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ANABOLIC ANDROGENIC RELATION AND ADVERSE EFFECTS OF 5A-HYDROXY LAXOGENIN IN TRAINED RATS

RELAÇÃO ANABÓLICA ANDROGÊNICA E EFEITOS ADVERSOS DA 5A-HIDROXI LAXOGENINA EM RATOS TREINADOS

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ABSTRACT: 5α-hydroxy laxogenin (5α-HL) is being inadvertently used to improve performance, although reports of its anabolic and/or androgenic actions in healthy humans or animal models are lacking. In this study 5α-HL (group THL) was compared with Oxandrolone (OX, group TOX) or vehicle (mineral oil, group TCN), given orally for six weeks to male Wistar rats subjected to resistance training in vertical ladder. Regardless of the treatment, the training protocol improved performance, recorded as the maximal load. 5α-HL and OX affected plasma lipids, increased muscle mass gain (THL) and fiber diameter (TOX) without changing body mass, glucose homeostasis or adiposity. 5α-HL, unlike OX, did not cause atrophy of the reproductive organs. 5α-HL had a better anabolic androgenic relation than OX in this model. However, its adverse effects and its neutral effect on performance do not support its safe use by healthy humans.

KEYWORDS: Brassinosteroids. Muscle hypertrophy. Resistance training. Steroidal saponin. Synthetic anabolic steroids.

RESUMO: A 5α-hidroxi laxogenina (5α-HL) vem sendo inadvertidamente usada para melhoria de desempenho, embora relatos sobre suas ações anabólicas e/ou androgênicas em humanos sadios ou modelos animais não sejam encontrados. A 5α-HL (grupo THL) foi comparada com Oxandrolona (OX, grupo TOX) ou veículo (óleo mineral, grupo TCN), administrados oralmente por seis semanas a ratos Wistar machos submetidos a treinamento resistido em escada vertical. Independentemente do tratamento, o treinamento melhorou o desempenho, registrado pela carga máxima. A 5α-HL e a OX afetaram os lipídios plasmáticos, aumentaram o ganho de massa muscular (THL) e o diâmetro das fibras (TOX) sem afetar massa corporal, homeostase da glicose ou adiposidade. A 5α-HL, ao contrário da OX, não causou atrofia dos órgãos reprodutores. A 5α-HL teve uma relação anabólica androgênica melhor do que a OX neste modelo. Contudo, seus efeitos adversos, e seu efeito nulo sobre o desempenho, não sustentam seu uso seguro por humanos sadios.

PALAVRAS-CHAVE: Brassinosteroides. Esteroides anabólicos sintéticos. Hipertrofia muscular. Saponina esteroidal. Treinamento resistido.

INTRODUCTION

There is an intense marketing stimulating the use of laxogenin-containing products as a natural surrogate of synthetic anabolic steroids (SAS) for muscle strength and hypertrophy. However, these products are generally composed of 5α-hydroxy laxogenin (5α-HL), a spirostan-like steroidal saponin structurally similar to laxogenin, which is a brassinosteroid (BR); these are phytohormones known for their similarity with animal steroids, although with diverse action at the cellular level[1]. Laxogenin is found mainly in plants from the species *Smilax sieboldii*[2] and presents growth- and vigor-promoting properties[3].

There are no reports of the existence of 5α-HL in nature; it is considered a synthetic derivative of diosgenin[4,5], a steroidal saponin mainly from Dioscorea plants (e.g., yam). Diosgenin is the precursor of several synthetic steroids widely produced by the pharmaceutical industry[6] with many pharmacological properties, such as hypolipemic, hypoglycemic, antioxidant, anti-inflammatory, anti-microbial, anti-proliferative, androgenic, estrogenic and contraceptive[7,8].

Online broadcasting media mention, without evidence, that 5α-HL has an anabolic androgenic relation similar to oxandrolone (OX), a synthetic derivative of testosterone for oral administration. OX has advantages over testosterone because it is more resistant against liver degradative metabolism; the alkyl group on carbon 17α of the steroid nucleus (17α-alkylated compound) increases its action[9,10] and it has a higher – about 10:1 – anabolic:androgenic ratio[9,11]. Additionally, it is well absorbed after oral administration and has a high affinity for plasma proteins[12]. Its anabolic effect was observed both in young men[13] and in older men and women[14]. Nevertheless, OX use is approved only for the treatment of catabolic disorders and retarded pubertal growth[9] and not as an ergogenic substance[15].

The fact that 5α-HL has a structure similar to laxogenin and anabolic androgenic relation comparable to OX is prompting the use of this compound by practitioners of physical activity with the purpose of improving performance, especially because of its supposed anabolic effects, such as muscle mass gain5. However, reports confirming these allegations were not found in the literature.

The wide proclaiming and use of 5α-HL-containing supplements and the lack of evidence attesting its benefits on physical performance motivated this first investigation of the effects of 5α-HL on Wistar rats. High-intensity interval resistance training was used as anabolic stimulus. This is the first study comparing such effects in an animal model, which is important to outline the profile of 5α-HL and its possible anabolic and other systemic effects. The results are relevant because the use of substances without scientific testing may have a significant negative impact on human health.

