PONTIFÍCIA UNIVERSIDADE CATÓLICA DO PARANÁ

ESCOLA DE CIÊNCIAS DA VIDA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO BIOCIÊNCIAS

DÊNIS DE LIMA GREBOGGY

OXANDROLONE USE CAUSES DYSLIPIDEMIA IN RESISTANCE TRAINING PRACTITIONERS

Curitiba
2020

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Tese apresentada ao Programa de Pós-Graduação em Odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos para obtenção do título de Doutor em Odontologia, Área de Concentração em Biociências.

Orientadora: Profa. Dra. Aline Cristina Batista Rodrigues Johann
Coorientador: Prof. Dr. João Armando Brancher

Curitiba
2020

Dados da Catalogação na Publicação
Pontifícia Universidade Católica do Paraná
Sistema Integrado de Bibliotecas – SIBI/PUCPR
Biblioteca Central
Luci Eduarda Wielganczuk – CRB 9/1118

G788o
2020

Greboggy, Dênis de Lima
Oxandrolone use causes dyslipidemia in resistance training practitioners / Dênis de Lima Greboggy ; orientadora: Aline Cristina Batista Rodrigues Johann ; coorientador: João Armando Brancher. – 2020.
50 f. : il. ; 30 cm

Tese (doutorado) – Pontifícia Universidade Católica do Paraná, Curitiba, 2020
Bibliografia: f. 20-36

1. Odontologia. 2. Oxandrolona. 3. Sangue. 4. Saliva. 5. Treinamento de resistência. 6. Dislipidemias. I. Johann, Aline Cristina Batista Rodrigues. II. Blancher, João Armando. III. Universidade Católica do Paraná. Programa de Pós-Graduação em Odontologia. IV. Título.

CDD 20. ed. – 617.6

Pontifícia Universidade Católica do Paraná
Escola de Ciências da Vida
Programa de Pós-Graduação em Odontologia

TERMO DE APROVAÇÃO

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Curitiba, 06 de março de 2020.

AGRADECIMENTOS

Escrever agradecimentos é algo difícil para mim, pois no meu caso, são inúmeras às pessoas que auxiliaram nesta jornada, desde 2016 até agora, 2020. Seria injusto esquecer de alguém. Ainda, a ordem em que estão citados aqui não refere grau maior ou menor de importância. Entretanto, vamos lá!!!

Agradeço a essa força Universal, aos que para um é DEUS, ou energia, ou cosmos, enfim. Acredito à minha forma que Deus esteve sempre comigo nessa jornada, e se passei tudo o que passei, tudo teve sua hora, seu momento.

Aos meus pais Reinaldo, Selma, e ao meu irmão Deiver, por tudo o que fizeram e significam para mim. Eu me espelho em cada um de vocês e AMO DEMAIS todos. Obrigado minha família amada!

À minha orientadora Professora Doutora Aline Cristina Batista Rodrigues Johann, por ter me aceito como doutorando, e ao meu Coorientador Professor Doutor João Armando Brancher, por me auxiliarem em tudo, vocês são pessoas maravilhosas e estiveram sempre ao meu lado. Levo em meu coração, vida e profissão, o profissional e pessoa que vocês são.

Professora Aline, obrigado de coração por todos os conselhos e orientações que me destes, sempre com confiança, serenidade e me encorajando a jamais desistir, quando eu estava prestes. Certamente isso fará um Dênis bem diferente.

Professor João Armando, com todo seu jeito peculiar de ouvir e orientar, meu muito obrigado.

À PUCPR, por me proporcionar meu trabalho que tanto amo, juntamente ao PPGO, com a possibilidade de realizar o doutorado com uma bolsa. Isso foi preponderante para eu dar continuidade aos estudos. Muito obrigado.

Ao pessoal do laboratório de Bioquímica: Ana Cláudia, Larissa, Daiane e demais pessoas que me pegaram pela mão e me ajudaram, desde o dia em que pela primeira vez entrei para dar início à pesquisa.

Ao Doutor José Stori e ao pessoal do Laboran, por todo o carinho e consideração nos momentos em que precisei.

Ao Tiago Franco e Thiago Beltrami, pela ajuda. Sem vocês isto não teria acontecido. Considero vocês meus irmãos de doutorado. Aprendi muito. Valeu gente.!!!

À professora Maria Heloísa, Julia Couto, Gisele Paiva, Heloísa, Leonardo, Rafael, Jackson e Paulo, Pamela e Professora Doutora Flávia, por tudo o que fizeram por mim, nunca negaram me ajudar quando eu ligava desesperado para as coletas.

Aos professores da banca de Qualificação e Defesa, pelas considerações e apontamentos, que certamente darão maior robustez para esta pesquisa.

Ao meu amigo e irmão Professor Doutor Ericson Pereira. Um maluco que teve uma ideia sensacional, da qual eu pude herdar e dar sequência. Não cometa mais loucuras, por favor!!! (risos).

A todos os voluntários que participaram da pesquisa, desde as etapas primeiras até o último dia. Vocês moram no meu coração. Jamais negaram nada, e sempre me incentivaram a ir até o final.

Ao professor Sérgio Ignácio, um gênio na estatística, que NUNCA me negou ajuda. Admiro sua pessoa, o profissional e profissão. Adoro estatística e aprendi muito com o senhor. Além de companheiros de profissão, somos grandes amigos. Obrigado mestre.

Ao meu colega Yesudian, vulgo Iam. Agradeço por toda a força.

À Neide, sempre ajudando, sorridente, contanto eternamente meus créditos. (Risos)

Obrigado meu amigo Luis Bauer, por me apoiar várias vezes durante o processo para estudarmos juntos, desabafarmos e trocarmos ideias sobre inúmeras coisas da vida. Valeu Bauer, o grande mister!

Ao Renan, ex-aluno e colega de profissão, trocamos várias ideias e pudemos estudar e escrever juntos, cada um seu estudo, mas com apoio sempre. Valeu gurizinho!

Ao meu grande nobre atual coordenador, amigo, veterano, Professor Doutor Rafael Kanitz Braga, e ao Professor Doutor Paulo Cesar Barros, os quais me apoiam até hoje em minha jornada acadêmica e profissional. “Isto posto meus nobres, vejam como foi”!!!!! (Risos).

