

Oxandrolone use causes dyslipidemia in resistance-training practitioners

El uso de oxandrolona causa dislipidemia en practicantes de musculación

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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; OG, oxandrolone group; CG, control group; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; RDW, red blood cell distribution width; ACHT, adrenocorticotrophic hormone.

Abstract

Objective: This study investigated blood and salivary parameters in male resistance-training practitioners (RTP) using oxandrolone (OG) and compared with reference values and control group (CG), in triplicate. **Methods:** Blood, saliva, and urine were collected from 22 individuals (OG, n = 11 / CG, n = 11) and analyzed: before oxandrolone (OX) consumption (T1), at cessation of OX use (T2), and three months after cessation of OX use (T3). Complete blood count, lipid profile, metabolites, and enzymes were analyzed from blood samples. Salivary flow, pH, triglycerides, urea, aspartate aminotransferase, alanine aminotransferase, phosphorus, and calcium were analyzed from saliva. **Urinalysis** was used for toxicological screening. **Results:** A reduced hdl cholesterol level was observed in the og group (24 mg/dL) compared with the reference value (>40 mg/dL) at T2. HDL levels return to normal after cessation AT T3 (49 mg/dL, >40 mg/dL). A higher triglyceride level (177 mg/dL) was verified in OG group compared with the reference value (<175 mg/dL) at T3. **Conclusions:** The results of this study suggest that oxandrolone use is associated with changes in lipid profiles, including lower HDL levels and higher triglyceride levels, which are characteristic of dyslipidemia. however, while these findings indicate an association, they do not establish definitive causality. further research, including well-controlled longitudinal studies, is needed to confirm the causal relationship between oxandrolone use and dyslipidemia.

Keywords: Oxandrolone; Blood; Saliva; Resistance training; Anabolic agents; Dyslipidemia.

Resumen

Objetivo: Este estudio investigó los parámetros sanguíneos y salivales en practicantes masculinos de musculación que usan oxandrolona (OG) y los comparó con valores de referencia y con el grupo de control (GC), en triplicado. **Métodos:** Se recolectaron sangre, saliva y orina de 22 individuos (OG, n = 11 / CG, n = 11) y se analizaron: antes del consumo de oxandrolona (OX) (T1), al cese del uso de OX (T2) y tres meses después del cese del uso de OX (T3). Se analizaron hemograma completo, perfil lipídico, metabolitos y enzimas a partir de muestras de sangre. A partir de la saliva se analizaron el flujo salival, el pH, los triglicéridos, la urea, la aspartato aminotransferasa, la alanina aminotransferasa, el fósforo y el calcio. Se utilizó análisis de orina para el cribado toxicológico. **Resultados:** Se observó una reducción del nivel de colesterol HDL en el grupo OG (24 mg/dL) en comparación con el valor de referencia (>40 mg/dL) en T2. Los niveles de HDL vuelven a la normalidad después de suspender el tratamiento en T3 (49 mg/dL, >40 mg/dL). Se verificó un nivel de triglicéridos más alto (177 mg/dL) en el grupo OG en comparación con el valor de referencia (<175 mg/dL) en T3. **Conclusiones:** Los resultados de este estudio sugieren que el uso de oxandrolona se asocia con cambios en los perfiles lipídicos, incluidos niveles más bajos de HDL y niveles más altos de triglicéridos, que son característicos de la dislipidemia. sin embargo, si bien estos hallazgos indican una asociación, no establecen una causalidad definitiva. Se necesitan más investigaciones, incluidos estudios longitudinales bien controlados, para confirmar la relación causal entre el uso de oxandrolona y la dislipidemia.

Palabras clave: Oxandrolona; Sangre; Saliva; Musculación; Agentes anabólicos; Dislipidemia.