METHODOLOGY

MATERIALS

The compounds 5α-HL and OX (Figure 1), both from China, were purchased from the Brazilian distributors PHD Innovation Expertise and Infinity Pharma, respectively. Analyses and approvals for these products were attested through physical-chemical methods by the distributors. Mineral oil (Nujol®, Mantecorp, Brazil) was used as vehicle for the oral administration of 5α-HL and OX. Anhydrous D-glucose (Synth, Brazil) was used for the oral glucose tolerance test and regular insulin Novolin® (Novo Nordisk, Brazil) for the intraperitoneal insulin tolerance test. Commercial kits (Gold Analisa Diagnóstica, Brazil) were used for plasma, serum and liver biochemical analyses. Portable digital glucometer Optium Xceed®

and MediSense® test strips (both from Abbott, Brazil) were used for blood glucose measurements. A vertical ladder was used for the resistance training (dimensions: 80º inclination; 1.10 m high; 10 cm wide; 2 cm between steps). The load was a plastic tube attached to the animal´s tail and filled with fishing lead weights.

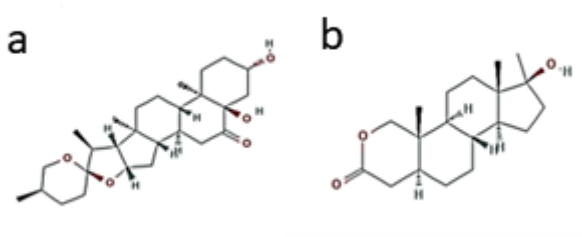

Figure 1. Molecular structures of 5α-hydroxy laxogenin (a) and oxandrolone (b).

ANIMALS AND EXPERIMENTAL MODEL

The experimental procedures were analyzed and approved by the Ethics Commission on Animal Use of the institution (CEUA protocol 8420230419). All measures were taken to assure safe and reliable animal handling and experimentation.

Male rats (*Rattus norvegicus*) of the Wistar strain aging 70 days and weighing 340 ± 15 g at the beginning of the experimental period were used. The animals were placed in pairs in plastic boxes. They had free access to water and rodent chow (Nuvilab®, Nuvital, Brazil) and were kept in a room with controlled temperature (23 ± 2 ºC) and photoperiod (12 h light/12 h dark).

After adapting for three days to the new environment, the animals were habituated with the vertical ladder for three alternate days by climbing it three times without load. On the following week they were randomly divided into three groups of 8-10 animals each. The rats were subjected to resistance training and were given either mineral oil (group TCN) or OX (group TOX) or 5α-HL (group THL) for six consecutive weeks. 5α-HL or OX, both at a dose of 20 mg/kg body mass (BM) or mineral oil (vehicle) were given intragastrically (gavage) in a volume of 0.1 mL/100 g BM once a day, in the afternoon (about 5 pm).

The dose of 20 mg/kg BM of 5α-HL was based on the study of Esposito *et al*.[16], which evaluated the effect of a BR on body and plasma profiles in untrained Wistar rats. For comparative purposes, the same dose of OX was chosen.

TRAINING PROTOCOL

The vertical ladder used for the training sessions had a chamber at the top where the rat could rest. The base was 10 cm distant from the floor to avoid contact with the load that was fixed at the animal's tail[17-21].

The training protocol was adapted from previous studies with mice[19-21]. This training format provides training volumes appropriate for hypertrophic responses[22]. Training was carried out for six weeks, in three weekly sessions on alternate days. The first session of each week was an incremental test to determine the individual maximal load (ML). The other two sessions were the training itself at 90% ML: this load characterized the protocol as high-intensity resistance training[20,21].

On the first week, the ML session began with 90% BM and on the following weeks with 100% ML of the previous week. At every complete climbing, there was a one-minute rest and then 50 g were added, until the rat could no longer reach the top chamber.

Each training session was composed of three rounds with continuous climbing attempts each, until exhaustion, and a one-minute interval between rounds. Exhaustion was settled as the incapacity of the animal to finish a climbing attempt even after non-painful manual stimuli on the tail.

DATA RECORDINGS

Body mass was recorded every week. Water and chow ingestion were obtained once a week by keeping the rats in individual cages for 24 h, and were expressed per 100 g BM. At the end of the intervention, naso-anal length was measured to calculate the body mass index (BMI, in g/cm^2).

During the ML sessions, the ML and the number of climbing attempts were recorded. The climbing attempts on each round of the two training sessions was also recorded.

ORAL GLUCOSE TOLERANCE TEST (GTT)

After six weeks of training and gavage, the rats were deprived of food for 12 h (overnight) and given glucose by gavage (1.5 g/kg BM diluted in water). Blood samples were collected from a puncture at the tip of the tail at 0, 5, 10, 15, 30, 45 and 60 min after the oral glucose, time zero being immediately before gavage and considered as fasting blood glucose. Glycemia was determined through test strips and glucometer and expressed as mg/dL. The variation of the glycemia of each animal during the 60-min test was calculated as incremental area under curve (iAUC) using the fasting blood glucose of each animal as baseline. After the collections, the animals were put back into their original boxes and given chow and water.