Aos meus doutores, que escutaram e me acalmaram, para eu sempre ter paciência e reduzir a ansiedade, Dra. Roze e Dr. Marlon, vocês fizeram o Dênis “entrar nos eixos”. Muito obrigado!!!!

Aos meus pais acadêmicos, Ninão, Claudio Tkac (vulgo tic-tac) e Ricardo Weigert Coelho. Sou o que sou porque estive sempre sobre os ombros de gigantes.

Aos meus colegas de profissão da PUCPR, professores, colaboradores, estudantes, pessoal do GECOM e demais pessoas que acreditaram sempre no meu potencial, a força de todos vocês fez um Dênis mais forte.

Aos que já se foram, familiares e amigos que tive, obrigado, vocês estão no meu coração.

SUMÁRIO

Title page

Oxandrolone use causes dyslipidemia in resistance training practitioners

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Abstract

The purposes of this study were to compare blood and salivary, parameters in only males resistance training practitioners, users of oxandrolone considering reference values, to compare these with a control group three times, and correlate salivary and blood parameters. In this prospective analytical observational study, in twenty-two individuals: Oxandrolone Group, n = 11 and Control Group n = 11 were collected blood, saliva, and urine, and it was analyzed at three moments: before, in cessation use, and three months after oxandrolone use. Complete blood count, lipid profile, metabolites, and enzymes were analyzed in the blood. In saliva were analyzed salivary flow, pH, triglycerides, urea, aspartate transaminase, alanine aminotransferase, phosphorus, calcium. Urine analysis was used for toxicological screening. U Mann-Whitney tests, Chi-Square, Friedman's ANOVA, and Spearman correlation tests were performed, with significance $p < 0.05$. It was found only lower blood HDL level for Oxandrolone Group (24 mg/dL) comparing with reference values (>40 mg/dL) immediately when its use ceased, returning to normal levels three months later (49 mg/dL, >40 mg/dL) and higher triglyceride level (177 mg/dL) comparing with reference value (<175 mg/dL) three months after use. Although there are distinct differences between groups and times, this did not show clinical relevance, as they were within typical values. There was no correlation between blood, saliva. Oxandrolone causes changes in the lipid profile of users.

Key-words: Oxandrolone, Blood, Saliva, Resistance Training, Anabolic Agents, Dyslipidemia

INTRODUCTION

Approximately 75% of adults make practice physical activity in compliance with WHO guidelines [1]. A 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 analogues with anabolizing activity [4–6]. These substances are used to therapeutically in different dosages (for example 20mg / day for 12 weeks) [7] for lack of aesthetics, ineffective weight maintenance [8], maintenance of skeletal muscles [9,10] and sexual functions [11], stabilization of body functionality, such as fundamental movements like walking and squatting in individuals who need weekly hemodialysis [12] and 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], the rapid loss of body fat [10] among other goals. Among several types of AAS, oxandrolone (OX) stands out is one of the most used [23,23–26] and have high-frequency use (current and former users) with 45.8% (N= 719; n= 329) in resistance training practice (RTP) [27]. OX is used to increase performance [28–31], as well as to improves the net protein balance and lean body mass in burned patients [32,33], Klinefelter Syndrome, metabolic diseases [34], as well as oral pathologies and treatment of individuals with HIV [33].

There are several AAS side effects 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, gynecomastia and

others [35], increased low-density lipoprotein (LDL), decreased high-density lipoprotein (HDL) [36–38] reduction in luteinizing hormone (LH) [39,40], follicle-stimulating hormone (FSH) [37,41,42] blood testosterone decreased [43,44], increase as well in liver enzymes such as alanine transaminase (ALT) [38], aspartate transaminase (AST) [45] and basal blood showing increased hemoglobin [18,46], high hematocrit counts [47], high inflammatory markers [48], including high white blood cell count [28], high neutrophil count [49], and C-reactive protein (CRP) [50,51]. Thus, these may generate costs with side effects treatment and therefore, a negative impact for public health [52].

While various biochemical parameters are evaluated in the blood, they are also measured in the evaluation of saliva and 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 that can be observed in the use of hypoglycemic drugs [55], antidepressants, hypothyroidism, and contraceptive [56], other effects are also observed, such, increased pH from green tea [57], decrease in salivary pH in crack users [58] and elevated and alanine aminotransferase (ALT) [59] in patients with kidney disease [60]. Even though these studies evaluating different drugs, none in the previous study has verified the salivary effect of OX. Therefore, even the present moment, for our knowledge, there are not studies that aimed to verify and correlate the blood and salivary effects in AAS users volunteered using only one isolated AAS with confirmation by urine metabolite. However, many studies do not measure the amount of AAS used by the individuals, by urine analysis, and there are self-report doses used, but nor compared with reference values [12,30,69,61–68], and still, to directly certify the OX use by urine.

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

MATERIAL AND METHODS

This is a prospective analytical observational study approved by the local Ethics Committee, under protocol number 2.556.109.

Inclusion and exclusion criteria

The subjects were recruited through the dissemination of the research on social networks, websites, WhatsApp, and snowball technique. Information about volunteers from resistance training programs were included in the study. They would be males, have to express intention of OX use, being six months in AAS washout or never have used AAS, self-report of no drug treatment and history of cardiovascular, respiratory, hepatic, renal, musculoskeletal and metabolic disorders [70]. It was included on this study individuals who participate in a resistance training program in the six months before the beginning of the evaluations; Self-report of no drug treatment and history of cardiovascular, respiratory, hepatic, renal, musculoskeletal, and metabolic disorders [70]; Never having used AAS [71].

Those individuals who did not complete any stage of the study were excluded from the research; any illness acquired during the training period and collections that interferes with the results; psychotropic drug users; Individuals who consume more than 15 doses of alcoholic beverage per week (≅ 30g/day); have fixed or mobile

orthodontic braces, removable total or partial denture, or fixed denture and being smoker. This is because these factors can interfere with the production of saliva [72].

