low drop-out rate was a strength of the study and greatly enhanced its internal validity. It also shows that subjects attached value to being enrolled. Although the study was purely observational, it may have created a sense of safety for subjects to have medical check-ups during and after AAS use. It could imply that the cohort was assembled of wary individuals who dealt with their health gingerly, and may therefore also use AAS in a more restrained manner. This effect was partly mitigated by disclosing results to subjects no sooner than after completion of the cycle, but some selection bias cannot be ruled out.

During follow-up, health analysis took place at the beginning and at the end of the cycle, as well as 3 months after the cycle and 1 year after enrollment. The purpose was to first assess the extent of health effects caused by AAS use between the baseline and the second visit, and to analyze the reversibility of those effects in the last two visits after a withdrawal period. In order to stick with this scheme, subjects were requested to perform just one cycle in the entire year. Nonetheless, fifteen subjects decided to use AAS throughout, or start a second cycle before the end of, the follow-up period. Their data could not be used to assess recovery but it surely characterizes the willfulness of some AAS users. Eventually, the follow-up period was extended, and all subjects were invited for a fifth and last clinic visit 24 months after enrollment. In the second year, subjects could use AAS in the way of their choice.

Serious adverse events

The number of serious adverse events during the HAARLEM study cohort total two year follow-up period was limited with only 4 cases in 203 person years, hence 1 per 51 person years. If only counting the time when subjects were actually performing a cycle, this is 1 in 17 years. The events, described in more detail in **CHAPTER 5** and also touched upon in **CHAPTER 10**, concerned heart failure, acute pancreatitis, exacerbation of ulcerative colitis, and suicidal ideation. The latter was strictly speaking not a serious adverse event but counted anyway due to gravity of the event. It is not possible to draw conclusions regarding causality between these events and AAS use. The strongest relationship is expected for the case with heart failure, as this event aligns with the known cardiac effects of AAS, also discussed in **CHAPTER 9**, and has been documented in literature before.[9-11]

Taking the pooled data of this thesis into account, there seems to be a proclivity for the occurrence of serious adverse events in individuals predisposed to certain conditions. This is illustrated by the case with heart failure, where cardiac decompensation probably occurred on a background of a hereditary hypertrophic cardiomyopathy, but also by the subject with suicidal ideation, who was previously known with a chronic mood disorder and was even institutionalized several years before, independent of any PIED use. It could be postulated that AAS use can unhinge a chronic illness or condition, in some cases one that was not disclosed previously. Serious adverse events which are directly and solely the result of the short term toxicity of AAS use are probably very rare.

Positive and negative side effects

From **CHAPTER 5** it becomes clear that, upon questioning subjects about positive and negative side effects, for most there was a favorable risk-benefit balance during an AAS cycle, at least on the short term. That is, positive effects occurred in every user, not only an increased muscle mass (95%) and strength (100%), but also an increase in energy (45%) and an improved concentration (29%). These effects contrasted with negative effects such as fluid retention (56%), painful injection sites (20%) and diaphoresis (17%). These effects were generally considered mild and transitory. Indeed, only 13% of subjects stopped or altered the AAS cycle due to side effects. Furthermore, physical examination at the end of the cycle revealed acne (13%) and gynecomastia (19%) in a subset of participants, but also was mild in most cases. After the cycle, positive effects quickly disappeared, and many subjects experienced a 'steroid dip', mainly consisting of a decreased libido (59%), sometimes even erectile dysfunction (14%). This combination of events forms an important motive for users to start a new AAS cycle or to perform a 'bridge' or maintenance dose of AAS between cycles.

According to literature, AAS exposure is associated with irritability, aggression, manic or hypomanic behavior and even psychosis, whereas withdrawal of AAS is linked to depression and anhedonia.[12] Nevertheless, findings from the HAARLEM study indicated that there was no significant disruption of psychological health in the vast majority of users. Questionnaires on depressive symptoms, quality of life, and wellbeing, did not show any relevant change during and after the cycle. Subjects did self-report a higher level of agitation and overall aggression, but there was no rise in physical aggression according to the Buss & Perry Aggression Questionnaire. The data pointed out, however, that body dysmorphic disorder, diagnosed with the BDD-YBOCS questionnaire, was a considerable comorbidity

in the cohort with a prevalence of 32%. The crux of this condition, being the disengagement of body image from true muscularity, was illustrated by the fact that the degree of body dysmorphic disorder did not change during the cycle despite the indisputable boost in brawn.