INTRAPERITONEAL INSULIN TOLERANCE TEST (ITT)

Two days after the GTT, the animals were deprived of food for 6 h and received an intraperitoneal injection of regular insulin (1 U/kg BM diluted in saline). Blood samples were collected from a puncture at the tip of the tail at times 0, 5, 10, 15, 20, 25, 30, 45 and 60 min, time zero min being immediately before insulin injection. Glycemia was determined with test strips and glucometer and expressed as mg/dL. The blood glucose decay index (kITT, expressed as %/min) was calculated for the first 30 min. At the end of the test the rats were returned to their original boxes and given chow and water.

TISSUE AND ORGAN COLLECTION

Two days after the ITT, the animals were deprived of food for 12 h and given an intraperitoneal injection of lethal dose of anesthetic (sodium thiopental 120 mg/kg BM after lidocaine 5 mg/kg BM). Blood samples were obtained through cardiac puncture; next, tissues and organs were removed and weighed: liver, heart, kidneys, testicles, seminal vesicles, prostate, white adipose tissues (WAT: retroperitoneal, epididymal, mesenteric, inguinal), brown adipose tissue and skeletal muscles (soleus and gastrocnemius). The adiposity index was the ratio between WAT and body mass. Tissue weights were expressed relative to body mass (g/100 g BM). Samples of liver, WAT and skeletal muscle were stored for further analyses.

BIOCHEMICAL DETERMINATIONS

Commercial kits were used to determine serum total cholesterol (CT), high-density lipoprotein (HDL) and triglycerides (TG) and the results were expressed as mg/dL. Low-density and very low-density lipoproteins (LDL and VLDL, respectively), also expressed as mg/dL, were calculated from Friedewald[23]. The CT/HDL ratio was taken as atherogenic index.

The plasma levels of fructosamine (in mmol/L), glutamic-pyruvic transaminase (GPT, in U/L), glutamic-oxalacetic transaminase (GOT, in U/L) and urea (in mg/dL) were also determined with commercial kits.

MORPHOMETRY OF MUSCLE FIBERS AND ADIPOCYTES

Samples of right gastrocnemius muscle and retroperitoneal WAT were dissected, weighed, and fixed in 4% paraformaldehyde for 24 h. The samples were dehydrated in ascending series of alcohol (from 70% to 100%), cleared in xylene and mounted in paraffin. Transverse histological sections (medial third of the gastrocnemius, 10 µm thick; retroperitoneal WAT, 5 µm thick) were made in automated microtome Leica RM 2145® (Leica, Germany) and stained with hematoxylin-eosin (HE). Images of the histological sections in random fields were obtained in Olympus BX 50® optical microscope coupled to an Olympus PBM 35 B® high-resolution camera (both from Olympus, Japan) using 20X objective.

Morphometric analysis was made using Image-Pro Plus® (Media Cybernetics, USA) image analyzer. The smallest diameter of 200 muscle fibers per animal per group was recorded, according to the method described by Aguiar[24]. The adipocytes diameter was determined in 10 fields per animal. All the adipocytes entirely seen in each field were measured. In both analyses the results were expressed as µm.

LIPID CONTENT OF THE LIVER

Liver samples previously stored at – 80 ºC were used for the analysis of lipid content through gravimetric method[25]. The lipids were extracted from the liver samples (about 1.0 g) in a mixture of chloroform:methanol (2:1, v:v). After lipid suspension in 2% Triton, agitation and warming at 55 °C, the contents of CT and TG were determined with commercial kits and expressed as mg/dL. Total lipid content was expressed as g fat/100 g liver, while CT and TG were expressed as mg/g total lipids.

STATISTICS

The results of the three groups are shown as mean and standard error (mean ± SE) unless stated otherwise; in figures individual values are also shown. Data sets were subjected to Kolmogorov-Smirnov normality test and compared through one-way ANOVA with Tukey's *post hoc* considering the significance level as 5%. Sequential data within a group were compared with repeated measures ANOVA with Dunnett's *post hoc*. The software Prism 8.0 (GraphPad, USA) was employed for statistical analyses and preparation of the figures.

RESULTS

TRAINING

Table 1 presents the variables of the training protocol. The number of climbing attempts, relative ML (g/100 g BM) and training volume were not different across the groups at each week recorded ($p>0.05$).

The groups were analyzed individually as well. There was a gradual increase of the relative ML from week 1 to week 6 in all the groups: about 17 g/100 g for group TCN, 14 for group TOX and 11 for THL. As for the number of climbing attempts, group THL had a significant decrease at week 6 when compared with week 1; the diminished values of groups TCN and TOX did not attain significance ($p>0.05$). Finally, group TCN had an increase in training volume at weeks 3 and 6 compared with week 1, while groups TOX and THL did not have significant differences on this training parameter ($p>0.05$).

Table 1. Training variables of rats from groups TCN, TOX and THL.

