

Oxandrolone Use Causes Dyslipidemia in Resistance-Training Practitioners

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Abstract

Background: Several of young people and adults make use of anabolic-androgenic steroid (AAS), during resistance training. The purposes of this study were to compare blood and salivary parameters in male resistance training practitioners using oxandrolone with reference values, compare these with a control group in triplicate, and correlate salivary and blood parameters.

Methods: In this prospective analytical observational study, blood, saliva, and urine were collected from 22 individuals (oxandrolone group, OG, n = 11 and control group, CG, n = 11), and these samples were analyzed at three time points: before oxandrolone consumption, at cessation of oxandrolone use, and three months after cessation of oxandrolone use. Complete blood count, lipid profile, metabolites, and enzymes were analyzed from blood samples. Salivary flow, pH, triglycerides, urea, aspartate transaminase, alanine aminotransferase, phosphorus, and calcium were analyzed from saliva. Urinalysis was used for toxicological screening. Mann-Whitney U tests, chi-square analysis, Friedman's ANOVA, and Spearman's correlation tests were performed, with significance $p < 0.05$.

Results: We found a lower blood HDL level for the oxandrolone group (24 mg/dL) compared with the reference value (>40 mg/dL), as soon as its use ceased, and a return to normal HDL levels three months later (49 mg/dL, >40 mg/dL). We also found higher triglyceride level (177 mg/dL) in this group compared with the reference value (<175 mg/dL), three months after use.

Conclusions: Although there were distinct differences between the groups and timepoints, these did not show clinical relevance, as they were within typical values. There was no correlation between blood and salivary parameters, but it is clear that oxandrolone causes changes in the lipid profile of users.

Background

Approximately 75% of adults engage in physical activity in compliance with WHO guidelines [1]. One type of physical exercise chosen by young people and adults is resistance training, and its prevalence varies, for example, from 12 to 18% in gyms in the USA [2, 3]. Several of these individuals make use of anabolic-androgenic steroid (AAS), a synthetic testosterone analog with anabolizing activity [4–6]. These substances are used therapeutically in different dosages (for example 20 mg/day for 12 weeks) [7] for aesthetic purposes, ineffectively for weight maintenance [8], maintenance of skeletal muscles [9, 10], and sexual function [11]. In addition, AAS may be used for stabilization of body functionality, in fundamental movements like walking and squatting in individuals who need weekly hemodialysis [12] or who have hypogonadism [13].

Despite its therapeutic use, AAS are also indiscriminately used in supraphysiological doses (more than 600 mg/week) [14, 15] by many professional or recreational athletes aiming to increase sports performance [16–18], rapid gains in lean mass [13, 16–18, 19], and rapid loss of body fat [10], among other goals. Among several types of AAS, oxandrolone (OX) is one of the most used [23, 23–26]; high-frequency use (current and former users) made up 45.8% of one sample (n = 719; n = 329) in resistance-training practice (RTP) [27]. OX is used to increase performance [28–31], as well as to improve net protein balance and lean body mass in patients with serious burns [32, 33], Klinefelter Syndrome, metabolic diseases [34], oral pathologies, and HIV [33].

Several AAS side effects have been reported, such as hypercoagulability, venous thromboembolism, arterial thromboembolism, stroke, ligamentous injuries, disc herniation, arrhythmia, steatosis, renal failure, decreased testis volume, suppressed spermatogenesis, infertility, loss of libido, erectile dysfunction, and gynecomastia [35]. AAS have

been shown to increase low-density lipoprotein (LDL), decrease high-density lipoprotein (HDL) [36–38], reduce luteinizing hormone (LH) [39, 40] and follicle-stimulating hormone (FSH) [37, 41, 42], decrease blood testosterone [43, 44], and increase alanine transaminase (ALT) and aspartate transaminase (AST) [45], as well as other liver enzymes [38]. In those using AAS, basal blood shows increased hemoglobin [18, 46], high hematocrit counts [47], and high inflammatory markers [48], including high white blood cell count [28], high neutrophil count [49], and high C-reactive protein (CRP) [50, 51]. These side-effects may result in costly treatment, and therefore a negative impact for public health [52].