Resumo

Objetivo: Este estudo investigou parâmetros sanguíneos e salivares em praticantes masculinos de musculação que utilizam oxandrolona (OG) e comparou com valores de referência e com o grupo controle (GC), em triplicata. **Métodos:** Sangue, saliva e urina foram coletados de 22 indivíduos (GO, n = 11 / GC, n = 11) e analisados: antes do consumo de oxandrolona (OX) (T1), ao término do uso de OX (T2) e três meses após cessação do uso de OX (T3). Hemograma completo, perfil lipídico, metabólitos e enzimas foram analisados a partir de amostras de sangue. Fluxo salivar, pH, triglicérides, uréia, aspartato aminotransferase, alanina aminotransferase, fósforo e cálcio foram analisados na saliva. O exame de urina foi utilizado para triagem toxicológica. **Resultados:** Observou-se redução do nível de colesterol HDL no grupo GO (24 mg/dL) em comparação com o valor de referência (>40 mg/dL) em T2. Os níveis de HDL voltam ao normal após a interrupção em T3 (49 mg/dL, >40 mg/dL). Verificou-se maior nível de triglicérides (177 mg/dL) no grupo GO em comparação ao valor de referência (<175 mg/dL) em T3. **Conclusões:** Os resultados deste estudo sugerem que o uso de oxandrolona está associado a alterações no perfil lipídico, incluindo níveis mais baixos de HDL e níveis mais elevados de triglicérides, característicos da dislipidemia. no entanto, embora estes resultados indiquem uma associação, não estabelecem uma causalidade definitiva. mais pesquisas, incluindo estudos longitudinais bem controlados, são necessárias para confirmar a relação causal entre o uso de oxandrolona e a dislipidemia.

Palavras-chave: Oxandrolona; Sangue; Saliva; Musculação; Agentes anabólicos; Dislipidemia.

1. Introduction

Many individuals make use of anabolic-androgenic steroid (AAS), a synthetic testosterone analog with anabolizing activity. These substances are used therapeutically in different dosages (for example: 20 mg/day for 12 weeks) mainly for maintenance of skeletal muscles and sexual function (Llewellyn's, 2010; Schroeder et al., 2004)

AAS are indiscriminately used in supraphysiological doses (more than 600 mg/week) for rapid gains in lean mass, rapid loss of body fat and other goals (Cheung & Grossmann, 2018). Among several types of AAS, oxandrolone (OX) is one of the most used with high-frequency use (current and former users) made up 45.8% of one sample (n = 719; n = 329) in resistance-training practitioners (RTP) (Pereira et al., 2019) OX is used to increase performance as well as to improve net protein balance and lean body mass Klinefelter Syndrome, metabolic diseases (Davis et al., 2017a), oral pathologies, and HIV treatment (Grunfeld et al., 2006).

Several side effects of AAS (Anabolic-Androgenic Steroids) have been reported, including hypercoagulability, venous and arterial thromboembolism, stroke, ligamentous injuries, and disc herniation. Cardiovascular issues like arrhythmia, as well as liver problems such as steatosis and renal failure, have also been observed. Furthermore, AAS users may experience reproductive system side effects, such as decreased testis volume, suppressed spermatogenesis, infertility, loss of libido, and erectile dysfunction. Additional concerns include gynecomastia, increased LDL (low-density lipoprotein), decreased HDL

(high-density lipoprotein), and hormonal imbalances like reduced luteinizing hormone (LH), follicle-stimulating hormone (FSH), and blood testosterone levels.

Moreover, AAS use can elevate liver enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). In those using AAS, basal blood tests often show increased hemoglobin, high white blood cell and neutrophil counts, and elevated C-reactive protein (CRP) levels (Hartgens & Kuipers, 2004a). These side effects may lead to costly treatments, ultimately posing a significant burden on public health (Horwitz et al., 2019).