Kidney and liver toxicity

The HAARLEM study could not demonstrate that high doses of AAS lead to kidney damage on the short term.[13] In **CHAPTER 5** results describe a small and reversible change of creatine concentrations during an AAS cycle. This may be attributed to an increase in muscle mass, a high protein diet, and in some cases to the use of creatine ethyl ester supplements.[14] There are several indicators, however, that AAS users may be at risk for chronic kidney damage. Urine dipstick analysis showed an increased rate of microalbuminuria which may indicate glomerular toxicity. The observed rise in blood pressure may increase the risk for nephrosclerosis. Other clinical studies have highlighted different pathways through which AAS use may lead to forms of chronic kidney damage, for example focal segmental glomerular sclerosis, the potentiation of the renin-angiotensin-aldosterone system, enhancement endothelin production, and induction of inflammatory cytokines and profibrotic mediators.[15] Current clinical data do not convincingly nominate the use of AAS as a cause for kidney damage, but definite interpretations cannot be made without long-term prospective data.

Similar to kidney damage, the occurrence of (sub)acute liver damage is a rare finding, despite the widespread use of oral AAS compounds. Hepatotoxicity has been reported as, among others, acute cholestatic syndromes, chronic vascular injury and fatty liver disease.[16] **CHAPTER 2** describes a bodybuilder with a spontaneous hemorrhage in a hepatic adenoma. This liver tumor is induced by estrogens, derived from aromatization of AAS, in a dose-dependent fashion. The incidence of hepatic adenomas in users of AAS is unknown, but probably most go unnoticed as they usually do not cause symptoms and may remit after cessation of AAS use. Barring these relatively exceptional cases, for the large majority of users, AAS use does not lead to considerable liver damage. As the data of the HAARLEM study point out, there is only a mild rise in concentrations of ALT, AST and LDH during the cycle. This may reflect minor hepatic injury but in many cases represents the muscle damage from heavy workouts, evidenced by the dramatic rise in CK concentrations in most subjects.

Testicular dysfunction

During AAS use there is an apparent disruption of testicular function. Details of how AAS interfere with endogenous testosterone production and spermatogenesis are described in **CHAPTER 6**. Briefly worded, the administration of supraphysiological doses of exogenous androgens led to very high total testosterone and estrogen concentrations in most cases and complete suppression of the hypothalamic-pituitary-gonadal axis. The latter was recognizable by undetectable LH and FSH concentrations in nearly all subjects. In concordance with these changes, testicular volume declined and spermatogenesis decreased. At the end of the cycle, three-quarters of subjects had a total sperm count below 40 million, and one-quarter had azoospermia. Interestingly, a longer duration of AAS use was not associated with a higher degree of suppression of spermatogenesis.

Recovery of testicular function after cessation of AAS use was less straightforward. A distinction could be made between subjects with a normal gonadal function at baseline, signifying a normal serum testosterone concentration and total sperm count, and those subjects without. Provided that gonadal function before the initiation of a cycle was normal, the chance of normalization of total testosterone concentration was 90% after 3 months of recovery and 100% at the end of follow-up. This observation was also independent of cycle dose or duration. Contrarily, 37% of cohort subjects had signs of abnormal gonadal function at baseline, and their recovery rate was slower with 24% not having a normal total testosterone concentration after 1 year. This group was composed of subjects with a higher cumulative past exposure to AAS, positing previous AAS as a risk factor for chronic disruption of testicular function.

