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ORIGINAL ARTICLE

Prospective study on blood pressure, lipid metabolism and erythrocytosis during and after androgen abuse

Diederik L. Smit[1] | Aldo Grefhorst[2] | Madelon M. Buijs[3] | Olivier de Hon[4] | Martin den Heijer[5] | Willem de Ronde[1]

[1]Department of Internal Medicine, Spaarne Gasthuis, Haarlem, The Netherlands

[2]Department of Experimental Vascular Medicine, Amsterdam UMC, Amsterdam, The Netherlands

[3]Atalmedial Diagnostics Centre, Spaarne Gasthuis, Hoofddorp, The Netherlands

[4]Doping Authority Netherlands, Capelle aan den IJssel, The Netherlands

[5]Department of Internal Medicine, Section of Endocrinology, Amsterdam UMC, Amsterdam, The Netherlands

Correspondence
Diederik L. Smit, PO box 417, 2000 AK, Haarlem, The Netherlands.
Email: smit_diederik@hotmail.com,

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Abstract

Androgen abuse is associated with unfavourable changes in blood pressure, lipid metabolism and erythrocytosis. Most knowledge is based on cross-sectional studies sensitive to bias. We assessed the magnitude of these effects and their recovery in a prospective cohort study which included 100 men (≥18 years) performing an androgen cycle. Clinic visits took place before the cycle, at the end, 3 months after and 1 year after start of the cycle and included measurement of blood pressure, lipid parameters and haematocrit. During androgen use, systolic and diastolic blood pressure increased 6.87 (95% CI 4.34–9.40) and 3.17 mmHg (1.29–5.04) compared to baseline respectively. LDL cholesterol and ApoB increased 0.45 mmol/L (0.29–0.61) 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 to 0.35), 36.6 mg/dl (30.2–42.9) and 37.6% (13.9–61.3). ANGPTL3 increased 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 the start, these parameters returned to baseline. In conclusion, androgen abuse induces small but clinically relevant adverse changes in blood pressure, lipid metabolism and erythrocytosis which are rapidly reversible after cessation. As follow-up was limited to 1 year, the impact of androgen abuse on cardiovascular disease remains uncertain.

KEYWORDS
anabolic steroids, androgen abuse, blood pressure, bodybuilding, lipid metabolism

1 | 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% (Sagoe et al., 2014; Tanner et al., 1995). 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 (Smit et al., 2019; Weber et al., 2017).

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 (Baggish et al., 2017; Thiblin et al., 2015). The mechanisms through which androgen abuse may lead to cardiovascular morbidity comprise high blood pressure (Giorgi et al., 1999; Kuipers et al., 1991) and unfavourable

changes in lipoprotein metabolism (Shankara-Narayana et al., 2020; Thompson et al., 1989). 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 (Liu & Wu, 2019). 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 injectable ones (Giorgi et al., 1999), 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 1-year follow-up period: before, during and after a cycle of androgens.

2 | 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 attending fitness centres of at least 18 years old intending to start a self-initiated androgen cycle on short notice (i.e. within 2 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 3 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.

2.1 | Clinic visits

Subjects visited the study clinic four times during a 1-year study period. A medical investigation was performed at baseline within 2 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), 3 months after the end of the cycle (T_2) and 1 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.

2.2 | 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).

2.3 | 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.

3 | RESULTS

Follow-up of 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 (Smit et al., 2019).

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.

3.1 | Baseline values

Mean systolic and diastolic blood pressure were 129 (SD ± 14) and 79 (SD ± 9) mmHg at baseline (T_0; see Figure 1a,b) 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

TABLE 1 Overview of follow-up period of the 100 subjects included in the HAARLEM study. 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

Subjects	T_0	T_1	T_2	T_3
76				
10*				◇
5**			◇	◇
3				◇
2		◇		
2			Consent withdrawn	
1			◇	
1			◇	◇
100	100	98	91	79

Note: 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.

TABLE 2 Results of blood analysis comprising blood cell count and lipid parameters during clinic visits. The conversion factor for haemoglobin from mmol/l to g/l is 0.6206

	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 ($\times 10^9$/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 ($\times 10^9$/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		

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 T0. 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 =0.01-0.05.

‡p<0.01.

systolic (β = 0.95, 95% CI 0.41–1.48, $p < 0.01$) and diastolic blood pressure (β = 1.03, 95% CI 0.48–1.58, $p < 0.01$).