While various biochemical parameters are evaluated in the blood, they are also measured from the saliva, a non-invasive method. Some substances can alter parameters, such as salivary flow reduction observed with use of hypoglycemic drugs antidepressants, hypothyroidism, or contraceptives (Castro-Silva et al., 2017). Other effects are also observed, such as increased pH from green tea consumption, decrease in salivary pH in crack cocaine users (Woyceichoski et al., 2013) and elevated alanine aminotransferase (ALT) in patients with kidney disease (Alpdemir et al., 2018). 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 blood and salivary effects in AAS users using only AAS (confirmation by urine screening). Furthermore, several studies do not measure AAS used by urinalysis; doses are self-reported, but not compared with reference values.

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

The article has already been published as a preprint on Research Square with the following registration: <https://doi.org/10.21203/rs.3.rs-50105/v1>. The title is "Oxandrolone Use Causes Dyslipidemia in Resistance Training Practitioners," and it is available at <https://assets-eu.researchsquare.com/files/rs-50105/v1/f4fc94e5-1f25-4f9c-86d3-72980046dd60.pdf?c=1631850727>

2. Methods

Study Design

According to Gil (2017), this is a prospective, analytical, observational study of a comparative, quantitative nature.

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: 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. This study included individuals who participated in a resistance-training program in the six months before the beginning of the evaluations.

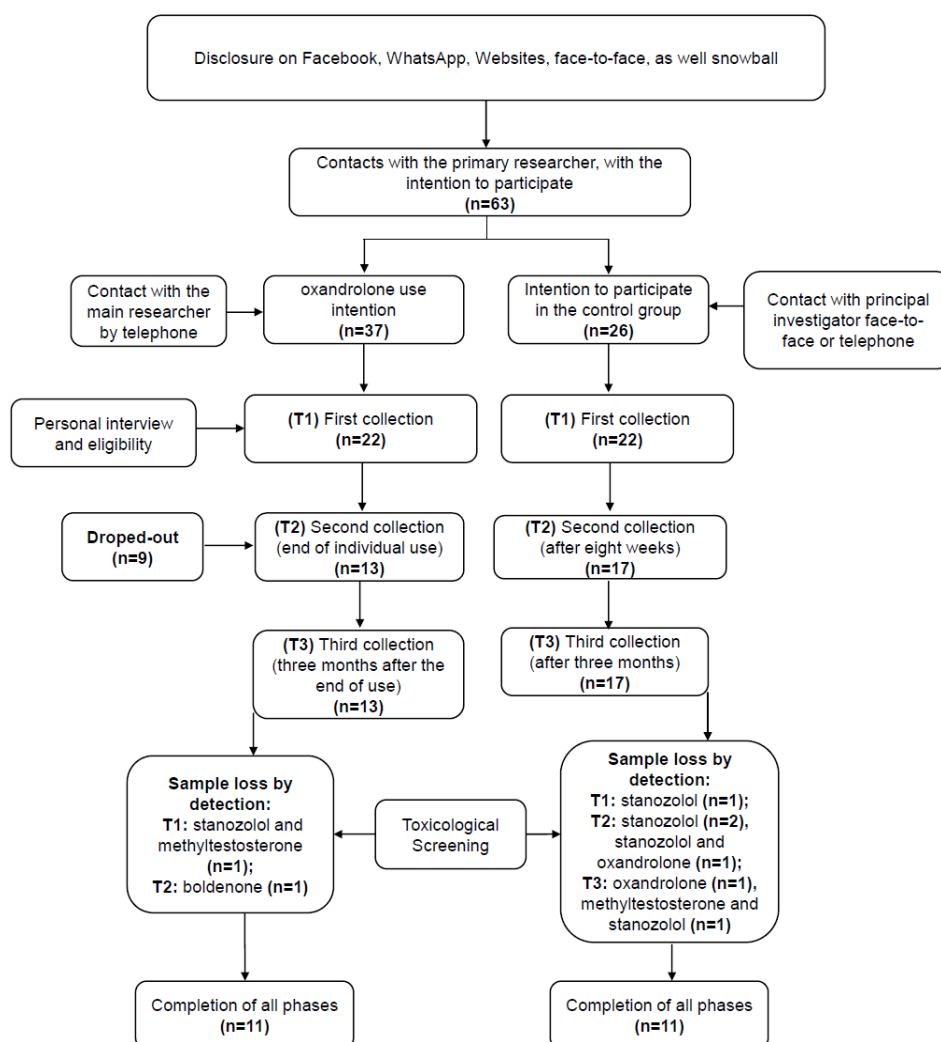
Those individuals who did not complete one or more stages of the study were excluded from the research. Other exclusion criteria were: any illness acquired during the training period and collections that interfered with the results; psychotropic drug use; consumption of more than 15 alcoholic beverages 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 saliva production.