While various biochemical parameters are evaluated in the blood, they are also measured from the saliva, a non-invasive method. Measurement of metabolites in the salivary fluid is considered a reliable method [53, 54]. Some substances can alter parameters, such as salivary flow reduction observed with use of hypoglycemic drugs [55], antidepressants, hypothyroidism, or contraceptives [56]. Other effects are also observed, such as increased pH from green tea consumption [57], decrease in salivary pH in crack cocaine users [58] and elevated alanine aminotransferase (ALT) [59] in patients with kidney disease [60]. Even though these studies evaluate different drugs and conditions, none has verified the salivary effect of OX. As far as we currently know, there are no studies that have aimed to verify and correlate blood and salivary effects in AAS users using a single AAS (confirmation by urine metabolite). Furthermore, many studies do not measure AAS used by urinalysis; doses are self-reported, but not compared with reference values [12, 30, 61–69].

Goals

The primary purpose of this study was to verify the effects of OX on blood and saliva compared with reference values, to compare parameters before, immediately after, and three months after volunteers had finished the OX cycle, and to correlate salivary and blood parameters.

Methods

Study Design

This is a prospective analytical observational study.

Ethical Procedures

The project was approved by the Ethics and Research Committee of the Pontifical Catholic University of Paraná under protocol number 2.556.109 and followed the STROBE Statement guideline. All participants signed an appropriate informed consent paperwork. The study was conducted with a high ethical standard, even when formal approval has been obtained.

Inclusion and exclusion criteria

Subjects were recruited through the dissemination of research on social networks, websites, WhatsApp, and the snowball technique. Information about volunteers from resistance-training programs was included in the study. Requirements were as follows: male, expressed intention of OX use, six months in AAS washout or never used AAS, and self-report of no drug treatment or history of cardiovascular, respiratory, hepatic, renal, musculoskeletal, or metabolic disorders [70, 71]. This study included individuals who participated in a resistance-training program in the six months before the beginning of the evaluations.

Those individuals who did not complete one or more stages of the study were excluded from the research. Other exclusion criteria were: any illness acquired during the training period and collections that interfered with the results; psychotropic drug use; consumption of more than 15 alcoholic beverages per week ($\cong 30$ g/day); fixed or mobile orthodontic braces, removable total or partial denture or fixed denture; and smoking. All of these factors can interfere with the production of saliva [72].

Sample selection

Thirty-seven male subjects were initially recruited by social networks via the snowball method [63, 73–76]. The researcher was approached by subjects (by telephone) who intended to use OX, while the 26 subjects in the CG contacted the researcher. Forty-four subjects attended a personal interview, during which it was explained that no one, at no time, would provide any AAS substance, and that information on the time of use or dosages was likewise not to be prescribed. Participants gave their consent to participate under these conditions. Sociodemographic data were provided in face-to-face interviews based on the criteria of the Brazilian Association of Research Companies (ABEP) from Brazil's economic classification criterion [77], and information about age, weight, height, body mass index, total time (years) and frequency (days/week) of resistance-training practice, as well as training session time per week (minutes/day). In the end, 22 individuals remained in the study; reasons dropping out are shown in figure A.

Twenty-two subjects participated in all stages of the study: 11 OG and 11 CG (Table 1). Among OX users, all used an oral form, and dosages varied from 0.04 to 0.97 mg/kg (supplementary Table 2) (from 4 to 12 weeks, mean = 7.4 SD ± 2.2) (progressive or regressive), with no participant having a similar pattern of use. Nine ($n = 9$) individuals acquired AAS in drugstores, and two ($n = 2$) purchased the product via the Internet, ready to use and with registered trademarks.

Sample collection timepoints

Samples were collected and analyzed at three timepoints. For the OG, these timepoints were: T1, before drug use; T2, after OX use (mean of 7.4 SD 2.2 weeks); and T3, three months after use, as previously indicated [78]. Samples from the CG were collected at T1 in the same week as the OG, and T2 was collected eight weeks later, according to previous studies [79, 80]. The collection of CG T3 occurred three months after T2. Blood, saliva, and urine were all collected from 2 pm to 5 pm, from March to November of 2018.