Spermatogenesis recovered more slowly than endogenous testosterone production, which is no surprise as it takes about 90 days before spermatozoa appear in the ejaculate after spermatogenesis resumes.[17] For the total study population, time to recovery of spermatogenesis, defined as a return of the total sperm count to baseline levels, was calculated at 48 to 69 weeks, or 47 to 56 weeks when only considering subjects with a normal gonadal function at baseline. This is consistent with the 14 months as estimated by a recent large cross-sectional study.[18] In the majority of cases, inside and outside the context of the study, it is common that AAS users start a new cycle before recovery of spermatogenesis is complete. It is presumable that repeated exposure to AAS extends the required recovery time and may eventually even cause permanent distortion of fertility.

Against popular belief in the bodybuilding community, the performance of PCT did not show any beneficial effect on the recovery of endogenous testosterone production or total sperm count. It is conceivable that PCT is truly ineffective and not able to counteract the strong suppressive effects of AAS on gonadotropin production. When exogenous testosterone concentrations fall below normal levels, the amplification of the negative feedback signal on the pituitary as induced by PCT could well be redundant. However, it is imaginable that PCT does have some efficacy, but that its effect was too minute to detect with the limited number of blood and semen measurements after the cycle during the HAARLEM study. In addition, subjects may have performed the PCT improperly, as quite many started anti-estrogens and aromatase inhibitors within 1 to 4 weeks after the end of the cycle when serum exogenous testosterone concentrations were probably still high due to the long half-life of intramuscular depots. Similarly, the use of hCG during the cycle or as part of a PCT regimen had no detectable effect on testicular size or total sperm count. The lack of this effect, in all likelihood, is due to impure products and inadequate dosage regimens, as normally hCG would be expected to lead to an enhancement of spermatogenesis in hypogonadotropic hypogonadism.[19]

Cardiovascular effects

Another health domain in which AAS play havoc is the cardiovascular system. Short term adverse effects on blood pressure, lipid profile and hematocrit are described in **CHAPTER 7**. Although the absolute increase in blood pressure is modest, 41% of subjects was hypertensive during the cycle. There is a similar picture for erythrocytosis, where AAS led to a hematocrit of 50% or higher in one third of subjects. Both effects unfavorably impact cardiovascular disease risk.[20,21] Interestingly, there was no observed dose-dependent effect between AAS and blood pressure and hematocrit. However,

the aforementioned limitations regarding estimation of cycle dose may have clouded this analysis. As is the case with basically all androgen-induced effects, the untoward changes rapidly reversed back to baseline after cessation of AAS use.

Table 1. Summary of the main health effects of AAS use as observed during the HAARLEM study. Results from electrocardiography are not reported in any of the chapters in this thesis but are added for the sake of completeness. 95%CI = 95% confidence interval.

Health parameter	**Effect of AAS use**
Serious adverse events	*Incidence rate*
All serious adverse events	1 in 17 person years (of AAS use)
Heart failure, acute pancreatitis, suicidal ideation, exacerbation of ulcerative colitis	1 in 68 person years (of AAS use)
Reported health effects	*Prevalence*
Increased strength	100%
Increased energy	45%
Decreased libido (after the cycle)	58%
Acne	52%
Agitation	36%
Gynecomastia	26%
Aggressiveness	23%
Fatigue	16%
Tendon injuries	7%
Physical examination	*Mean change relative to baseline [95%CI]*
Weight (kg)	+4,9 [4.0 – 5.8]
Systolic blood pressure (mmHg)	+6.8 [4.3 – 9.4]
Diastolic blood pressure (mmHg)	+3.2 [1.3 – 5.0]
Testicular volume (ml)	-4.4 [-4.8 – -3.7]
Laboratory analysis	*Mean change relative to baseline [95%CI]*
Hematocrit (%)	+3% [2 – 4]
Platelet count ($x10^9$)	+47 [38 – 55]
Creatinine (µmol/l)	+4.8 [2.5 – 6.8]
Alanine transaminase (U/l)	+19 [12 – 26]
LDL-cholesterol (mmol/l)	+0.45 [0.29 – 0.61]
ApoB (mg/dl)	+18 [14 – 23]
HDL-cholesterol (mmol/l)	-0.40 [-0.46 – -0.35]
ApoA1 (mg/dl)	-37 [-43 – -30]
Lp(a) (mg/dl)	-9.6 [-13 – -6.6]
ANGPTL3 (ng/nl)	+63 [-34 – 161]