Sample selection

Thirty-seven male subjects were initially recruited by social networks via the snowball method. 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. Finally, 22 individuals remained in the study; reasons dropping out are shown in Figure 1. We can observe some subjects dropped out from the research or, were excluded because they used AAS other than OX.

Figure 1 - Study design and allocation of the study population.



Source: Authors.

It was collected 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) (Table 1).

Table 1 - Anthropometrics and training characteristics among OG and CG (columns).

Anthropometrics and Training characteristics	OG (n=11) Mean (SD)	CG (n=11) 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)

Notes: SD: Stand Deviation; RT = Resistance Training; BMI = Body Mass Index Source: Authors.

Among OX users, all used an oral form, and dosages varied from 0.04 to 0.97 mg/kg (from 4 to 12 weeks, mean = 7.4 SD ± 2,2) (Table 2) with no participant having a similar pattern of use. Twenty-two subjects participated in all stages of the study: 11 OG and 11 CG . It is important remembering that at no time any subject was instructed regarding the amount, frequency, and time of OX use. All subjects seek the investigator of free desire. 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.

Table 2 - Individual oxandrolone use (OG), according to self-report.

Subjects	Min. Ox. (mg/kg/day)	Max. Ox. (mg/kg/day)	Weight (kg)	Weeks
1	0,75	1,00	80	7
2	0,73	0,73	82	9
3	0,39	0,39	103	9
4	0,47	0,47	85,5	7,5
5	0,47	0,47	84,6	7,5
6	0,50	0,50	80	7
9	0,51	0,51	79	4,5
8	0,56	0,56	72	6
9	0,24	1,20	83,5	4
10	0,16	0,66	122	12
11	0,06	0,45	88,4	8

Ox: oxandrolone; min: minimum; max: maximum. Calculations based on volunteers' self-report. Source: Authors.

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 (Hartgens et al., 2004). 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 study. The collection of CG T3 occurred three months after T2. Blood, saliva, and urine were all collected from 2 pm to 5 pm.

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). Hemogram 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 3-Hemogram). 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), alanine aminotransferase (ALT) (Table 4. Biochemical analysis).

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.

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 5).

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 (OX) for the three timepoints of the study. We also detected 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 test followed Wilcoxon peer comparison were made. The *p*-value adopted was < 0.05. The software used was IBM SPSS 25.0 for Windows.

3. Results

In comparisons Among groups (OG X CG) at the three timepoints (T1, T2, and T3), significant increased OG hemogram were found for: band neutrophils at T2, monocytes at T1 and platelets at T1 and T2. 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 (Table 3).

Table 3 - Hemogram comparison groups (columns), within times (rows) and reference values.