Blood collection and analyses

Blood collection took place in the Biochemistry Laboratory at the School of Life Sciences located in the Pontifical Catholic University of Paraná. There was no indication of fasting. A nurse collected blood by radial artery puncture, and it was processed on the same day. Plasma biochemical analyses were performed on an AU480 Chemistry Analyzer (Beckman Coulter, Pasadena, CA), except for glucose measurement, which was performed using the Cobas Mira Plus device (Roche Diagnostic Systems, Basel, Switzerland). The reagents used were analysis-grade (Kovalent, São Gonçalo, Brazil). Hormones were measured on the UniCel DxI 800 Access Immunoassay System (Beckman Coulter, Pasadena, CA), and blood count on the Coulter LH 750 Hematology Analyzer (Beckman Coulter, Pasadena, CA). Blood counts included: erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red blood cell distribution width (RDW), leukocytes, basophils, eosinophils, band neutrophils, segmented neutrophils, lymphocytes, monocytes and platelets (Table 2). We also observed glucose, follicle-stimulating hormone (FSH), luteinizing hormone (LH), adrenocorticotrophic hormone (ACTH), total testosterone, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, estradiol, c-reactive protein (CRP), urea, amylase, albumin, calcium, creatinine, alkaline phosphatase (ALP), phosphorus and aspartate transaminase (AST) (Table 3).

Saliva collection and analyses

Saliva was collected by masticatory stimulation using a 1 cm sterile piece of rubber stimulator, with for 1 minute for oral self-cleaning and 5 minutes for chewing. All saliva produced was captured in an 80 ml universal collection flask and immediately assessed for pH with a digital pH-meter (Digimed, Analytical Instrumentation, model DM 20, São Paulo, Brazil). Sialometry was obtained by the gravimetry method. Samples were weighed with a precision analytical balance (Gehaka AG-200, São Paulo, Brazil). The calculated salivary flow is represented in mL per minute. For preparation and storage, samples were centrifuged at 2000 *g* for 5 minutes at room temperature to remove debris. The supernatant was separated and frozen until the time of analysis [81].

The equipment used for sialochemical analysis was the Cobas Mira Plus (Roche Diagnostic Systems, Basel, Switzerland). Bioclin reagents (Bioclin/Quibasa, Belo Horizonte, Brazil) were also used for analysis. The methodology followed was according to the manufacturer's recommendations. The sensitivity and method linearity are described in supplementary Table 1. Salivary variables evaluated were salivary flow, pH, triglycerides, urea, AST, alanine aminotransferase (ALT), phosphorus, and calcium (Table 4).

Urine collection and AAS Screening

Urine samples were self-collected and analyzed liquid chromatography system coupled to a quadrupole time-of-flight mass spectrometer (LC-QTOF) to quantify oxandrolone metabolites (OX) and testosterone for the three timepoints of the study (Table 5). We also to detect other AAS (Supplementary Text 1).

Statistical analysis

For statistical analysis, data normality was assessed using the Shapiro-Wilk test; where the data did not present a normal distribution, nonparametric statistics were used. Mann-Whitney U and chi-square tests were performed to compare anthropometric, demographic, blood, salivary, and urine variables between groups. On timepoints within groups, Friedman's ANOVA followed by the peer comparison test were made, with median compared to reference values. Spearman's correlation test was used to calculate the relationship between blood, saliva, and urine testosterone. The *p*-value adopted was < 0.05. The software used was IBM SPSS 25.0 for Windows.

Results

With relation to anthropometrics and sociodemographic characteristics, only schooling was different between groups (Table 1).

Table 1
Anthropometrics, training, and sociodemographic characteristics

Anthropometrics and Training characteristics	OG (n = 11)		CG (n = 11)	
	Mean (SD)		Mean (SD)	
Age (year)	27,5(± 6,6)		23,7(± 2,8)	
Weight (kg)	87,2(± 13,8)		76,0(± 10,9)	
Height (m)	1,78(± 0,05)		1,76 (± 0,04)	
BMI (kg/m ²)	27,3(± 3,4)		24,4(± 2,6)	
Total RT practice (year)	6,3(± 1,7)		5,2(± 3,4)	
RT frequency (days/week)	4,7(± 1,0)		4,0(± 1,3)	
Training session time (min)	67,7(± 31,4)		59,0(± 12,2)	
Sociodemographic characteristics	n	%	n	%
Marital Status				
Single	11	100	10	90,9
Married	0	0	1	9,1
Schooling				
Complete higher education	4	36,4 ^A	0	0 ^B
Incomplete higher education	5	45,5 ^A	11	100 ^B
Complete secondary	2	18,2	0	0
Socioeconomic class				
A	4	36,4	4	36,4
B1	2	18,2	5	45,5
B2	3	27,3	0	0
C1	2	18,2	2	18,2
Notes: Socioeconomic class is an average household income according to ABEP [77] A: 5.477,71 US\$;B1: 2.446,97 US\$; B2: 1.263,51 US\$; C1: 698,68 US\$; SD: Stand Deviation; RT = Resistance Training; BMI = Body Mass Index Uppercase letters: Chi-square p < 0,05. Values without letters mean no significant difference.				