PCSK9 (ng/ml)	-36 [-66 – -5.3]
Prostate-specific antigen (µg/l)	+0.21 [0.13 – 0.31]
Total sperm count (x10^6)	-115 [92.3 – 147]
Electrocardiography	*Mean change relative to baseline*
Heart rate (/min)	+8.9 [6.7 – 11]
Corrected QT interval (ms)	-14 [-10 – -18]
Early repolarization	1.9 [1.1 – 3.2]
Sokolow-Lyon index	+2.2 [1.1 – 3.2]
Echocardiography	
Left ventricular mass (g)	+28 [14 to 42]
Left ventricular ejection fraction 3D (%)	-4.9 [-2.5 – -7.2]
E/A-ratio	-0.45 [-0.21 – -0.45]
Left atrial volume 3D (ml)	+9.2 [2.9 – 15.4]

The effect of AAS on lipid metabolism and related cardiovascular risk was more intricate. On the one hand, there was a clear adverse effect on LDL cholesterol and ApoB, and HDL cholesterol and ApoA1. These effects were worse in subjects using oral in addition to injectable compounds. The increase of ANGPTL3, an important regulator of lipid metabolism, can be considered disadvantageous as well. On the other hand, the lipid parameter Lp(a), which is strongly associated with cardiovascular disease,[19] showed a mean decrease of 50% at the end of the cycle. The observed change of Lp(a) was inversely correlated with cycle dose and duration. The main question remains whether the observed beneficial reduction of Lp(a) can counterbalance the unfavorable effects induced by the other lipid parameters. Current evidence suggests this is not likely to be the case, as AAS use is associated with premature atherosclerosis and coronary artery disease.[11,23]

The most important lesson learnt with the observations on cardiovascular parameters during the HAARLEM study is probably that the cardiovascular risk of AAS use will not so much be determined by the magnitude of the changes of these parameters, but predominantly by the duration of AAS use. In other words, the longer a strength athlete uses AAS in his sporting career, the worse his risk profile for cardiovascular disease becomes. The study cohort, which is composed of mainstream androgen abusing amateur strength athletes in the Netherlands, used AAS for a cumulative 68 years during 203 person years. With this statistic, one may just figure the long-term cardiovascular burden on society if such a lifestyle were to be continued for a long time by an increasing group of AAS users.

Coagulation

Although clinically less apparent than cardiovascular disease, previous literature has also raised the suspicion there may be a relationship between AAS use and thromboembolic disease. This evidence is based on coagulation studies and small epidemiological studies.[24] Indeed, as discussed in **CHAPTER 8**, the data from the HAARLEM study showed a significant increase in concentration of coagulation parameters during the cycle, both procoagulant (factor II, IV, and XI) and anticoagulant (protein S). The net effect of these changes on coagulation was hard to estimate, but, curiously, as opposed to the

hypothesis, the outcome of the thrombin-generation assay, which reflects the overall coagulation potential, clearly leaned towards an anticoagulant change. Furthermore, clot lysis time was longer which indicated impaired fibrinolysis. Oral AAS had a stronger impact on the coagulation parameters levels than did injectables. Cycle dose and duration only modified protein S concentrations and did not affect the other parameters significantly. All changes were completely reversible after cessation of AAS use, even after multiple cycles were performed.

These results notwithstanding, it remains to be determined whether, clinically, there is an inclination for thrombosis in AAS users. None of the subjects in the 68 years of androgen exposure during the HAARLEM study showed any signs of thrombotic disease. On the other hand, a retrospective cohort study showed a hazard ratio of approximately 5.0 for venous thromboembolism in abusers of AAS, although selection bias was an important issue.[25] True thrombosis risk in this population of strength athletes, whether or not in some degree attributable to AAS use, may well be overshadowed by other factors such as the widespread use of other recreational drugs, the gaining popularity of auxiliary PIEDs such as growth hormone,[26] and self-medication such as tamoxifen and anastrozol.[27]