Variables	TCN	TOX	THL
Relative maximal load			
Week 1	12.03 ± 0.72^{a}	12.75 ± 1.25^{a}	11.71 ± 0.37^{a}
Week 3	20.90 ± 2.24^{ab}	17.55 ± 1.58^{a}	21.69 ± 1.67^{b}
Week 6	29.62 ± 4.24^{b}	26.39 ± 2.21^{b}	22.60 ± 1.75^{b}
Series			
Week 1	5.67 ± 1.07^{a}	5.89 ± 1.10^{a}	8.80 ± 1.21^{a}
Week 3	6.11 ± 0.73^{a}	6.78 ± 0.97^{a}	6.10 ± 0.79^{ab}
Week 6	3.67 ± 0.58^{a}	4.22 ± 0.46^{a}	3.50 ± 0.95^{b}
Training volume*			
Week 1	66.41 ± 11.90^{a}	76.88 ± 15.30^{a}	104.90 ± 16.00^{a}
Week 3	132.80 ± 27.41^{b}	124.03 ± 20.95^{a}	129.60 ± 18.22^{a}
Week 6	116.00 ± 28.73^{ab}	113.40 ± 18.05^{a}	82.21 ± 23.29^{a}

Results expressed as mean ± SE (n = 8/group). For the comparison across weeks within each group, different superscript letters represent statistical difference ($p<0.05$, repeated measures ANOVA/Dunnett). *climbing attempts multiplied by the training load of the week.

BIOMETRIC PARAMETERS

Figure 2a shows the profile of body mass during the six weeks of the interventions for the three groups. The inset demonstrates that there was no difference across the groups in body mass gain ($p>0.05$).

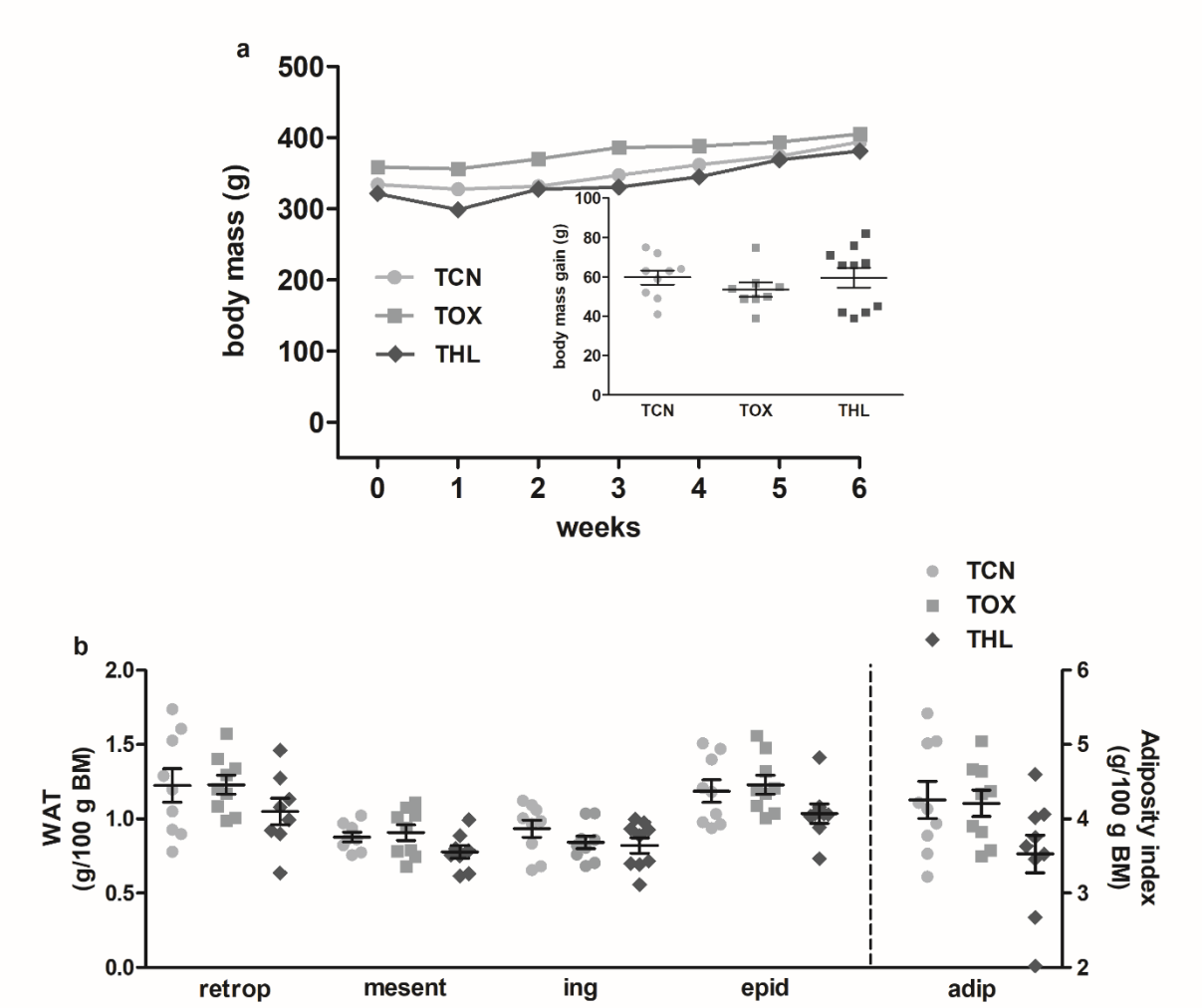

Figure 2. Body mass (a) during the six weeks of intervention and white adipose tissues (b) of rats from groups TCN, TOX and THL. Results expressed as mean and mean ± SE (inset) (n = 8-10/group). Retroperitoneal (retrop), mesenteric (mesent), inguinal (ing), epididymal (epid) white adipose tissues (WAT); adiposity index (adip).