Concerning reference values, the OG presented a higher HDL level at T2, returning to normal levels at T3, and a triglyceride level that remained within a typical value range at T1 and T2, but went above this range at T3 (Table 3). For blood, salivary, and urinary testosterone variables, values found were in accordance with reference values (Tables 2, 4 and 5).

In comparisons between groups (OG X CG) at the three timepoints (T1, T2, and T3), significant increased OG blood values were found for: band neutrophils at T2, monocytes at T1, platelets at T1 and T2, LH at T1, and triglycerides at T2 and T3. In contrast, some variables showed a reduction in OG: total testosterone at T1 and T2, calcium at T3, and ALP at T1 and T2. For salivary metabolites, only the pH in OG was decreased, at T3. Moreover, OX urinary levels in OG increased at T2 but were then reduced at T3. Comparing HDL and triglycerides from the stratified oxandrolone group by instances of use per week, there were no significant differences (Supplementary Table 3).

When blood variables were compared between timepoints (T1 x T2; T2 x T3; T1 x T3) within each treatment group separately, OG showed: hemoglobin count reduced from T1 to T2; FSH increased from T1 to T3; HDL increased from T2 to T3; triglycerides increased from T1 and T2 to T3; estradiol increased from T1 and T2 to T3; calcium reduced from T1 and T2 to T3.

Table 2
Hemogram count (Group X Time)

		OG (n = 11)	CG (n = 11)	
Hemogram variables				
Variables	Times	Median	Median	Reference value [83]
Erythrocytes	T1	5,30 ^a	5,36 ^a	4,60–6,20 µL
	T2	5,07 ^a	5,20 ^b	
	T3	5,12 ^a	5,47 ^{ab}	
Hemoglobin	T1	15,90 ^a	15,70 ^{ac}	14,0–18,0 (g/dL)
	T2	15,60 ^b	15,80 ^b	
	T3	15,60 ^{ab}	16,40 ^c	
Hematocrit	T1	45,10 ^a	48,50 ^a	40,0–54,0 (mL/dL)
	T2	45,10 ^a	45,50 ^b	
	T3	45,10 ^a	47,30 ^{ab}	
MCV	T1	86,90 ^a	88,81 ^a	80,0–96,0 (fL)
	T2	86,80 ^a	87,82 ^{ab}	
	T3	86,69 ^a	87,31 ^b	
MCH	T1	29,51	29,77	26,0–34,0 (pg)
	T2	29,78	30,18	
	T3	30,47	30,19	
R.D.W.	T1	12,80	13,30	11,0–14,5 (%)
	T2	13,20	13,00	
	T3	12,90	12,70	
Leukocytes	T1	7.000,00 ^a	6.700,00 ^a	3.600–11.000 /µL
	T2	6.900,00 ^a	6.445,00 ^b	
	T3	7.200,00 ^a	7.500,00 ^{ac}	
Basophils	T1	0,00	0,00	0–100 /µL
	T2	44	46,00	
	T3	0,00	0,00	
Eosinophils	T1	146,00	138,00	50–400 /µL

		OG (n = 11)	CG (n = 11)	
	T2	124,00	164,00	
	T3	216,00	97,00	
Band neutrophils	T1	70,00 ^a	79,00 ^a	0–700
	T2	69,00 ^{Aa}	46,00 ^{Bb}	/μL
	T3	72,00 ^a	75,00 ^{ac}	
Segmented neutrophils	T1	4.218,00 ^a	4.187,00 ^a	1.400–6.600
	T2	3.933,00 ^a	3.692,00 ^b	/μL
	T3	3.762,00 ^a	3.740,00 ^{ab}	
Lymphocytes	T1	2.075,00	2.415,00	1.200–3.200
	T2	2.070,00	2.067,00	/μL
	T3	2.025,00	2.130,00	
Monocytes	T1	490,00 ^{Aa}	232,00 ^{Ba}	300–900
	T2	504,00 ^a	516,00 ^b	/μL
	T3	574,00 ^a	497,00 ^b	
Platelets	T1	268.000,00 ^{Aa}	212.000,00 ^{Ba}	150.000–450.000
	T2	269.000,00 ^{Aa}	207.000,00 ^{Bb}	/μL
	T3	238.000,00 ^a	211.111,00 ^{ac}	