Cardiac structure and function

A subset of 31 athletes partaking in the HAARLEM study was also included for a comprehensive 3D echocardiographic examination, the results of which are discussed in **CHAPTER 9**. The process of enrolment and inclusion criteria was the same as for the main observational study. Because all subjects who were offered the analysis eventually signed informed consent and none of them dropped out, the disposition of this group of subjects did not magnify any form of existing selection bias. Two different operators performed all the analyses, and the same operator carried out the echocardiography in the same subject. This reduced interobserver variability to a minimum. The study cardiologist who interpreted the images was blinded for subject and the timing of clinic visit with respect to AAS use.

The findings strongly confirmed the cardiotoxic nature of AAS and were in accordance with a recent large cross-sectional study.[11] There was a clear increase in left ventricular mass, due to an enlarged interventricular septum and posterior wall. In addition, left ventricular ejection fraction decreased by 5% and left ventricular stiffness increased as illustrated by a significantly lower E/A-ratio. There was a positive relationship between average weekly dose of AAS during the cycle and left ventricular mass. The increase in blood pressure and training time in study subjects did not interact with the observed changes in cardiac parameters. When AAS were discontinued, after a median recovery time of 8 months, all parameters returned to their baseline values.

Although the study provided strong evidence for an impaired cardiac function due to AAS use, the impairment did not lead to symptoms, such as dyspnea or peripheral oedema, in any of the investigated subjects. The aforementioned single subject with heart failure in the HAARLEM study and several other cases in literature[28] nevertheless point out that AAS can be the straw that effectually breaks the camel's back for the heart of an athlete who suffers from a pre-existing, possibly undiagnosed, cardiomyopathy. In addition, it is possible that cumulative cardiac damage may follow repeated AAS use when recovery time in between cycles is too short or when AAS are used

continuously. It may well explain why several renowned bodybuilders with a long-standing history of AAS use have succumbed to heart disease at a particularly young age.[29]

Patterns and determinants of AAS use

Subjects remained in follow-up after their fourth and last clinic visit of the first year. From that point onwards they were allowed to use AAS in the way of their choice and therefore probably better exhibited the true patterns of AAS use by amateur strength athletes. The results are discussed in **CHAPTER 10**. Approximately two-thirds of subjects engaged in AAS use in the second year again. Of the repeat users, 25% used continuously throughout the extended follow-up period, mainly in a blast and cruise fashion, but also as TRT or a non-stop cycle. This subgroup of continuous users raised the mean duration of AAS use from 18 weeks in the first year to 27 weeks in the second year, if not counting the subjects who did not use AAS again. Their longer duration of AAS use poses a larger burden on health and this group probably accumulates the most risk for long-term health problems.

The design of the study allowed to identify baseline characteristics of athletes that predicted future AAS use. The single most important factor that correlated with future AAS use, and especially for continuous AAS use, was cycle duration in the first year. This may portray the hesitancy experienced by subjects to terminate their cycle, for example due to the perceived benefits of AAS use or fear for withdrawal symptoms. An important predictor for the blast and cruise strategy in the second year was taking part in bodybuilding competitions, body dysmorphia and self-reported dependence on AAS. These features have been recognized in AAS users before and are thought to perpetuate AAS use.[30-32]

Concluding remarks and future research

This thesis has provided convincing and detailed information about the short-term hazards of AAS use. It is plausible that, besides a variety of side effects and adverse cardiovascular and hormonal effects, there is no meaningful risk of acute toxicity or overdose of AAS. Athletes who use AAS may consequently do so unhindered for many years and not run into serious medical trouble. Moreover, they take into account, and self-medicate, the possible wide range of mild and reversible symptoms. Sometimes it is necessary for them to adjust the cycle in a minor way, but it does not often lead to the decision to quit AAS use altogether. Even when a serious adverse event is experienced, subjects seldom feel inhibited to engage into a new cycle afterwards.