Subjects with a history of androgen abuse had a higher total and LDL cholesterol by 0.54 mmol/L (95% CI 0.03–1.05, p = 0.04) and 0.67 mmol/L (95% CI 0.15–1.19, p = 0.01) respectively, and a lower HDL cholesterol by 0.15 mmol/L (95% CI −0.28 to −0.02, p = 0.03), at baseline. There was a positive association between BMI and total (β = 0.08, 95% CI 0.03–0.13, $p < 0.01$) and LDL cholesterol (β = 0.09, 95% CI 0.04–0.15, $p < 0.01$) and ApoB (β = 3.22, 95% CI 1.57–4.89, $p < 0.01$). 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 = 0.46) between previous users and new users.

3.2 | Changes during androgen abuse

At the end of the cycle (T_1), mean systolic and diastolic blood pressure were 6.87 (95% CI 4.34–9.40, $p < 0.01$) and 3.17 mmHg (95% CI 1.29–5.04, $p < 0.01$) higher respectively, compared to baseline (T_0;

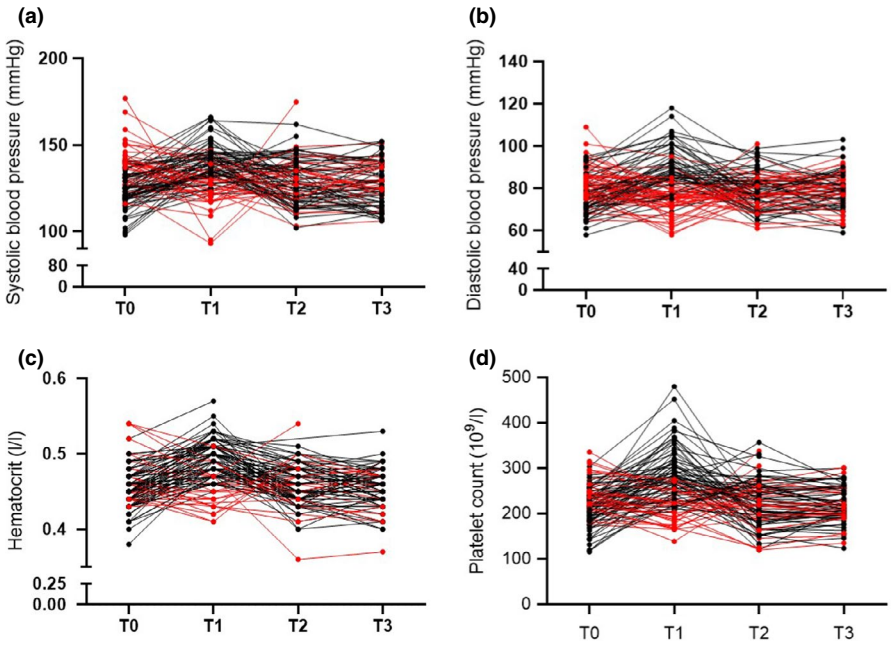

FIGURE 1 Connected scatter plots of systolic (a) and diastolic (b) blood pressure, (c) haematocrit and (d) platelet count. Black lines indicate subjects with a rise of the variable between T_0 and T_1, whereas red lines indicate subjects in whom a decline took place. T_0 = before the start of the cycle, 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