In Table 2 are the biometric values during and at the end of the intervention. The daily intakes of chow and water, recorded once a week for six weeks, were similar across the groups, as was the BMI and the mass of kidneys, soleus muscle and brown adipose tissue ($p>0.05$).

The OX-treated group had higher liver mass and lower mass of testicles, seminal vesicles and prostate compared with the control, mineral oil treated, group (TCN). In contrast, these changes were not observed in the animals that were given 5α-HL, which caused only a greater mass of the gastrocnemius and a lower mass of the heart relative to the other groups.

The visceral WATs (retroperitoneal, mesenteric and epididymal) and subcutaneous (inguinal) WAT were not different across the groups (Figure 2b), neither was the adiposity index. Although the administration of 5α-HL tended to decrease these values, the differences were not significant ($p>0.05$).

Table 2. Biometric values and biochemical parameters of rats from groups TCN, TOX and THL.

Variables	TCN	TOX	THL
Daily chow ingestion (g/100 g BM)	8.07 ± 0.14[a]	7.91 ± 0.19[a]	7.66 ± 0.18[a]
Daily water ingestion (mL/100 g BM)	13.75 ± 0.36[a]	13.63 ± 0.30[a]	13.36 ± 0.28[a]
BMI (g/cm^2)	0.68 ± 0.01[a]	0.68 ± 0.01[a]	0.65 ± 0.01[a]
Kidneys (g/100 g BM)	0.64 ± 0.01[a]	0.63 ± 0.01[a]	0.62 ± 0.01[a]
Testicles (g/100 g BM)	0.86 ± 0.01[a]	0.77 ± 0.03[b]	0.88 ± 0.02[a]
Seminal vesicles (g/100 g BM)	0.45 ± 0.02[a]	0.33 ± 0.02[b]	0.43 ± 0.01[a]
Prostate (g/100 g BM)	0.13 ± 0.01[a]	0.08 ± 0.01[b]	0.12 ± 0.01[a]
Soleus muscle (g/100 g BM)	0.08 ± 0.00[a]	0.09 ± 0.00[a]	0.09 ± 0.00[a]
Gastrocnemius muscle (g/100 g BM)	1.04 ± 0.02[a]	1.00 ± 0.02[a]	1.13 ± 0.02[b]
Heart (g/100 g BM)	0.39 ± 0.01[a]	0.38 ± 0.01[a]	0.35 ± 0.01[b]
Liver (g/100 g BM)	3.04 ± 0.06[a]	3.31 ± 0.07[b]	2.95 ± 0.06[a]
Brown adipose tissue (g/100 g BM)	0.06 ± 0.01[a]	0.05 ± 0.00[a]	0.06 ± 0.00[a]
Plasma Biochemistry	**TCN**	**TOX**	**THL**
Fasting glucose (mg/dL)	84.38 ± 2.91[a]	90.88 ± 1.57[a]	89.75 ± 1.88[a]
Triglycerides (mg/dL)	55.62 ± 2.56[a]	74.75 ± 4.78[b]	40.56 ± 2.82[c]
Total cholesterol (mg/dL)	66.63 ± 2.69[a]	70.75 ± 2.37[a]	72.66 ± 3.50[a]
HDL (mg/dL)	40.00 ± 2.48[a. b]	45.38 ± 2.42[a]	32.87 ± 2.58[b]
LDL (mg/dL)	15.50 ± 2.55[a]	11.85 ± 2.55[a]	31.67 ± 2.26[b]
VLDL (mg/dL)	11.12 ± 0.51[a]	14.95 ± 0.95[b]	8.11 ± 0.56[c]
Atherogenic index	1.69 ± 0.07[a]	1.59 ± 0.10[a]	2.26 ± 0.12[b]
GOT (U/L)	82.62 ± 8.82[a]	78.31 ± 2.49[a]	80.44 ± 4.35[a]
GPT (U/L)	34.50 ± 1.72[a]	50.00 ± 1.08[b]	41.56 ± 1.65[c]
Fructosamine (mmol/L)	0.86 ± 0.05[a]	0.95 ± 0.02[a]	0.71 ± 0.01[b]
Urea (mg/dL)	38.81 ± 1.68[a]	38.88 ± 1.41[a]	39.56 ± 0.99[a]

Results expressed as mean ± SE (n = 8/group). Different superscript letters on the same row represent statistical difference ($p<0.05$, one-way ANOVA/Tukey). BM: body mass; BMI: body mass index.

IN VIVO TESTS

In Figure 3a is presented the profile of blood glucose during the 60 min after glucose gavage. The AUC showed that there was no difference on the glucose tolerance test across the groups (Figure 3b).

During the intraperitoneal ITT (Figure 3c) the three groups had similar blood glucose decays (kITT) for the first 30 min of the test (Figure 3d).