After an AAS cycle is terminated the recovery appears to be complete for most of the side effects, laboratory values and cardiac parameters. The hazardous effects of AAS generally reverse within three months after the end of the cycle, to an important extent determined by the half-life of the androgen esters last used. Endocrine parameters need more time to mend. Adequate endogenous testosterone production picks up within 3 months in most, but takes longer if pre-existing gonadal function was suboptimal, for instance because recovery from a previous cycle was still not complete. The return of a normal sperm count is the slowest and takes approximately one year. These time frames for recovery are important to know for clinicians, as they can be used to inform users and plan laboratory research, and also determine when repeat investigation or treatment would be appropriate.

Thus, the short-term damage of AAS use and rate of recovery has become quite perceptible. It is nonetheless by no means certain what repeated AAS use conveys in the long run. Especially worrisome is the cardiovascular risk that AAS use may bring along,[11,13] but the exact contribution of AAS to cardiovascular disease later in life remains largely undetermined. Future research should bridge this knowledge gap. This should be done by prospectively following groups of previous AAS users or performing large cross-sectional studies. The proper conduction of such research is challenged by ethical principles, which limit the availability of subjects and their willingness to participate, especially in countries where doping regulations are very strict. This unavoidably introduces selection bias. Lack of funding may also stand in the way of gathering the large sample sizes needed to investigate these issues reliably.

Despite the uncertainty about the long term, the potential for health damage by AAS use arguably forms a spectrum, ranging from negligible in one or a few short cycles, to large in case an athlete performs dozens of cycles or uses AAS non-stop for many years. A comparison can be drawn with smoking and its associated risk of cardiovascular and pulmonary disease. That is, one year of smoking will not contribute significantly, if at all, as opposed to a lifespan of packyears. Therefore every user should be assessed and judged – not prejudiced – individually, taking into account the history of AAS use and likelihood of ongoing use. It can be contended that physicians may condone an occasional short cycle by a recreational athlete – e.g., once every year, provided that the risk of escalating use and addiction is low.

For future research a more innovative approach than mapping the harmfulness of AAS use would be to investigate methods by which damage caused by AAS can be moderated. It can be assumed that AAS use has deleterious effects on health and therefore it could be beneficial to impel athletes to use no, or less, AAS than they would have done otherwise without an intervention. Obviously the most desirable outcome for a clinician or health care worker would be to intercept the AAS use by an athlete entirely, i.e. primary prevention. This is currently endeavored by anti-doping organizations with large-scale education programs and restrictive regulations. These measures could surely be effective but will never rule out AAS use by a still sizeable group of strength athletes.

Secondary prevention could therefore be complimentary and may take the form of harm reduction strategies, for instance the face-to-face counseling of current users by health professionals and education through online discussion forums.[33] Such an approach would be suitable for the outpatient AAS clinic, considering its opportunity to provide ambulatory care and the expertise of the health care providers that work in the clinic. It would however be illegitimate to operate such a strategy without properly testing its efficacy. Also, offering such a strategy could persuade strength athletes who contemplate AAS use to sign up and start a cycle under the aegis of the provided care. A well-designed trial that investigates the efficacy of a harm reduction strategy is needed to determine whether such an approach could be put into practice.

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SUMMARY

An estimated 20.000 amateur athletes use anabolic androgenic steroids (AAS) in the Netherlands. These drugs are illicitly obtained and usually of very low quality. The harmfulness of AAS has hitherto not been studied systematically. Therefore knowledge of the unwanted somatic and psychological effects of AAS is based on rather low-level evidence, such as expert opinion, case reports and cross-sectional studies. The outpatient AAS clinic in Haarlem was founded to gain more insight into health risks associated with AAS use. Patients interviewed in this clinic helped to envisage the typical AAS user and what side effects and health issues are mostly encountered.