Sample selection

Thirty-seven male subjects were recruited initially by social networks, as snowball method [62,73–76] The researcher was approached by the subjects (by telephone) who had intention to make OX use, while the 26 subjects in the CG contacted the researcher. Forty-four subjects first performed a personal interview, and they were clarified that nobody and at no time would provide any AAS substance, information on the time of use, and dosages. The participants gave their consent to participate and would have no information about places of purchase, dosages, and time of use. Sociodemographic data were 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 and frequency about resistance training practice (years), as well training session time per week (minutes/day). Finally, 22 individuals remained in the study, being the reasons for sample dropped-out, as shown in figure 1.

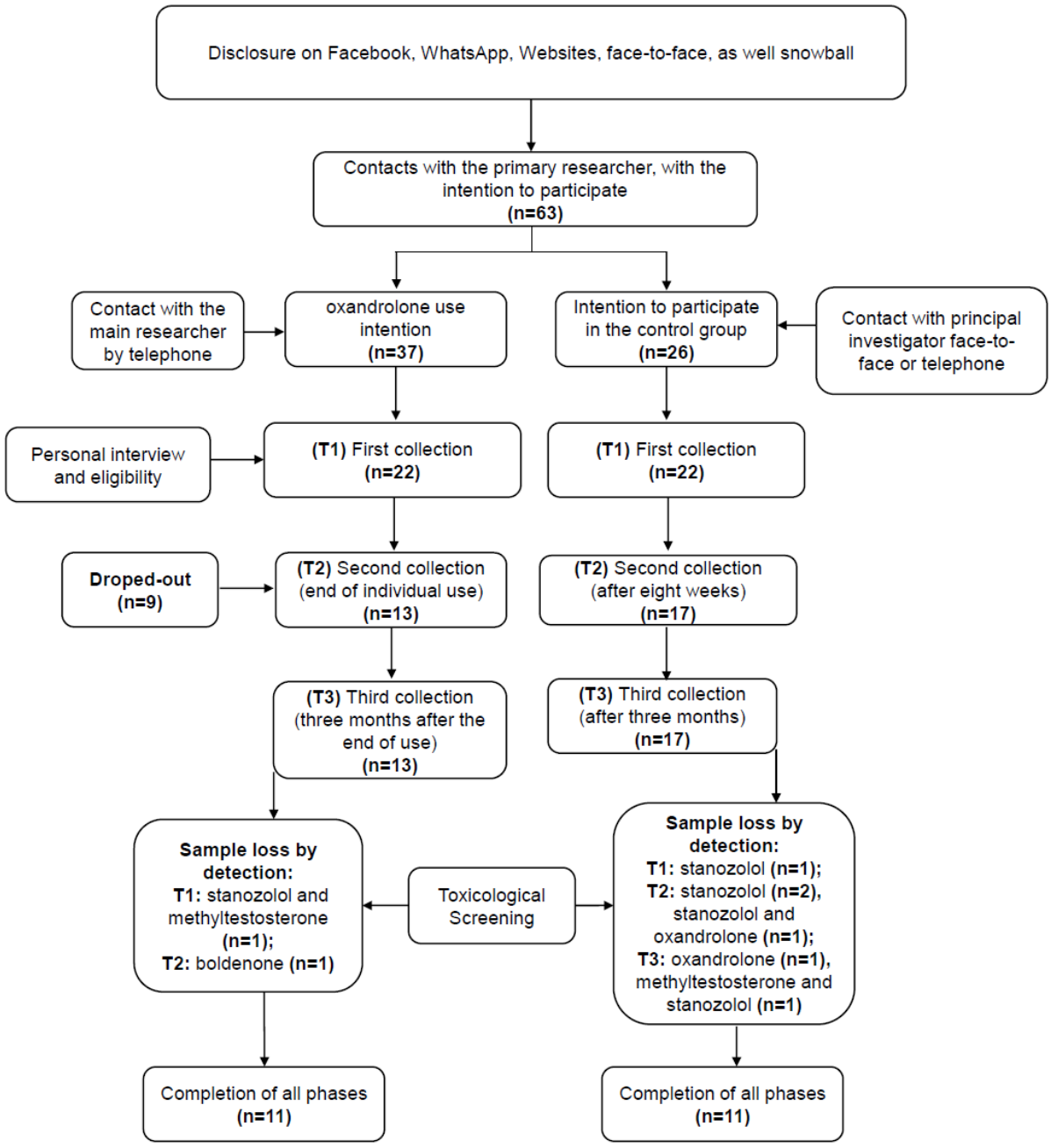

Figure 1. Study design and allocation of the study population.

Twenty-two subjects participated in all stages of study: 11 OG; 11 CG (Table 1). Among OX users, all of them used oral form, and dosages varied enter 0.04 and 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 internet, ready to use and with registered trademarks.

Samples collection moments

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

Blood collection and analyses

The blood collection took place at the Biochemistry laboratory, located at the School of Life Sciences of Biochemical Laboratory located at Pontifical Catholic University of Paraná and there was no indication of fasting. A nurse collected blood by radial artery puncture and processed on the same day. Plasma biochemical analyses were performed on a AU480 Chemistry Analyzer (Beckman Coulter, Pasadena, CA), except for the glucose measurement that was performed by the Cobas Mira Plus device (Roche Diagnostic Systems, Basel, Switzerland). The reagents used were pro analysis grade (Kovalent, São Gonçalo, Brazil). The hormones were measured on the UniCel DxI 800 Access Immunoassay System (Beckman Coulter, Pasadena, CA) and the blood count on the Coulter LH 750 Hematology Analyzer (Beckman Coulter, Pasadena, CA). For blood, counting was done: erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, red blood cell distribution width, leukocytes, basophils, eosinophils, band neutrophils, segmented neutrophils,

lymphocytes, monocytes, platelets, glucose, follicle-stimulating hormone, luteinizing hormone, adrenocorticotropic hormone, total testosterone, total cholesterol, high-density lipoprotein, low-density lipoproteins, triglycerides, estradiol, c-reactive protein, urea, amylase, albumin, calcium, creatinine, alkaline phosphatase, phosphorus, aspartate transaminase, alanine aminotransferase.