VARIABLES	TIMES	MEDIAN OG (n=11)	MEDIAN CG (n=11)	REFERENCE VALUE (Bick, 1993)
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
	T2	124,00	164,00	
	T3	216,00	97,00	
Band neutrophils	T1	70,00 ^a	79,00 ^a	0 – 700 /μL
	T2	69,00 ^{Aa}	46,00 ^{Bb}	
	T3	72,00 ^a	75,00 ^{ac}	
Segmented neutrophils	T1	4.218,00 ^a	4.187,00 ^a	1.400 – 6.600 /μL
	T2	3.933,00 ^a	3.692,00 ^b	
	T3	3.762,00 ^a	3.740,00 ^{ab}	
Lymphocytes	T1	2.075,00	2.415,00	1.200 – 3.200 /μL
	T2	2.070,00	2.067,00	
	T3	2.025,00	2.130,00	
Monocytes	T1	490,00 ^{Aa}	232,00 ^{Ba}	300 – 900 /μL
	T2	504,00 ^a	516,00 ^b	
	T3	574,00 ^a	497,00 ^b	
Platelets	T1	268.000,00 ^{Aa}	212.000,00 ^{Ba}	150.000 – 450.000 /μL
	T2	269.000,00 ^{Aa}	207.000,00 ^{Bb}	
	T3	238.000,00 ^a	211.111,00 ^{ac}	

Uppercase letters represent differences in time between groups by Mann-Whitney U test.

Lowercase letters represent differences between the moments in both groups by Wilcoxon test 2x2 comparison; Median values without letters mention the absence of any statistically significant difference. Source: Authors.

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 was above this range at T3.

There were groups (OG X CG) at the three timepoints (T1, T2, and T3), significant increased LH at T1 and triglycerides at T2 and T3. In contrast, some variables showed a reduction in OG: total testosterone at T2 and T3, calcium at T3, and ALP at T1 and T2. When blood variables were compared among timepoints (T1 x T2; T2 x T3; T1 x T3) within each treatment group separately, OG showed: 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 4).

Table 4 - Blood variables comparison groups (columns), within times (rows) and reference values.

VARIABLES		MEDIAN OG (n=11)	MEDIAN CG (n=11)	REFERENCE VALUE*
Glucose	T1	73,00	72,00	65 – 99 (mg/dL) (Oliveira et al., 2018)*
	T2	86,00	83,00	
	T3	85,00	81,00	
FSH	T1	3,38 ^a	3,01 ^a	1,27 – 19,26 (mUI/m) (Beckman Coulter, 2020)*
	T2	4,41 ^{ab}	4,91 ^b	
	T3	4,36 ^b	4,78 ^b	
LH	T1	4,26 ^A	3,00 ^B	1,24 – 8,62 (mUI/mL) (Beckman Coulter, 2020)*
	T2	3,35	4,70	
	T3	5,22	4,35	
ACHT	T1	22,00	16,00	< 46,0 (pg/mL) (Siemens Healthcare Diagnósticos S.A., 2020)*
	T2	16,90	17,10	
	T3	21,40	16,30	
Total Testosterone	T1	358,45	414,23	175,00 – 781,00 (ng/dL) (Beckman Coulter, 2020)*
	T2	240,94 ^A	414,35 ^B	
	T3	295,56 ^A	489,81 ^B	
Total cholesterol	T1	183,00	154,00	< 190 (mg/dL) (Scartezini et al., 2017)*
	T2	185,00	150,00	
	T3	159,00	143,00	
HDL †	T1	47,00^{ab}	50,00^a	>40(mg/dL) (Scartezini et al., 2017)*
	T2	24,00^a	45,00^a	
	T3	49,00^b	47,00^a	
LDL	T1	102,00 ^a	95,00 ^a	< 130 (mg/dL) (Scartezini et al., 2017)*
	T2	111,00 ^a	91,00 ^{ab}	
	T3	101,00 ^a	79,00 ^b	
Triglycerides ‡	T1	133,00^a	75,00^a	< 175 (mg/dL) (Scartezini et al., 2017)*
	T2	91,00^{Aa}	69,00^{Ba}	
	T3	177,00^{Ab}	90,00^{Ba}	
Estradiol	T1	20,00 ^a	20,00 ^a	< 47 (pg/mL) (Pierre & Ciriades, 2009)*
	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) (Pearson et al., 2003)*
	T2	0,56 ^a	0,53 ^b	
	T3	0,70 ^a	0,51 ^{ab}	
Urea	T1	42,00	39,00	17 – 43 (mg/dL) (Pierre & Ciriades, 2009)*
	T2	42,00	36,00	
	T3	39,00	40,00	
Amylase	T1	49,00	62,00	22 – 80 (U/L) (Basílio et al., 2016)*
	T2	52,00	67,00	
	T3	53,00	57,00	
Albumin	T1	4,90 ^a	4,90 ^{ab}	3,5 – 5,2 (g/dL) (Beckman Coulter, 2020)*
	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) (Pierre & Ciriades, 2009)*
	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) (Pierre & Ciriades, 2009)*
	T2	1,12	1,06	
	T3	1,03	1,01	
ALP	T1	56,00 ^A	75,00 ^B	30 – 120 (U/L) (Beckman Coulter, 2020)*
	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) (Wu, 2006)*
	T2	3,90	3,80	
	T3	3,90	4,10	
AST	T1	26,00	26,00	< 50 (U/L) (Beckman Coulter, 2020)*
	T2	27,00	25,00	
	T3	26,00	23,00	
ALT	T1	26,00	24,00	< 50 (U/L) (Beckman Coulter, 2020)*
	T2	34,00	24,00	
	T3	28,00	24,00	