To generate more reliable data less susceptible to bias, the HAARLEM study was initiated, which prospectively analyzed a cohort of 100 amateur athletes during and after an AAS cycle. The cohort was assembled of strength athletes mostly in their 20s and 30s, and one-fifth was competitor in a bodybuilding competition. The majority used AAS to gain muscle mass and to improve their level of strength. The cycle performed during the first year of the study had a median duration of 13 weeks, average weekly androgen dose of 901 mg and consisted of 5 different AAS types. All of the subjects reported positive effects as well as a wide range of transient and mild negative side effects, such as acne, gynecomastia and erectile dysfunction. Four subjects encountered a serious adverse event, among others congestive heart failure, but all recovered with treatment. Liver and kidney function were only slightly affected by AAS use. Psychological questionnaires revealed no major changes during and after the cycle. A body dysmorphic disorder was present in one-fourth of participants.

During the cycle testicular dysfunction was observed with suppression of endogenous testosterone production as well as spermatogenesis. Both recovered in most subjects after the cycle, although a long history of AAS use was associated with a limited rate and extent of recovery. The performance of PCT did not affect recovery in any positive way. Changes of cardiovascular parameters observed during AAS use were an elevated blood pressure, rise in hematocrit and platelet count, and a mostly inimical change of lipid profile. The extent of these changes was not associated with cycle dose or duration, but oral AAS had a worse impact on lipid profile than injectables alone. Electrocardiography revealed a shortened corrected QT interval, increased rate of early repolarization, and higher indices for left ventricular hypertrophy. The latter was confirmed with echocardiography, which not only showed an increased left ventricular mass but also a reduced ejection fraction, an increased ventricular stiffness and larger left atrial volume. After discontinuation of AAS use, all of the mentioned changes reversed back to baseline levels.

The cohort was followed-up for an additional year after the first. Two-thirds of subjects used AAS again this year, and one-quarter of them used AAS all year long, mainly in a blast and cruise fashion. Continuous use was determined in some degree by taking part in bodybuilding competitions and training time. Future research should particularly focus on how to effectively reduce harm in this group of avid users. A trial assessing a harm reduction strategy with face-to-face counseling by health professionals in the outpatient AAS clinic will soon be under way.

SAMENVATTING

In Nederland gebruiken naar schatting 20.000 amateursporters anabole androgene steroïden (AAS). Deze middelen worden illegaal verkregen en zijn meestal van zeer lage kwaliteit. De schadelijkheid van AAS is tot nog toe niet systematisch onderzocht. Daarom is de huidige kennis over de somatische en psychologische bijwerkingen gebaseerd op bewijs van beperkte kwaliteit, zoals de mening van deskundigen, casusrapporten en transversaal onderzoek. De anabolenpoli in Haarlem is opgericht om meer inzicht te verkrijgen in gezondheidsrisico's die samenhangen met AAS-gebruik. Op basis van de patiënten die in deze kliniek zijn gezien, is een beeld ontstaan van de typische AAS-gebruiker en welke bijwerkingen en gezondheidsproblemen het meeste voorkomen.

Om betrouwbaardere en minder voor bias vatbare gegevens te verkrijgen, werd de HAARLEM-studie gestart. Dit was een prospectief cohortonderzoek die 100 amateursporters tijdens en na een kuur met AAS heeft onderzocht. Het cohort bestond uit krachtsporters van in de 20 en 30, en één-vijfde was deelnemer aan een bodybuildingcompetitie. De meerderheid gebruikte AAS om spiermassa op te bouwen en kracht te verbeteren. De kuur in het eerste jaar van de studie had een mediane duur van 13 weken, een gemiddelde wekelijkse androgeendosis van 901 mg en bevatte 5 verschillende AAS-types. Alle proefpersonen meldden positieve effecten, alsook een breed scala aan voorbijgaande milde bijwerkingen, zoals acne, gynaecomastie en erectiestoornissen. Vier proefpersonen maakten een ernstige bijwerking mee, waaronder hartfalen, maar herstelden na een behandeling. De lever- en nierfunctie werden slechts in geringe mate beïnvloed door het gebruik van AAS. Psychologische vragenlijsten lieten geen grote veranderingen zien tijdens en na de kuur. Bij een kwart van de deelnemers was er sprake van een stoornis in de lichaamsbeleving.