Table 3
Blood variables (Group X Time)

Blood variables				
Glucose	T1	73,00	72,00	65–99
	T2	86,00	83,00	(mg/dL) [84]
	T3	85,00	81,00	
FSH	T1	3,38 ^a	3,01 ^a	1,27–19,26
	T2	4,41 ^{ab}	4,91 ^b	(mUI/mL) [85]
	T3	4,36 ^b	4,78 ^b	
LH	T1	4,26 ^A	3,00 ^B	1,24–8,62
	T2	3,35	4,70	(mUI/mL) [85]
	T3	5,22	4,35	
ACHT	T1	22,00	16,00	< 46,0
	T2	16,90	17,10	(pg/mL) [86]
	T3	21,40	16,30	
Total Testosterone	T1	358,45	414,23	175,00–781,00
	T2	240,94 ^A	414,35 ^B	(ng/dL) [85]
	T3	295,56 ^A	489,81 ^B	
Total cholesterol	T1	183,00	154,00	< 190
	T2	185,00	150,00	(mg/dL) [87]
	T3	159,00	143,00	
HDL	T1	47,00 ^{ab}	50,00 ^a	> 40
	T2	24,00 ^a	45,00 ^a	(mg/dL) [87]
	T3	49,00 ^b	47,00 ^a	
LDL	T1	102,00 ^a	95,00 ^a	< 130
	T2	111,00 ^a	91,00 ^{ab}	(mg/dL) [87]
	T3	101,00 ^a	79,00 ^b	
Triglycerides	T1	133,00 ^a	75,00 ^a	< 175
	T2	91,00 ^{Aa}	69,00 ^{Ba}	(mg/dL) [87]
	T3	177,00 ^{Ab}	90,00 ^{Ba}	

Blood variables				
Estradiol	T1	20,00 ^a	20,00 ^a	< 47 (pg/mL) [88]
	T2	23,00 ^a	21,00 ^{ab}	
	T3	39,00 ^b	34,00 ^b	
CRP	T1	0,67 ^a	0,32 ^a	< 5,00 (mg/L) [89]
	T2	0,56 ^a	0,53 ^b	
	T3	0,70 ^a	0,51 ^{ab}	
Urea	T1	42,00	39,00	17–43 (mg/dL) [88]
	T2	42,00	36,00	
	T3	39,00	40,00	
Amylase	T1	49,00	62,00	22–80 (U/L) [90]
	T2	52,00	67,00	
	T3	53,00	57,00	
Albumin	T1	4,90 ^a	4,90 ^{ab}	3,5–5,2 (g/dL) [85]
	T2	4,90 ^a	5,00 ^a	
	T3	4,70 ^a	4,80 ^b	
Calcium	T1	9,80 ^a	9,80 ^a	8,9–10,7 (mg/dL) [88]
	T2	9,60 ^a	9,50 ^b	
	T3	9,30 ^{Ab}	9,70 ^{Bab}	
Creatinine	T1	1,03	1,20	0,90–1,30 (mg/dL) [88]
	T2	1,12	1,06	
	T3	1,03	1,01	
ALP	T1	56,00 ^A	75,00 ^B	30–120 (U/L) [85]
	T2	49,00 ^A	69,00 ^B	
	T3	57,00	68,00	
Blood phosphorus	T1	3,60	3,80	2,7–4,5 (mg/dL) [91]
	T2	3,90	3,80	
	T3	3,90	4,10	
AST	T1	26,00	26,00	< 50 (U/L) [85]
	T2	27,00	25,00	

Blood variables				
	T3	26,00	23,00	
ALT	T1	26,00	24,00	< 50
	T2	34,00	24,00	(U/L) [85]
	T3	28,00	24,00	

Table 4
Salivary variables (Group X Time)