Saliva collection and analyses

The saliva was collected by masticatory stimulation using a 1 cm sterile piece of rubber stimulator, occurring for 1 minute for oral self-cleaning and 5 minutes for chewing to deposit all the saliva produced in an 80ml universal collection flask and right after pH measurement with 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, the 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 Cobas Mira Plus (Roche Diagnostic Systems, Basel, Switzerland). Bioclin reagents (Bioclin/Quibasa, Belo Horizonte, Brazil) were used for analysis. The methodology followed was according to the manufacturer's recommendations. The sensitivity and method linearity are described in supplementary Table 1. The salivary variables evaluated were salivary flow, pH, triglycerides, urea, aspartate transaminase, alanine aminotransferase, phosphorus, calcium.

Urine collection and AAS Screening

Urine samples were self-collected and analyzed according to Campos et al., (2005) [82], with modifications. Is was used UHPLC-MS/MS to quantify oxandrolone metabolites in the three moments of the study and also to detect other AAS (see Supplementary Text 1).

Statistical analysis

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

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, Oxandrolone group (OG) presented a higher level for HDL in T2, returning to normal levels in T3, and triglyceride level remained with typical values in T1 and T2 and above in T3 (Table 2). For other blood, salivary, and urinary testosterone variables, values found are in accordance with reference values.

Comparisons only between groups (OG X CG) at the three times (T1, T2, and T3), significant increased OG blood values were found for: band neutrophils at T2; monocytes at T1; platelets in T1 and T2; LH at T1; and triglycerides in T2 and T3. On the other hand, some variables showed a reduction in OG for total testosterone at T1 and T2, calcium in T3, and ALP in T1 and T2. For salivary metabolites, only pH in OG decreased at T3. Moreover, OX urinary levels in OG increased in T2, reducing at T3.

Comparing HDL and triglycerides from the stratified oxandrolone group by moments of use in weeks, there were no significant differences (see Supplementary Table 3).

When blood variables were compared between times (T1 x T2; T2 x T3; T1 x T3) within each group separately, the OG showed: counts hemoglobin reduction from T1 to T2; FSH increased from T1 to T3; HDL increase from T2 to T3; increased triglycerides from T1 and T2 to T3; increased estradiol from T1 and T2 to T3 and calcium reducing from T1 and T2 to T3.

Table 2. Results of Hemogram count, blood, salivary and urine variables (Groups X Times)

		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
	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}**	
Blood variables				
Glucose	T1	73,00	72,00	65 – 99 (mg/dL) [84]
	T2	86,00	83,00	
	T3	85,00	81,00	
FSH	T1	**3,38^{a}**	**3,01^{a}**	1,27 – 19,26 (mUI/mL) [85]
	T2	**4,41^{ab}**	**4,91^{b}**	
	T3	**4,36^{b}**	**4,78^{b}**	

Continuation Table 2

LH	T1	**4,26^{A}**	**3,00^{B}**	1,24 – 8,62 (mUI/mL) [85]
	T2	3,35	4,70	
	T3	5,22	4,35	
ACHT	T1	22,00	16,00	< 46,0 (pg/mL) [86]
	T2	16,90	17,10	
	T3	21,40	16,30	
Total Testosterone	T1	**358,45**	**414,23**	175,00 – 781,00 (ng/dL) [85]
	T2	**240,94^{A}**	**414,35^{B}**	
	T3	**295,56^{A}**	**489,81^{B}**	
Total cholesterol	T1	183,00	154,00	< 190 (mg/dL) [87]
	T2	185,00	150,00	
	T3	159,00	143,00	
HDL	T1	**47,00^{ab}**	**50,00^{a}**	> 40 (mg/dL) [87]
	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) [87]
	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) [87]
	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) [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	
	T3	26,00	23,00	
ALT	T1	26,00	24,00	< 50 (U/L) [85]
	T2	34,00	24,00	
	T3	28,00	24,00	

Continuation Table 2

Salive variables				
Salivary flow	T1 T2 T3	0,82 0,95 1,09	1,11 1,22 1,17	>0,7 ml/min [92]*
pH	T1 T2 T3	7,68 7,88 **7,50^{A}**	7,66 7,67 **7,84^{B}**	(6,1 – 8,0) [93]*
Triglycerides	T1 T2 T3	5,00 2,58 2,58	2,58 2,58 2,58	4,88 ±0,21 (mg/dL) [94]*
Urea	T1 T2 T3	30,40 23,40 25,90	26,30 20,90 22,40	30,75 ±9,63 (mg/dL) [95]*
AST	T1 T2 T3	14,00 15,00 9,00	12,00 8,00 9,00	20,8 ±23,9 (U/L) [60]*
ALT	T1 T2 T3	3,00 4,00 0,99	2,00 3,00 0,99	8,67 ±12,9 (U/L) [60]*
Phosphorus	T1 T2 T3	8,37 10,96 5,70	7,10 5,67 6,73	6,6 ±5,1 (mg/dL) [94] *
Calcium	T1 T2 T3	**0,48^{a}** **0,35^{a}** **0,51^{a}**	**0,53^{ab}** **0,23^{a}** **0,38^{b}**	3,4 ±2,7 (mg/dL) [96]*
Urine variables				
Testosterone	T1 T2 T3	22,18 12,89 19,15	11,19 13,55 12,83	41.7 ± 34.8 (ng/mL) [97]*
OX	T1 T2 T3	**0,00^{a}** **31,35^{Ab}** **0,00^{ab}**	**0,00^{a}** **0,00^{Ba}** **0,00^{a}**	(ng/mL)

NOTES: Uppercase letters represent differences in time between groups by U Mann Whitney test.
Lowercase letters represent differences between the moments in both groups by Friedman's 2x2 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 (see Supplementary Table 4)

DISCUSSION

To our knowledge, this is the first study that evaluates the blood and salivary parameters in OX users, with confirmation by urine metabolite quantification.

Although there are differences between groups and times, the values of the dosed components were within the reference limits described in the literature. The main results show that there was no correlation amongst blood, saliva, and urine in OG. However, higher presence of the inflammatory cells and lipids in OG are suggestive of the inflammation and dyslipidemia.