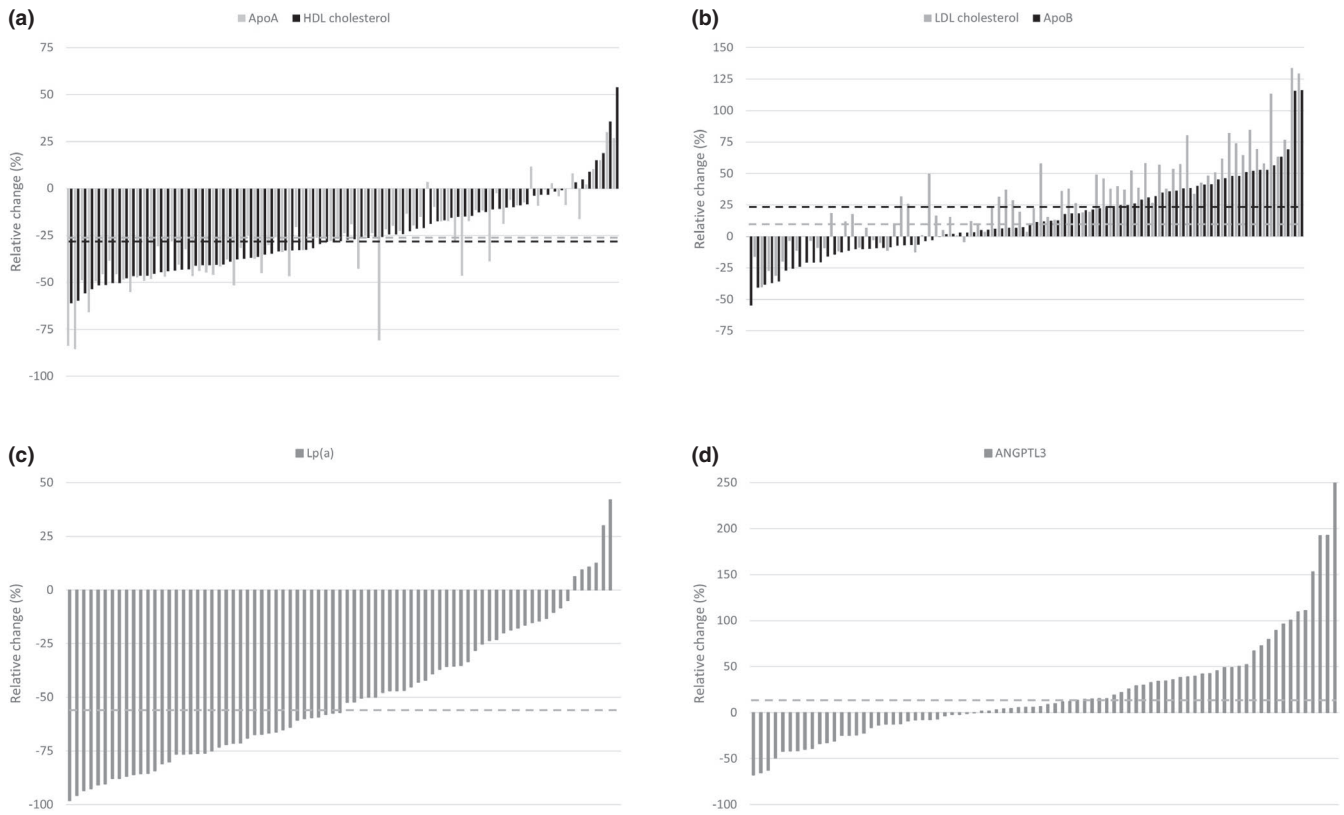

FIGURE 2 Waterfall plots of (a) HDL cholesterol and ApoA, (b) LDL cholesterol and ApoB, and (c) Lp(a) and (d) ANGPTL3, showing the relative change of these parameters during androgen abuse (T_1) in comparison to baseline (T_0) values. The bars display paired data of subjects from whom data were available from both clinic visits. The horizontal dashed lines indicate the median relative change in the entire cohort for the corresponding variable

see Figure 1a,b). The number of subjects with a systolic and diastolic blood pressure above 140 or 90 mmHg, respectively, were 35 (OR 5.65, 95% CI 2.28–14.0, $p < 0.01$) and 23 (OR 3.42, 95% CI 1.24–9.44, $p = 0.02$).

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 0.29–0.61, $p < 0.01$) and 18.2 mg/dl (95% CI 13.5–22.8, $p < 0.01$), respectively, whereas mean HDL cholesterol and ApoA1 decreased with

0.40 mmol/L (95% CI −0.45 to −0.35, $p < 0.01$) and 36.6 mg/dl (95% CI 30.2–42.9, $p < 0.01$). Waterfall plots of relative change of HDL cholesterol and ApoA1 as well as LDL cholesterol and ApoB are shown in Figure 2a,b 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 ($\beta = 0.53$, 95% CI 0.15–0.91, $p < 0.01$) and ApoB ($\beta = 19.9$, 95% CI 9.65–30.2, $p < 0.01$), and a greater decrease of ApoA1 ($\beta = -19.8$, 95% CI −33.9 to −5.66, $p < 0.01$), 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 0.06–0.31, $p < 0.01$) was observed at the end of the cycle (T_1) compared to baseline (T_0).