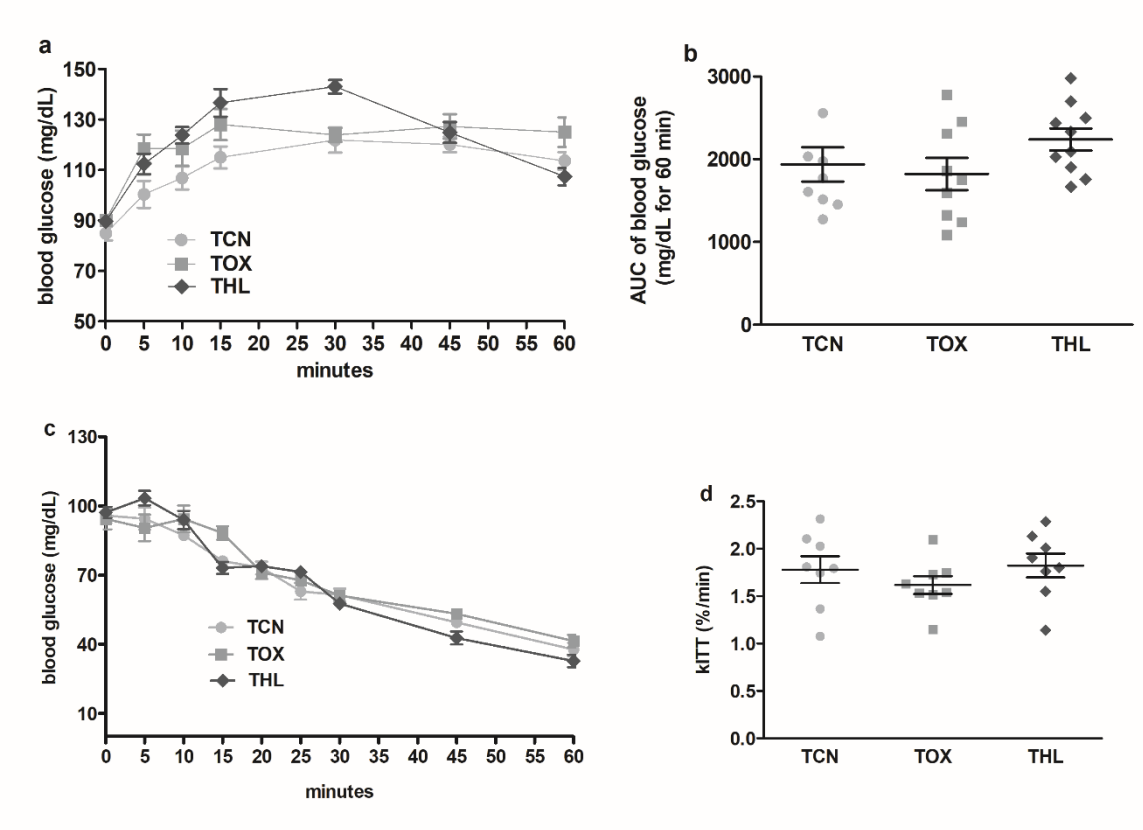

Figure 3. GTT profiles (a) and iAUCs (b); ITT profiles (c) and kITTs (d) of rats from groups TCN, TOX and THL. Results expressed as mean (a) and mean ± SE (b) (n = 8-10/group).

BIOCHEMICAL PARAMETERS

After euthanasia, blood was collected for biochemical analysis. Table 2 presents fasting blood glucose, lipid profile, urea, markers of liver damage and of protein glycation. There were no differences across the groups for fasting blood glucose, total cholesterol, GOT and urea.

The OX-treated rats had higher levels of TG and VLDL than the control group (TCN), while in group THL these values were lower. As for HDL and LDL, the OX-treated was similar to the control group, but 5α-HL increased LDL compared with the other two groups and decreased HDL compared with TOX, resulting in a higher atherogenic index in this group than in the others.

Both OX and 5α-HL administration resulted in higher GPT levels compared with the control, although the effect of 5α-HL was smaller than that of OX. Fructosamine was lower only in the 5α-HL-treated animals.

Differences were not found in the total lipid content (Figure 4a), total cholesterol (Figure 4b) and triglycerides (Figure 4c) in samples of liver from animals of the three groups.

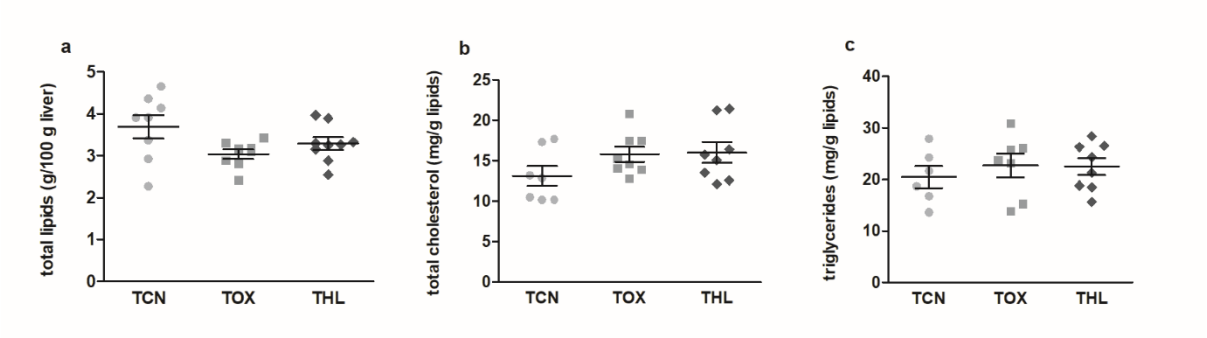

Figure 4. Liver lipid content of rats from groups TCN, TOX and THL. (a) Total lipids, (b) total cholesterol and (c) triglycerides. Results expressed as mean ± SE (n = 8/group).