Regarding the education levels of the subjects, our results differ from previous others [98], where most of the subjects studied have completed high school, followed by higher education in progress. Our study showed that part of the subjects had complete higher education. However, the current research is in line with other studies, which revealed a higher prevalence among people with complete secondary and higher education, with specialists in the profession, with aesthetic aspects as the primary motivation for the use of AAS [99,100]. Therefore, the 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, 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 returned to normal, users had isolated hypertriglyceridemia three months after use OX. These data point to dyslipidemia in OX users.

Interesting, in a previous study, 1980 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. At same time, observed high cholesterol levels [103]. Another study with subjects with metabolic syndrome used oral OX with doses of 10 mg/day for one week also found reductions in HDL levels and marked increases in hepatic ketogenesis. This fact is due to an increase in the 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]. A study aimed at treatment with Oral OX 0.06 mg/kg/day or placebo for two years, HDL was lower in Klinefelter Syndrome treatment [105]. In our study, the reduction in HDL was observed, but was also seen that immediately after cessation of OX use, HDL levels return to normal, despite the differences of the research subjects. Meantime, triglyceride levels remained high pointing to the action of OX on fat metabolism and impacting negatively on the cardiovascular system.

Although the use of anabolic steroids in high doses is used for short periods of time [106], many athletes abuse these anabolic steroids and self-administer doses up to 100 - to even 1000-fold more than safe doses, producing circulating testosterone levels two to three orders of magnitude above the healthy male reference range, and 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], cause dyslipidemia secondary to drugs increasing total cholesterol, without changes in triglycerides and a decrease in HDL [108,109]. In the present study we were unable to determine the exact dose of OX used by the volunteers, but we can suggest that they

were supraphysiological doses and they caused a consistent effect of the OX on HDL production.

It's not new that OX is used for therapeutic purposes in different situations [78,79,110–112]. A study with HIV-infected men without controlling antiretroviral intake provided that used of OX daily, for 12 weeks, provided a reduction in HDL for all doses of OX studied [33]. Even in another animal model, OX elevated triglycerides, reduced HDL , and also decreased hepatic triglycerides, as well as tending to elevate levels of non-esterified fatty acids 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, increased triglycerides [114]. For the purpose of comparison, we used in this study the reference values to Brazilian population for triglycerides and the results points to a significant increased, almost borderline, for cardiovascular disease after ceasing Ox use. It is important to mention that, according WHO classification, individuals who have triglyceride level higher than 180 mg/dl are risk groups for cardiovascular disease. Besides that, the results found here for triglycerides were consistent with other studies, this is, increased lipolysis and liberation of free fatty acid with the use of OX [78,79,110–112], however, three months after ending use, this variable was higher than the reference standards.

Maybe this contradictory result could be explained by the management of OX, including different doses, different monitoring times, and the practice of resistance exercises, days of use, types of the cycle, and others. Also, the short time of evaluation after use could be a variable. Washout of three or more months probably take these parameters back to normal [78,115]. Despite all these biases, this research presented relevant methodological criteria that were used in the study, for example, comparison

with reference values, and the detection of metabolite in urine. Studies shows that AAS cycle (duration use) [116] can last from 10 to 12 weeks [4,117–119], which close to this study. It is important as well as evaluating just one AAS. Other studies have not reported standardization in blood sample collection times [70,120], in contrast to a previous study (10 to 12 a.m.) [51].

Limitations of our study have to be considered. The results found in our study were, therefore, different from the studies mentioned above, where they did not follow the subjects several months after ending the use of AAS. We did not make a standardized dose, or cycles limited because the OX is a controlled drug, and its prescription just is performed by physicians. Other limitation is a family history of atherosclerotic disease. The micro 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, however, taken from previous research, for comparative purposes about what OX can change, and what implications this can have. Besides, even though there is no consensus in the literature on these values, the use of salivary markers would be a non-invasive method, cheaper than blood tests, and that could be a facilitator for future studies.

CONCLUSION

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

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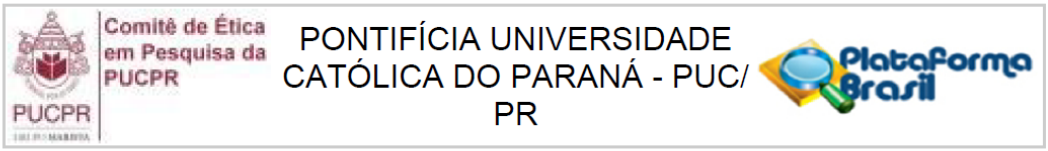
ANEXOS

Parecer de comitê de ética



PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: PARÂMETROS SÉRICOS E SALIVARES EM PRATICANTES DE MUSCULAÇÃO USUÁRIOS OU NÃO DE ESTEROIDES ANABOLIZANTES

Pesquisador: Ericson Pereira

Área Temática:

Versão: 2

CAAE: 55365016.2.0000.0020

Instituição Proponente: Pontifícia Universidade Católica do Parana - PUCPR

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.556.109

Apresentação do Projeto:

A pesquisa está dividida em 2 etapas, na primeira será realizado um estudo transversal para identificar a prevalência de usuários de anabolizantes praticantes de musculação na cidade de Curitiba. Na segunda etapa será realizado um estudo longitudinal (caso-controle) comparando os parâmetros séricos e salivares pré ciclo e pós ciclo.

Objetivo da Pesquisa:

Objetivo Primário:

Este estudo tem como objetivo geral verificar parâmetros salivares e sanguíneos em praticantes de musculação usuários e não usuários de EA

Objetivo Secundário:

• Identificar a prevalência do uso de EA nas academias de musculação da cidade de Curitiba, bem como as características desses indivíduos;• Identificar os níveis séricos e salivares: de testosterona, hormônio luteinizante (LH), hormônio folículo estimulante (FSH), hormônio adrenocorticotrófico (ACTH), Estrogênio, amilase, proteína reativa C, fosfato inorgânico, cálcio, ureia, creatinina, plaquetas, eritrócitos, colesterol total, HDL, LDL, Glicemia, Alanina Aminotransferase (ALT), Aspartato Aminotransferase (AST), Fosfatase Aminotransferase (ALP), o tipo e a quantidade de EA;• Quantificar e qualificar os microrganismos presentes na saliva desses indivíduos;• Comparar essas variáveis entre praticantes de musculação (usuários e não usuários de

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EA), pré e pós ciclo;• Verificar a associação entre os parâmetros séricos e os parâmetros sialoquímicos e sialométricos.