Saliva variables				
Salivary flow	T1	0,82	1,11	> 0,7 ml/min
	T2	0,95	1,22	[92]*
	T3	1,09	1,17	
pH	T1	7,68	7,66	(6,1–8,0)
	T2	7,88	7,67	[93]*
	T3	7,50 ^A	7,84 ^B	
Triglycerides	T1	5,00	2,58	4,88 ± 0,21 (mg/dL)
	T2	2,58	2,58	[94]*
	T3	2,58	2,58	
Urea	T1	30,40	26,30	30,75 ± 9,63 (mg/dL)
	T2	23,40	20,90	[95]*
	T3	25,90	22,40	
AST	T1	14,00	12,00	20,8 ± 23,9 (U/L)
	T2	15,00	8,00	[60]*
	T3	9,00	9,00	
ALT	T1	3,00	2,00	8,67 ± 12,9 (U/L)
	T2	4,00	3,00	[60]*
	T3	0,99	0,99	
Phosphorus	T1	8,37	7,10	6,6 ± 5,1 (mg/dL)
	T2	10,96	5,67	[94] *
	T3	5,70	6,73	
Calcium	T1	0,48 ^a	0,53 ^{ab}	3,4 ± 2,7 (mg/dL)
	T2	0,35 ^a	0,23 ^a	[96]*
	T3	0,51 ^a	0,38 ^b	

Table 5
Urine variables (Group X Time)

Urine variables				
Testosterone	T1	22,18	11,19	41.7 ± 34.8
	T2	12,89	13,55	(ng/mL)
	T3	19,15	12,83	[97]*
OX	T1	0,00 ^a	0,00 ^a	(ng/mL)
	T2	31,35 ^{Ab}	0,00 ^{Ba}	
	T3	0,00 ^{ab}	0,00 ^a	
Uppercase letters represent differences in time between groups by Mann-Whitney U test.				
Lowercase letters represent differences between the moments in both groups by Friedman's 2 × 2 comparison; Median values without letters mention the absence of any statistically significant difference				
* The reference values for the saliva and urine variables were taken from studies, but there is no consensus on standardized methodologies				

In the present study, there were no statistically significant correlations between blood, salivary, and urinary variables (Supplementary Table 4).

Discussion

To our knowledge, this is the first study that evaluates blood and salivary parameters in OX users, with confirmation by urine metabolite quantification. Although there are differences between groups and timepoints, values of the dosed components were within the reference limits described in the literature. The main results show that there was no correlation among blood, saliva, and urine in OG. However, higher levels of inflammatory cells and lipids in OG are suggestive of inflammation and dyslipidemia.

Regarding education levels of the subjects, our results differ from previous studies [98], in which most subjects had completed high school, followed by higher education in progress. Our study showed that some of the subjects had completed higher education. However, the current research is in line with other studies, which revealed a higher prevalence of use among people with complete secondary and higher education, as well as those who are specialists in the profession, with aesthetic aspects as the primary motivation for use of AAS [99, 100]. Use of AAS does not depend on educational level; that is, having more or less education is not a determining factor for the use of AAS.

According to the WHO [101], low levels of HDL cholesterol, as well as high levels of triglycerides and LDL cholesterol, are important risk factors for cardiovascular disease. In fact, improving the lipid profile of individuals is the main preventive measure for cardiovascular diseases. [102]. In the present study, it was found that OX users had reduced levels of HDL cholesterol shortly after use of OX, returning to normal after three months. Despite HDL returning to normal, users had isolated hypertriglyceridemia three months after using OX. These data point to dyslipidemia in OX users.

Interestingly, in a previous study, OX was tested as a cholesterol-lowering drug. The authors proposed 7.5 mg/day for three months, followed by washout for two months, and showed that HDL decreased significantly in patients under these conditions. At the same time, they observed high cholesterol levels [103]. Another study focusing on subjects with metabolic syndrome used oral OX at doses of 10 mg/day for one week, and also found reductions in HDL levels, with marked increases in hepatic ketogenesis. This was due to increased influx of fatty acids into the liver. However, it is not possible to exclude the possibility that short-term administration of OX acts directly on the liver to stimulate the oxidation of fatty acids [104]. In a study aimed at treatment of Klinefelter syndrome with oral OX 0.06 mg/kg/day or placebo for two years, HDL was lowered [105]. In our study, the reduction in HDL was observed, but it was also observed that, immediately after cessation of OX use, HDL levels return to normal, despite differences between the research subjects. Meanwhile, triglyceride levels remained high, pointing to the action of OX on fat metabolism and negative impacts on the cardiovascular system.