DIAMETER OF MUSCLE FIBERS AND ADIPOCYTES

The analysis of the gastrocnemius muscle showed that fiber diameter was larger with OX administration than in group TCN, while 5α-HL did not yield the same result (Figure 5a).

When the retroperitoneal white adipose tissue was analyzed, there were no differences ($p>0.05$) in adipocyte diameter across the groups (Figure 5b).

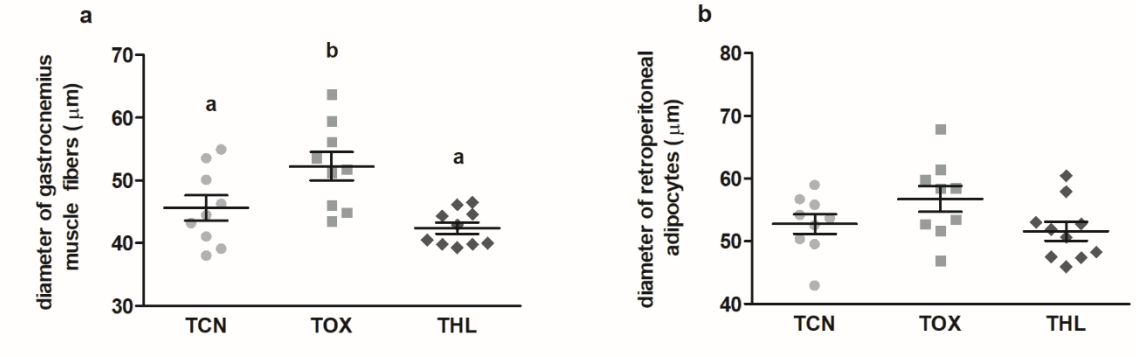

Figure 5. Diameter of muscle fibers (a) and adipocytes (b) of rats from groups TCN, TOX and THL. Results expressed as mean ± SE (n = 9-10/group). Different superscript letters represent statistical difference ($p<0.05$, one-way ANOVA/Tukey)..

DISCUSSION

Careful experimental analyses in animal models are an initial, but essential step for the safe use of any chemical substance, either natural or synthetic, including phythormones with alleged anabolic action. The inappropriate use of these substances may bring serious risks to human health, because they may have unknown biodisponibility, chemical structures and biological activities. Their attractiveness resides in the fact that they promise fewer collateral effects compared to synthetic steroids already widely – and sometimes dangerously – employed.

This study proposed the first experimental evaluation of 5α-HL effects on Wistar rats subjected to a resistance training protocol. The wide use of this substance for the improvement of physical performance is due to its homology with laxogenin, a phytosteroid acting on plant growth through cell

membrane receptors[26]. The results showed that 5α-HL had a better anabolic androgenic relation when compared to the synthetic anabolic steroid OX, as 5α-HL did not cause atrophy of the reproductive organs. However, both promoted dyslipidemia, increased markers of cardiovascular risk and of liver damage, and did not improve performance on the resistance training. Although preliminary, these results raise an alert against the indiscriminate use of 5α-HL for performance improvement by the general population.

The training protocol of this investigation, although using strength instead of endurance, was designed as high-intensity interval training, which is characterized by sessions of high-intensity workouts intercalated with periods of lower intensity or rest[27]. The efficacy was shown by the progressive increase of the ML – and therefore strength – of the three groups during the training period of six weeks, in accordance with studies in mice[20,21], rats[28] and young humans[29]. Training volume did not change in any of the groups, which can be explained by the diminishing climbing attempts in each session as ML increased, especially in the last weeks. However, there was no difference in ML, total climbing repeats or training volume across groups, demonstrating that the improved performance was due only to the resistance training protocol, and not to OX or 5α-HL themselves. Rats given injectable SAS did not change their maximal load as well when subjected to resistance training[30].

Although the treatments did not improve exercise performance, there was a small but significant increase of the gastrocnemius mass promoted by 5α-HL, and of gastrocnemius fiber diameter promoted by OX. These results suggest different mechanisms of action of these products on muscle hypertrophy, that can be both intracellular, such as increase of myofibrils and sarcoplasmic components, and extracellular, such as increase of matrix elements[31]. Studies of cultured rat skeletal muscle fibers show that BRs are effective in modulating protein synthesis through the activation of Akt phosphorylation[18]. Akt is a kinase that acts on several intracellular signaling pathways, including those involved in protein synthesis via mTOR (a protein kinase) and those inhibiting proteolysis and skeletal muscle apoptosis via phosphorylation of transcriptions factors of the FoxO family[32]. In addition, Akt stimulates glucose uptake and glycogen synthesis[33]. Specifically, a study has shown the action of OX through intracellular androgen receptors and subsequent genomic effects; this improves muscle efficiency in synthetizing proteins by enhancing the influx and use of amino acids, in addition to enhancing the expression of androgen receptors[12]. Changes of the extracellular matrix because of physical activity, such as collagen remodeling, were described[34], but corresponding investigations using 5α-HL, OX or even BRs were not found.