Avaliação dos Riscos e Benefícios:

Riscos:

Na primeira etapa os participantes podem sentir algum desconforto ao responder ao questionário por se tratar de um tema polêmico, o qual pode expor a intimidade do participante. Mas o questionário preserva o sigilo do participante, a não ser que o mesmo tenha interesse em participar da segunda etapa, daí ele terá a opção de se identificar. Também, será esclarecida a importância do estudo e que o participante não está sendo julgado por seus atos, mas que a colaboração no estudo pode ajudar a compreender as questões em torno do uso de EA na sociedade.Na segunda etapa os participantes terão o desconforto da coleta das amostras de sangue, os quais podem sentir uma dor como uma picada de abelha.

Benefícios:

Na primeira etapa terão o benefício de contribuir para identificar a prevalência do uso de EA em academias de musculação na cidade de Curitiba.Na segunda etapa os mesmos terão acesso ao seu exame, bem como aqueles que apresentarem alguma alteração nos resultados dos exames serão encaminhados para avaliação no ambulatório de endocrinologia do Hospital Nossa Senhora da Luz.

Comentários e Considerações sobre a Pesquisa:

Projeto de pesquisa relevante, metodologicamente adequado.

Considerações sobre os Termos de apresentação obrigatória:

TCLE claro, objetivo, preserva o sujeito de pesquisa.

Recomendações:

nenhuma

Conclusões ou Pendências e Lista de Inadequações:

Projeto aprovado

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_1074307_E1.pdf	16/02/2018 09:57:36		Aceito

Outros	Divulgacao_pesquisa.jpeg	16/02/2018 09:47:11	Dênis de Lima Greboggy	Aceito
Outros	Metodologia_Urina.docx	16/02/2018 09:45:44	Dênis de Lima Greboggy	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_2_CORRIGIDO.doc	16/02/2018 09:34:49	Dênis de Lima Greboggy	Aceito
Outros	Quest_EA_1.doc	18/04/2016 17:05:02	Ericson Pereira	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_2.doc	18/04/2016 17:02:43	Ericson Pereira	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_1.docx	18/04/2016 17:02:27	Ericson Pereira	Aceito
Outros	Metodologia.doc	18/04/2016 17:02:06	Ericson Pereira	Aceito
Outros	Aut_Inst.doc	18/04/2016 17:01:44	Ericson Pereira	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO_Ericson5.doc	18/04/2016 17:00:30	Ericson Pereira	Aceito
Folha de Rosto	FR.pdf	18/04/2016 16:59:49	Ericson Pereira	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CURITIBA, 22 de Março de 2018

Assinado por:
NAIM AKEL FILHO
(Coordenador)

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Bairro: Prado Velho **CEP:** 80.215-901
UF: PR **Município:** CURITIBA
Telefone: (41)3271-2103 **Fax:** (41)3271-2103 **E-mail:** nep@pucpr.br

TCLE - Termo de consentimento livre e esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Você está sendo convidado(a) como voluntário(a) a participar do estudo *"Aplicação de Testes Sialoquímicos e Sialométricos em Praticantes de Musculação Usuários e Não Usuários de Esteroides Anabolizantes"* e que tem como objetivo verificar parâmetros salivares e sanguíneos em praticantes de musculação usuários ou não usuários de esteroides anabolizantes (EA). Acreditamos que ela seja importante porque podem ocorrer alterações na saúde de pessoas que fazem o uso desses EA, principalmente em parâmetros cardíacos, renais e hepáticos.

PARTICIPAÇÃO NO ESTUDO

A minha participação no referido estudo será de (1) realizar uma coleta de amostra salivar, que consiste em cuspir em um tubo de ensaio; (2) realizar uma coleta de amostra de sangue, que consiste em fornecer uma pequena quantidade de sangue retirada do meu braço; (3) essas coletas serão realizadas em dois momentos, com intervalo de aproximadamente 9 semanas; (4) realizar uma coleta de amostra de urina, que consiste em fornecer uma pequena quantidade de urina num frasco de coleta.

RISCOS E BENEFÍCIOS

Fui alertado de que, da pesquisa a se realizar, posso esperar alguns benefícios, tais como: avaliar minha condição de saúde por meio dos testes bioquímicos identificando se os indicadores cardíacos, renais e hepáticos estão dentro dos padrões de normalidade, além de ter acesso ao resultado do meu exame. Recebi, também que é possível que aconteçam os seguintes desconfortos ou riscos como: sentir durante a coleta de sangue o desconforto de uma leve dor como uma picada de abelha durante a coleta da amostra de sangue. Também caso eu seja usuário de EA, estou ciente dos riscos que isso pode causar a minha saúde no futuro, e fui alertado sobre isso pelos pesquisadores. Das quais medidas serão tomadas para sua redução, tais como: Caso seja identificada alguma alteração nos indicadores bioquímicos, o mesmo será encaminhado para uma avaliação no ambulatório de endocrinologia do Hospital Nossa Senhora da Luz.

SIGILO E PRIVACIDADE

Estou ciente de que minha privacidade será respeitada, ou seja, meu nome ou qualquer outro dado ou elemento que possa, de qualquer forma, me identificar, será mantido em sigilo. Os pesquisadores se responsabilizam pela guarda e confidencialidade dos dados, bem como a não exposição dos dados de pesquisa.

AUTONOMIA

É assegurada a assistência durante toda pesquisa, bem como me é garantido o livre acesso a todas as informações e esclarecimentos adicionais sobre o estudo e suas consequências, enfim, tudo o que eu queira saber antes, durante e depois da minha participação. Também fui informado de que posso me recusar a participar do estudo, ou retirar meu consentimento a qualquer momento, sem precisar justificar, e de, por desejar sair da pesquisa, não sofrerei qualquer prejuízo à assistência que venho recebendo.