Uppercase letters represent differences in time between groups by Mann-Whitney U test.

Lowercase letters represent differences between the moments in both groups by Wilcoxon test 2x2 comparison; Median values without letters mention the absence of any statistically significant differences.

* Reference value for the blood variables

† HDL (mg/dL) minimum-maximum (OG T1: 34-75; T2: 11-72; T3: 22-72)

‡ HDL (mg/dL) minimum-maximum (CG T1: 26-65; T2: 29-70; T3: 25-74)

§ Triglycerides (mg/dL) minimum-maximum (OG T1: 45-317; T2: 37-241; T3: 49-528)

‡ Triglycerides (mg/dL) minimum-maximum (CG T1: 36-127; T2: 36-93; T3: 47-140). Source: Authors.

For salivary variables, only the pH in OG was decreased, at T3 (Table 5).

Table 5 - Salivary variables comparison groups (columns) within times (rows).

VARIABLES		MEDIAN OG (n=11)*	MEDIAN CG (n=11)*
Salivary flow	T1	0,82	1,11
	T2	0,95	1,22
	T3	1,09	1,17
pH	T1	7,68	7,66
	T2	7,88	7,67
	T3	7,50 ^A	7,84 ^B
Triglycerides	T1	5,00	2,58
	T2	2,58	2,58
	T3	2,58	2,58
Urea	T1	30,40	26,30
	T2	23,40	20,90
	T3	25,90	22,40
AST	T1	14,00	12,00
	T2	15,00	8,00
	T3	9,00	9,00
ALT	T1	3,00	2,00
	T2	4,00	3,00
	T3	0,99	0,99
Phosphorus	T1	8,37	7,10
	T2	10,96	5,67
	T3	5,70	6,73
Calcium	T1	0,48 ^a	0,53 ^{ab}
	T2	0,35 ^a	0,23 ^a
	T3	0,51 ^a	0,38 ^b

Median values without letters mention the absence of any statistically significant difference. *Reference values for the saliva variables there are no consensus on literature or standardized methodologies. Source: Authors.

4. Discussion

This study explored the effects of OX on blood and saliva compared with reference values during and after the OX cycle. To our knowledge, this is the first study that evaluated blood and salivary parameters in RTP that used only OX validated by urine screening.

Despite the alteration on lipidic profile by several AAS concomitant use has been established in the literature (Arazi, 2018), concerning the only use of OX, we found reducing levels of HDL cholesterol shortly after use of OX, returning to normal after three months.

According to (Bates et al., 2019), low levels of HDL cholesterol, as well as high levels of triglycerides and LDL cholesterol, are important risk factors for cardiovascular disease. Considering only HDL cholesterol, our results indicate the effect of OX use can be returned after three months washout. Despite HDL returning to normal, users had hypertriglyceridemia three months after using OX. These data point to dyslipidemia in OX users.