Although anabolic steroids in high doses are used for short periods of time [106], many athletes abuse these anabolic steroids and self-administer doses up to 100- and even 1000-fold more than safe doses, producing circulating testosterone levels two to three orders of magnitude above the healthy male reference range, often for prolonged periods. The maximal anabolic dose of testosterone is not known, but almost certainly vastly exceeds 600 mg of testosterone a week [107]. Excess doses cause dyslipidemia secondary to drugs increasing total cholesterol, create no changes in triglycerides, and decrease HDL [108, 109]. In the present study, we were unable to determine the exact dose of OX used by the volunteers, but we suggest that they were supraphysiological doses, and that they caused a consistent effect of OX on HDL production.

OX has been used for therapeutic purposes in many different situations [78, 79, 110–112]. A study of HIV-infected men that was not controlled for antiretroviral intake treated with OX daily for 12 weeks and reduced HDL for all doses of OX studied [33]. In an animal model, OX elevated triglycerides, reduced HDL, decreased hepatic triglycerides, and elevated levels of non-esterified fatty acids produced by the liver, possibly leading to increased lipidic synthesis of hepatic triglycerides [113]. Additionally, OX may reduce blood triglycerides by further activation of the triglyceride lipase that results in hydrolysis of peripheral triglycerides. Once the intake of OX ceases, triglycerides increase [114]. For the purpose of comparison, we used in this study the reference values of the Brazilian population for triglycerides; the results indicate a significantly increased, almost borderline, risk for cardiovascular disease after ceasing OX use. According WHO classification, individuals who have triglyceride level higher than 180 mg/dl are risk groups for cardiovascular disease. In addition, the results found here for triglycerides were consistent with other studies; that is, increased lipolysis and liberation of free fatty acid with the use of OX [78, 79, 110–112]. However, three months after ending OX use, this variable was higher than the reference standards.

This contradictory result can perhaps be explained by the management of OX, including different doses, different monitoring times, the practice of resistance exercises, days of use, types of cycle, and other variables. Also, the short time of evaluation after use could be a variable. Washout of three or more months may take these parameters back to normal [78, 115]. Despite this, our research presents relevant methodological criteria that were used in the study: comparison with reference values and the detection of metabolite in urine, among others. Studies show that the AAS cycle (duration use) [116] can last from 10 to 12 weeks [4, 117–119], close to the timeframe of this study. It is important that we evaluated a single, isolated AAS. Other studies have not reported standardization in blood sample collection times [70, 120], in contrast to a previous study that reported collection between 10 and 12 a.m. [51].

Limitations of our study have to be considered. The results found in our study were different from the studies mentioned above, perhaps because they did not follow the subjects several months after ending the use of AAS. We did not make a standardized dose, or limit cycles, because OX is a controlled drug, and its prescription is provided

only by physicians. Another limitation is that the subjects' family history of atherosclerotic disease was unknown. Micronutrient and macronutrient intake, training intensity, and endurance also were not analyzed. The selection of subjects and randomness of groups would be necessary for subsequent studies.

This study showed, for the first time, reference values of saliva taken from previous research, for comparative purposes about what OX can change, as well as what implications this can have. Additionally, even though there is no consensus in the literature on these values, the use of salivary markers is a non-invasive method, cheaper than blood tests, and may facilitate future studies.

Conclusions

This study provides evidence that the use of oxandrolone promotes dyslipidemia when used in non-therapeutic doses.

Abbreviations

AAS – Anabolic-androgenic steroid

OX – oxandrolone

RTP – resistance-training practice

LDL – low-density lipoprotein

HDL – high-density lipoprotein

LH – luteinizing hormone

FSH- follicle-stimulating hormone

ALT – alanine transaminase

AST – aspartate transaminase

CRP – C-reactive protein

ABEP - Brazilian Association of Research Companies

OG – oxandrolone group

CG – control group

MCV – mean corpuscular volume

MCH – mean corpuscular hemoglobin

RDW – red blood cell distribution width

ACHT – adrenocorticotrophic hormone

Declarations

Ethics approval and consent to participate:

The project was approved by the Ethics and Research Committee of the Pontifical Catholic University of Paraná under protocol number 2.556.109.

Consent for publication:

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The author declare that they have no competing interests

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Author's contributions

DLG performed most of the research and described their result appointments and as well drafting the manuscript. **EP** and **TFO** participated in the recruiting people as also reviewing the manuscript. **TBDB, SEFO, LB, LCSB** and **JSJ** was responsible for the clinical agenda, medicating contact with the participants. **PM, FA, MHMC** and **RIW** responsible by blood analysis. **FT** responsible for reviewing the manuscript. **MMTB** responsible saliva table. **EARR, MFS** and **SACP** supervising the clinical procedures. **EC, YYG** and **SAI** responsible by the statistical analysis. **JAB** did the oxandrolone reading sheets. **ACBRJ** as the group leader, gave the guidelines for the whole project execution. All authors have read and approved the manuscript.