RÚBRICA DO SUJEITO DE PESQUISA

RÚBRICA DO PESQUISADOR

RESSARCIMENTO E INDENIZAÇÃO

No entanto, caso eu tenha qualquer despesa decorrente da participação na pesquisa, tais como transporte, alimentação entre outros, haverá ressarcimento dos valores gastos na forma seguinte: dinheiro.
De igual maneira, caso ocorra algum dano decorrente da minha participação no estudo, serei devidamente indenizado, conforme determina a lei.

CONTATO

Os pesquisadores envolvidos com o referido projeto são Ericson Pereira (PUCPR), Dênis de Lima Greboggy (PUCPR) e Aline Cristina Batista Rodrigues Johann (PUCPR) e com eles poderei manter contato pelos telefones 3271-1561, 98730-7377 ou 99633-0754
O Comitê de Ética em Pesquisa em Seres Humanos (CEP) é composto por um grupo de pessoas que estão trabalhando para garantir que seus direitos como participante de pesquisa sejam respeitados. Ele tem a obrigação de avaliar se a pesquisa foi planejada e se está sendo executada de forma ética. Se você achar que a pesquisa não está sendo realizada da forma como você imaginou ou que está sendo prejudicado de alguma forma, você pode entrar em contato com o Comitê de Ética em Pesquisa da PUCPR (CEP) pelo telefone (41) 3271-2292 entre segunda e sexta-feira das 08h00 as 17h30 ou pelo e-mail nep@pucpr.br.

DECLARAÇÃO

Declaro que li e entendi todas as informações presentes neste Termo de Consentimento Livre e Esclarecido e tive a oportunidade de discutir as informações deste termo. Todas as minhas perguntas foram respondidas e eu estou satisfeito com as respostas. Entendo que receberei uma via assinada e datada deste documento e que outra via assinada e datada será arquivada nos pelo pesquisador responsável do estudo.
Enfim, tendo sido orientado quanto ao teor de todo o aqui mencionado e compreendido a natureza e o objetivo do já referido estudo, manifesto meu livre consentimento em participar, estando totalmente ciente de que não há nenhum valor econômico, a receber ou a pagar, por minha participação.

Dados do participante da pesquisa	
Nome:	
Telefone:	
e-mail:	

Curitiba, _____ de _____ de _____.

_____ _____
Assinatura do participante da pesquisa Assinatura do Pesquisador

RÚBRICA DO SUJEITO DE PESQUISA

RÚBRICA DO PESQUISADOR

Metodologia complementar

Supplementary Table 1. Sialochemical analysis description

Analyze	Method	Sensitivity	Linearity	Kit code
ALT	Ultraviolet Kinetic	0,998 U/L	400 U/L	**K049**
AST	Ultraviolet Kinetic	2,847 U/L	400 U/L	**K048**
Calcium	Endpoint Colorimetric Arsenazo III	0,074 mg/dL	20 mg/dL	**K051**
Phosphorus	Endpoint ultraviolet	0,11 mg/dL	15 mg/dL	**K068**
Triglycerides	Trinder Enzymatic-	2,58 mg/dL	900 mg/dL	**K117**
Urea	Fixed time kinetics	1,51 mg/dL	300 mg/dL	**K056**

Note: GOD = glucose oxidase; IFCC = International Federation of Clinical Chemistry. Observations: the term Quantification Limit (LOQ) can also be used instead of sensitivity.

Supplementary Table 2. Individual use (OG) (n =11)

Subjects	Min. Ox. (mg/kg/dia)	Max. Ox. (mg/kg/dia)	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	0,47	0,47	84,6	7
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

Note: ox: oxandrolone; min: minimum; max: maximum; Calculations based on the volunteers' self-report.

Supplementary Table Table 3. Comparison - HDL and Triglycerides stratifying by time of use in weeks

U Mann-Whitney test	Median 4 to 7 (n=4)	Median 7,5 to 12 (n=7)	p value
HDL T1	8,25	4,71	0,10
HDL T2	4,50	6,86	0,31
HDL T3	7,75	5,00	0,23
Triglycerides T1	5,13	6,50	0,52
Triglycerides T2	7,75	5,00	0,23
Triglycerides T3	6,25	5,83	0,92

Supplementary Table Table 4. Spearman's correlation matrix between blood, salivary and urine variables for OX Group

Correlation Blood x Saliva (on three times)			
	r value		r value
Blood x Saliva Triglycerides T1	0,31	**Blood x Saliva Calcium T1**	0,01
Blood x Saliva Triglycerides T2	-0,43	**Blood x Saliva Calcium T2**	-0,30
Blood x Saliva Triglycerides T3	-0,28	**Blood x Saliva Calcium T3**	0,16
Blood x Saliva Urea T1	0,53	**Blood x Saliva Phosphorus T1**	-0,50
Blood x Saliva Urea T2	0,05	**Blood x Saliva Phosphorus T2**	0,12
Blood x Saliva Urea T3	0,47	**Blood x Saliva Phosphorus T3**	-0,25
Blood x Saliva AST T1	-0,06	**Blood x Urine Testosterone T1**	-0,46
Blood x Saliva AST T2	0,18	**Blood x Urine Testosterone T2**	0,46
Blood x Saliva AST T3	0,01	**Blood x Urine Testosterone T3**	0,52
Blood x Saliva ALT T1	0,00		
Blood x Saliva ALT T2	-0,17		
Blood x Saliva ALT T3	-0,28		