In a previous study (Cheung et al., 1980) OX was tested as a cholesterol-lowering drug. The authors proposed 7.5 mg/day for three months, followed by two months washout showed HDL decreased significantly. An investigation focusing on metabolic syndrome, used 10 mg/day oral OX doses for one week and found reductions in HDL levels with marked increases in hepatic ketogenesis, increasing influx fatty acids into the liver. Other paper purposed Klinefelter syndrome treatment, with oral OX 0.06 mg/kg/day (therapeutic purpose) or placebo for two years, HDL was lowered (Davis et al., 2017). A study with HIV-infected men that was not controlled antiretroviral intake treated with OX daily for 12 weeks reduced HDL for all doses of OX used (Grunfeld et al., 2006).

Although high AAS doses are used for short periods of time, many athletes abuse these ones, self-administering doses up to 100-and even 1000-fold, more than safe doses, producing circulating testosterone levels two to three orders of magnitude above healthy male reference range, often for prolonged periods (Graham et al., 2008). The maximal anabolic dose of testosterone is not known, but almost certainly vastly exceeds 600 mg of testosterone a week (Cheung & Grossmann, 2018). Excess AAS dose cause dyslipidemia secondary, increasing total cholesterol, create no changes in triglycerides, and decrease HDL (Souza et al., 2019). 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.

Other lipid profile variable we observed was triglyceride, which remained high. This point is important, as the scientific literature points to the AAS action on fat metabolism (Rosca et al., 2019) and can promote negative impacts on the cardiovascular system.

In an animal model, OX elevated triglycerides, reduced HDL, decreased hepatic triglycerides, and increased levels of non-esterified fatty acids produced by the liver. This could possibly lead to increased lipid synthesis of hepatic triglycerides (Rosca et al., 2019). Additionally, OX may reduce blood triglycerides by activating triglyceride lipase, which results in the hydrolysis of peripheral triglycerides. However, once the intake of OX ceases, triglycerides tend to increase (Glueck et al., 1973).

For comparison, we used Brazilian reference values for triglycerides in this study. The results indicate a significant increase, almost to the borderline level, posing a risk for cardiovascular disease after stopping OX use (Oliveira et al., 2018). According to WHO classification, individuals with triglyceride levels higher than 180 mg/dL are considered at risk for cardiovascular disease.

Furthermore, the results for triglycerides in this study align with findings from other research, showing increased lipolysis and the release of free fatty acids with OX use. However, three months after discontinuing OX, triglyceride levels remained above the reference standards. This contradictory result may be explained by factors such as varying OX doses,

different monitoring periods, resistance exercise practices, duration of use, cycle types, and other variables. It is possible that a washout period of three or more months could bring these parameters back to normal (Hartgens & Kuipers, 2004).

Our research also observed some hemogram variation in hemoglobin, band neutrophils, monocytes and platelets (note Table 4). Assessed components were within the reference limits described in the consolidated literature as well blood variables (triglyceride exception at T3).

Despite this, our research presents relevant methodological criteria that were used in the study: comparison with reference values and the detection urine screening, among others. Studies show that the AAS cycle (duration use) can last from 10 to 12 weeks (Llewellyn's, 2010) close to the timeframe of this study. It is important that we evaluated a single, isolated AAS.

Limitations of our study must 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 substance, and its prescription is made only by physicians.

5. Conclusions

Oxandrolone users had a reduction in HDL levels after ending the use of OX, returning to reference values three months ending the use of OX, which was not observed for triglycerides, which remained high. The results of this study suggest that oxandrolone use is associated with changes in lipid profiles, including lower HDL levels and higher triglyceride levels, which are characteristic of dyslipidemia. However, while these findings indicate an association, they do not establish definitive causality. Further research, including well-controlled longitudinal studies, is needed to confirm the causal relationship between oxandrolone use and dyslipidemia.

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