Mean Lp(a) decreased 37.6% (95% CI 13.9–61.3, $p < 0.01$) between clinic visit T_0 and T_1 (see Figure 2c). Lp(a) was negatively associated with cycle duration ($\beta = -0.51$, 95% CI −0.89 to −0.14 $p < 0.01$) and weekly cycle dose ($\beta = -9.4e^{-3}$, 95% CI $-1.6e^{-2}$ to $3.1e^{-3}$, $p < 0.01$) during the clinic visit at the end of the cycle (T_1). ANGPTL3 increased 20.3% (95% CI 7.38–33.2, $p < 0.01$) between baseline (T_0) and the end of the cycle (T_1; see Figure 2d). There was a positive association between ANGPTL3 and total ($R^2 = 108.0$, $p < 0.01$) and LDL cholesterol ($R^2 = 108.7$, $p < 0.01$). Mean PCSK9 decreased 35.6 ng/ml (95% CI 5.27–66.0, $p = 0.02$) in this time period.

Mean haemoglobin and haematocrit increased with 0.33 mmol/L (or 0.53 g/L; 95% CI 0.22–0.44 mmol/L, or 0.35–0.71 g/L, $p < 0.01$) and 0.03 L/L (95% CI 0.02–0.03, $p < 0.01$; 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 5.53–134, $p < 0.01$). The increase of haematocrit was not associated with longer cycles or higher androgen dose. The mean platelet count increased by 46.3×10^9 (95% CI 37.5–55.0, $p < 0.01$; see Figure 1d) and was positively correlated with the use of oral androgens ($\beta = 50.7$, 95% CI 30.5–70.8, $p < 0.01$).

3.3 | Recovery after androgen abuse

Approximately 3 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 3 months after the cycle (T_2).

4 | 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. 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.

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 (Smit & Ronde, 2018) and participants of earlier survey studies (Petersson et al., 2010; Westerman et al., 2016) 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 (Smit & Ronde, 2018) 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 3 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 (Shankara-Narayana et al., 2020). 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 in 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.

4.1 | 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 (Giorgi et al., 1999; Kuipers et al., 1991; Shankara-Narayana et al., 2020). Forty-one per cent of our subjects were 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 (Fonseca-Reyes et al., 2009). One of the mechanisms by which androgen abuse may lead to elevated blood pressure is water and sodium retention (Achar et al., 2010), which is often reported by androgen users (Smit et al., 2020; Smit & Ronde, 2018).

4.2 | Effects of androgen abuse on lipid metabolism

Unfavourable changes in serum LDL and HDL cholesterol due to androgen abuse have been well documented before (Bhasin et al., 2001; Kuipers et al., 1991; Shankara-Narayana et al., 2020; Thompson et al., 1989). 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 (Collaboration, 2012). 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 (Hartgens et al., 2004; Marcovina et al., 1996; Zmuda et al., 1996). 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) (Berglund et al., 1996). It remains to be elucidated whether the reduction in Lp(a) could counterbalance the change of parameters which promote the development of atherosclerosis and cardiovascular disease.

Angiopoietin-like 3 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 (Su & Peng, 2018). 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 (Su & Peng, 2018; Wu et al., 2020). 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 (Nidhina Haridas et al., 2015). 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.

Proprotein convertase subtilisin/kexin type 9 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 (Cohen et al., 2006). In our subjects, PCSK9 was reduced by ~11% during the cycle which may be brought about by elevated oestradiol concentrations (Ooi et al., 2015) 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.

4.3 | 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 (Gagnon et al., 1994). We did not observe a dose-dependent effect on erythropoiesis, contrary to physiological testosterone replacement therapy in hypogonadal patients (Coviello et al., 2008). 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 (Ansell et al., 1993). In concert with a presumed enhanced platelet aggregability (Ajayi et al., 1995), 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 (Vanberg & Atar, 2010). An adjoining coagulation study will provide data into these mechanisms in order to create a better understanding of thrombosis risk in androgens users.

4.4 | 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 (Calvo et al., 2010).

4.5 | 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 (Smit et al., 2019). 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 3 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.

5 | CONCLUSION

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 a 1-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 3 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 despite with tremendous variation. The cumulative duration of androgen abuse should therefore be considered a risk factor for cardiovascular disease.

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CONFLICT OF INTEREST

We declare no conflicts of interest or commercial affiliations.

AUTHOR CONTRIBUTIONS

DLS, OdH and WdR conceived and designed the analysis. DLS, AG, MB and WdR collected the data. DLS and AG performed the analysis. DLS, AG, MB, OdH, MdH and WdR wrote the paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Diederik L. Smit https://orcid.org/0000-0003-1635-1203
Willem de Ronde https://orcid.org/0000-0003-2224-8659

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