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Figures

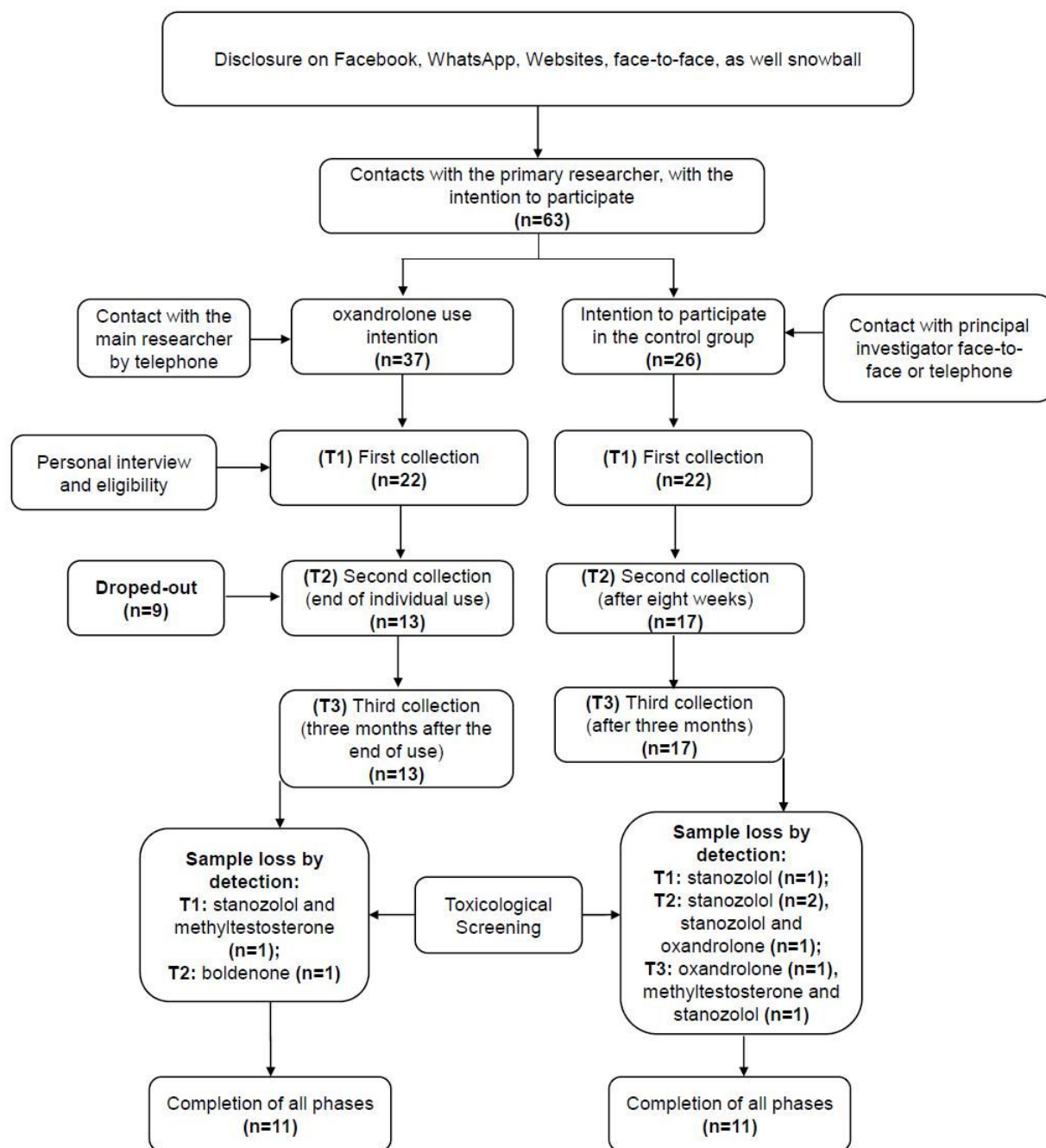


Figure 1

A. Study design and allocation of the study population.

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