Regardless of 5α-HL, OX or vehicle administration, the groups did not differ in body mass gain, BMI, adiposity index or size of retroperitoneal adipocytes. These results may be partially explained by the similar chow ingestion of the groups. In Wistar rats under resistance HIIT and given protein supplementation[28] and in BR-treated and trained rats fed normal or protein-enriched chow[16], these parameters were not altered either, suggesting that the lack of difference in the results presented here were not caused by protein deficit or by type/dose of the substances being tested. Despite the indiscriminate use of OX in the search for maximization of physical exercise and sport performance, literature data on these aspects are scarce, as OX is not recommended for this purpose. Its safety and applicability are best known in the treatment of catabolic disorders[35].

The androgenic effects of 5α-HL and OX were assessed by the relative mass of the reproductive organs (testicles, seminal vesicles and prostate), which showed a marked decrease when the animals were treated with OX but not with 5α-HL. A hypogonadotropic status, characterized by testicular atrophy, low levels of endogenous testosterone and reduced spermatogenesis, stems from the suppressive effects of exogenous SAS on the hypothalamus-pituitary-gonad axis and directly on the

testicles[35,36]. Low levels of plasma testosterone can be related with decreased muscle strength and mass[37], which could partially explain the lack of a more effective response of muscle hypertrophy and training volume to OX in this study. The androgenic effect of BRs was assessed in a single study in castrated rats; there was no difference in the relative mass of the reproductive organs[34], reinforcing the lack of androgenic effect of 5α-HL, probably due to the different mechanism of action from OX.

Resistance training did not impact glucose homeostasis, as the blood glucose profile was not affected by glucose (GTT) or insulin (ITT) challenges in any of the groups. A similar observation was made in trained rats under protein supplementation[28]. There is a paucity of studies assessing glucose homeostasis under OX and BRs administration, but they are unanimous in the lack of effect in rats and humans[16,38-42]. A hypoglycemic effect of BRs was reported in obese mice and was related to enhanced insulin sensitivity and inhibition of gluconeogenic enzymes[43]. Curiously, 5α-HL decreased fructosamine, which represents protein glycosylation and is widely used in the diagnosis and monitoring of blood glucose[44]; this suggests a possible beneficial effect of 5α-HL in conditions of persistent hyperglycemia.

An aspect apparent with both OX and 5α-HL was dyslipidemia, albeit with distinct presentations. OX-treated animals had higher levels of TG and VLDL, suggesting higher cardiovascular risk[45], while 5α-HL-treated rats had lower TG, VLDL and HDL, but higher LDL and atherogenic index. The levels of TG were not affected when untrained rats were given BR at the same dose used here[16]. However, when athletes use supra-physiological doses of SAS, lipid levels vary according to the type, combination and duration of use[46,47].

Although the literature emphasizes the atherogenic effect of SAS – especially the 17α-alkylated compounds for oral use – resulting from the increased LDL and decreased HDL in plasma[48], this effect was observed only with the administration of 5α-HL, revealing an adverse effect of this product. The atherogenic index[49], as well as a higher LDL/HDL ratio[50], is associated with greater risk for cardiovascular disease. Investigations are needed to approach the impact of these substances on cardiovascular function.

Liver toxicity was monitored through liver damage markers, GOT and GPT. In any species, including humans, GPT activity is higher in the liver, while GOT is higher in skeletal and cardiac muscle[51]. Animals given 5α-HL and especially OX had GPT levels higher than the controls. Kobayashi and co-workers[52] reported the relationship between high plasma levels of transaminases and lipid and/or glucose metabolism-modifying drugs; here, both 5α-HL and OX changed lipid plasma levels and increased plasma GPT. Assessments of liver function under BRs administration were not found, while rat liver lipid degeneration[53] and changes in transaminases levels[50,54,55] were noticed with the use of 17α-alkylated compounds. A marked rise of GPT was seen in older trained men using OX[56]. Studies point out that the plasma alterations of transaminases are usually transient and return to basal values when SAS use is interrupted[9,57].

In contrast with 5α-HL, OX increased the liver relative mass, being in accordance with studies pointing to histological changes, such as adenomas and hepatocellular hyperplasia[57], associated with the use of 17α-alkylated SAS, as well as the induction of cellular proliferation in rat liver[53]. Histological analyses are necessary to confirm the possible morphological alterations in the liver, because there is a relation between high plasma levels of transaminases and larger liver due to hepatocyte damage by the drugs[58].

CONCLUSION

This study has shown, as far as it could be checked, for the first time in healthy rats, that 5α-HL has a better anabolic androgenic relation, yet it is similar to OX in promoting dyslipidemia, signs of hepatotoxicity and risk of cardiovascular disease in rats subjected to resistance training. Together with the lack of effect of 5α-HL on exercise performance, these outcomes do not support either an anabolic effect or a safe use by healthy humans. Further investigations are needed to assess 5α-HL effects and risks to human health.

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