Supplementary Text 1. Urine collection and AAS Screening

Samples were processed according to Campos et al., (2005) [82], with modifications. In 500 µL of urine were added 20 µL of internal standard (testosterone-d_3; 1 µg/mL), 440 µL of 0.2 M ammonium acetate buffer (pH 6.8) and 40 µL of β-glucuronidase enzyme (200 U). The mix was vortexed for 30 seconds and incubated at 55 °C for 60 minutes. At the end of hydrolysis, liquid-liquid extraction was performed by adding 800 µL of methyl tert-butyl ether, followed by centrifugation at 3500 rpm for 10 minutes. After centrifugation, 600 µL of organic phase were collected, evaporated under nitrogen flow at 40 ºC and resuspended in 50 µL of metanol-water. Aliquots of 30 µL were injected into a system formed by a Prominence UFLC liquid chromatograph (Shimadzu, Kyoto, Japan) equipped with two binary pumps (LC-30AD, Shimadzu, Kyoto, Japan), a column oven (CTO-20A, Shimadzu, Kyoto, Japan) and an automatic injector (SIL-20A, Shimadzu, Japan). The UHPLC system is integrated with a micrOTOF-Q™ III mass spectrometer (Bruker Corporation, Billerica, MA) and data were processed using Data Analysis and HyStar™ software (Bruker Corporation, Billerica, MA). Chromatographic elution was performed on a 75 × 2.0 mm i.d., 2.2 µm column, Shim-pack XR-ODS II (Shimadzu, Kyoto, Japan), eluted with a water gradient (Solution A) and acetonitrile (Solution B), both solutions supplemented with 0,1% formic acid at 400 µL/min flow rate, at 45 °C, under the following conditions: 0 – 2.5 min, 40 – 100% of B; 2.5 – 3.5 min, 100% of B; 3.5 – 3.6 min, 100 – 40% of B; 3.6 – 5 min, 40% of B. Total running time was 5 minutes. Mass spectra were obtained in positive mode (ESI+, $[M+H]^+$) using following optimized parameters: capillary energy, 5500 V; nebulizer gas, 3 bar; drying gas, 6 L/min; temperature, 200 °C; quadrupole energy, 10 eV; hexapole radio frequency, 200 Vpp; and acquisition frequency, 1 Hz. System

was calibrated with ammonium formate solution, and the exclusion mass error adopted was 5 ppm. Monitoring was done in 50 - 600 m/z for retroactive data analysis. Calibration curves were constructed at 1 – 1000 $ng.mL^{-1}$ for 3-hydroxystanozolol (m/z 345.43), 16-β-hydroxystanozolol (m/z 345.43), 17-α-methyl 5-α-androstane-3-α-17-β-diol (m/z 289.16), androsterone (m/z 273.01), boldenone (m/z 287.32), epitestosterone (m/z 289.29), etiocolanolone (m/z 273.00), fluoxymesterone (m/z 337.38), methenienone (m/z 301.37), methenolone (m/z 303.38), methyltestosterone (m/z 303.32), nandrolone (m/z 275.32), norethiocolocolanolone (m/z 277.25), oxymesterone (m/z 319.32), oxandrolone (m/z 307.22) and testosterone (m/z 289.24).

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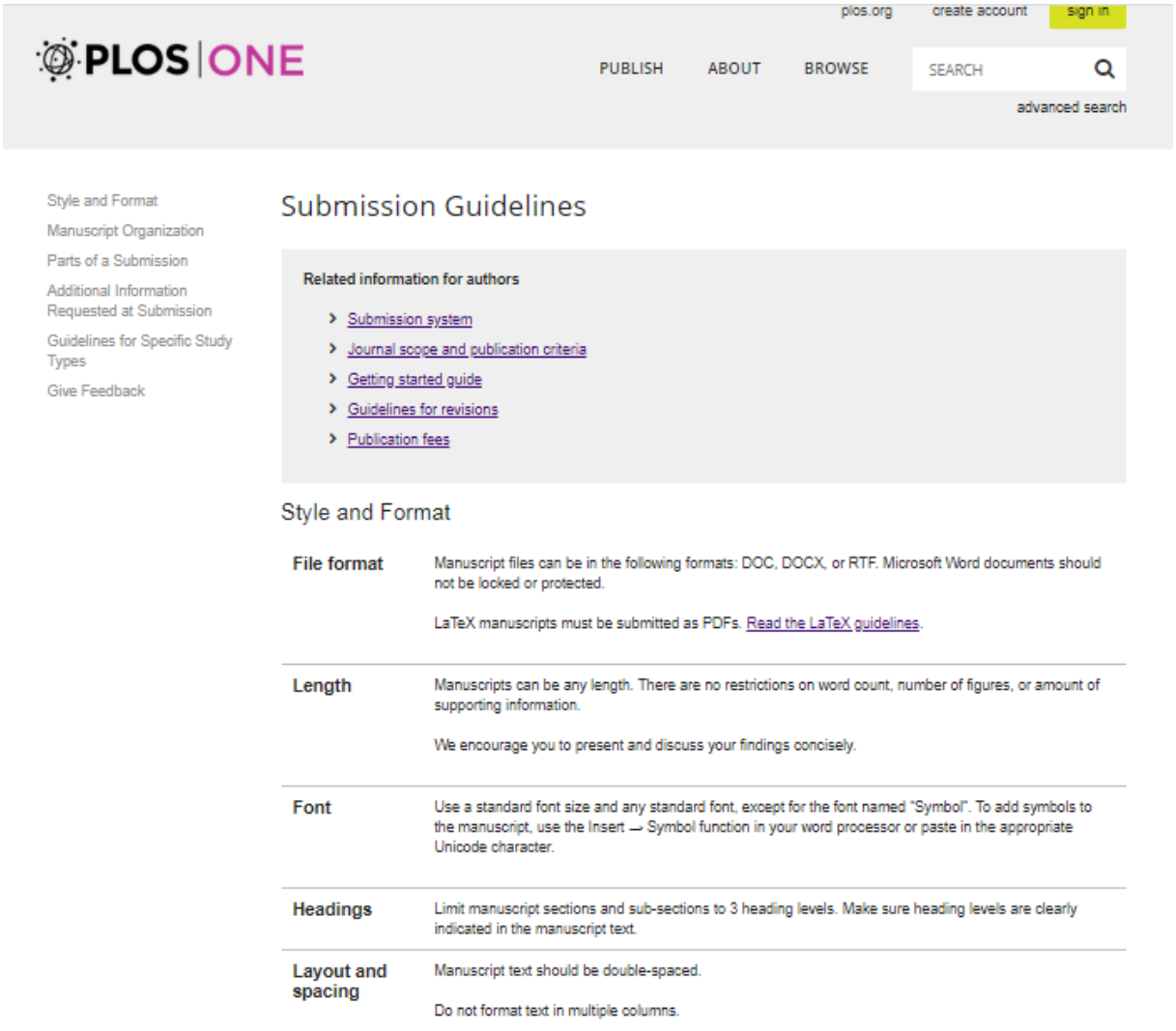

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