Tijdens de kuur ontstond testiculaire disfunctie met suppressie van endogene testosteronproductie en spermatogenese. Beide herstelden bij de meeste proefpersonen na de kuur, hoewel een lange historie van AAS-gebruik geassocieerd was met een onvolkomen en langzamer herstel. Een nakuur had geen positieve invloed op het herstel. Op cardiovasculair gebied ontstond tijdens de kuur een hogere bloeddruk, een stijging van het hematocriet en aantal bloedplaatjes, en een overwegend ongunstige verandering van het lipidenprofiel. Er was geen associatie tussen kuurdosis of -duur en de mate van deze veranderingen; orale middelen hadden wel een negatiever effect op het lipidenprofiel dan injecteerbare AAS. Elektrocardiografie toonde een korter gecorrigeerd QT-interval, het ontstaan van vroege repolarisatie, en hogere indices voor linkerventrikelhypertrofie. Dit laatste werd ook gezien met echocardiografie, die niet alleen een verhoogde linkerventrikelmassa toonde, maar ook een afname van ejectiefractie, een grotere ventriculaire stijfheid en een groter linkeratriumvolume. Na de kuur herstelden alle genoemde veranderingen tot het baselineniveau.

Het cohort werd ook een tweede jaar gevolgd. Twee-derde van de proefpersonen gebruikte dit jaar opnieuw AAS en een kwart van hen zelfs het hele jaar door. Determinanten van herhaald gebruik waren bodybuildingwedstrijden en trainingstijd. Toekomstig onderzoek zou zich met name moeten richten op hoe de schade bij deze groep fervente gebruikers te beperken. Binnenkort zal een trial worden gestart op de anabolenpoli die onderzoek doet naar de effectiviteit van een harm reduction-strategie waarbij gebruikers van AAS persoonlijk worden begeleid.

This thesis provides an academic insight into the health consequences of androgen abuse by amateur strength athletes in the Netherlands. From the growth in muscle size to shrinkage of testicles, and from heart failure to bleeding liver tumors. The scientific data come from the anabolic steroid clinic in Haarlem, and the HAARLEM study, which is the largest prospective research initiative conducted in this field to date.

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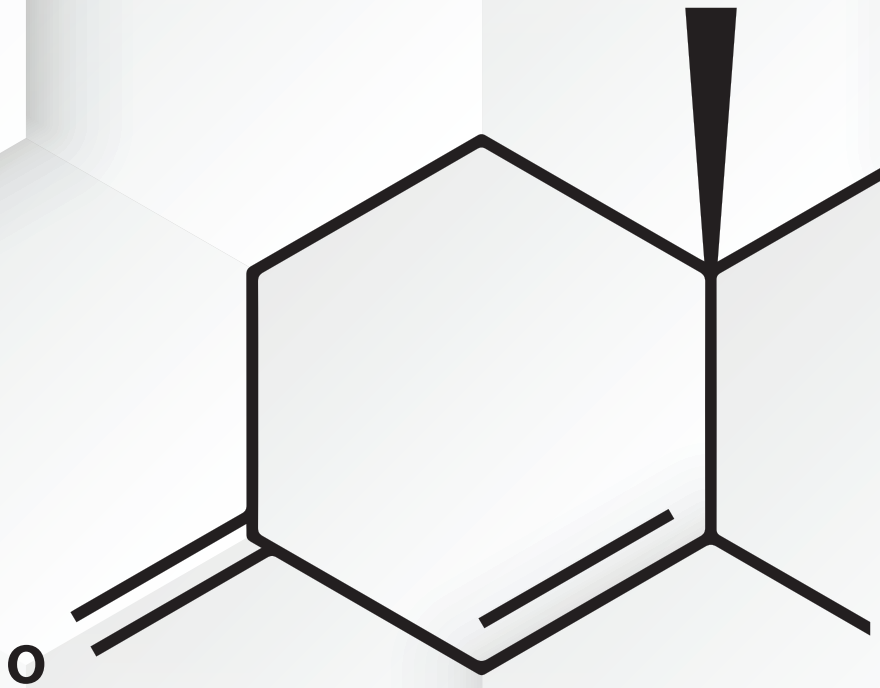

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Spaarne Gasthuis