



Original/Alimentos funcionales

# The action of avocado oil on the lipidogram of wistar rats submitted to prolonged androgenic stimulum

Renato de Souza Abboud<sup>1</sup>, Vivian Alves Pereira<sup>1</sup>, Carlos Alberto Soares da Costa<sup>2</sup>,  
Gilson Teles Boaventura<sup>2</sup> and Mauricio Alves Chagas<sup>1</sup>

<sup>1</sup>Department of Morphology, Laboratory of Cellular and Extracellular Biomorphology Biomedic Institute, Federal Fluminense University. <sup>2</sup>Laboratory of Experimental Nutrition. Department of Nutrition and Dietetics. Fluminense Federal University. Rio de Janeiro, Brazil.

## Abstract

**Introduction:** the abuse of steroid hormones administered in chronic form may cause alterations in the lipidic profile, conveying an increase in the levels of LDL, and reduction in the levels of HDL. In average, 53.44% of the lipidic composition of the avocado core is composed of oleic acid (which is a phytosterol) and the study of the hypolipemiant effect of these substances has been performed aiming at the prevention and control of dyslipidemias.

**Objective:** to assess the potential hypolipemiant power of the avocado oil on the lipidogram of adult male Wistar rats submitted to prolonged androgenic hiperestimulation.

**Materials and methods:** twenty eight Wistar rats were divided in 4 groups of 7 animals: the control group (CG); Avocado Oil Group (AOG) fed with a staple based on Avocado Oil; Induced Grupo (IG); and the Induced Grupo fed with a staple based on Avocado Oil (AOIG). The inducing was performed through surgery to subcutaneously implant silicon pellets suffed with 1ml of testosterone propionate which were replaced at every 4 weeks.

**Results:** VLDL (AOIG:  $28.14 \pm 4.45$ ; IG:  $36.83 \pm 5.56$  mg/ml); Triglicerides (AOIG:  $140.07 \pm 22.66$ ; IG:  $187.2 \pm 27$  mg/ml); HDL (AOIG:  $40, 67 \pm 1.2$ ; GI:  $35.09 \pm 0.8$ ; AOG:  $32.31 \pm 2.61$  e CG:  $32.36 \pm 4.93$ mg/ml) Testosterone (AOIG:  $1.42 \pm 0.46$ ; GI:  $2.14 \pm 0.88$ ; AOG:  $2.97 \pm 1.34$  e CG:  $1.86 \pm 0.79$ ng/ml).

**Conclusion:** avocado Oil exerted a direct regulating effect on the lipidic profile, acting efficiently on animals submitted to androgenic stimulation through a prolonged period.

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Key words: *Steroid hormones. Dyslipidemia. Phytosteroids. Hypolipemiant power.*

**Correspondence:** Mauricio Alves Chagas.  
Department of Morphology.  
Laboratory of Cellular and Extracellular  
Biomorphology Biomedic Institute.  
Federal Fluminense University. Brasil.  
E-mail: chagas.m@gmail.com

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## ACCIÓN DEL ACEITE DE AGUACATE SOBRE EL LIPIDOGRAMA DE RATONES WISTAR SOMETIDOS A ESTIMULACIÓN ANDROGÉNICA PROLONGADA

### Resumen

**Introducción:** el uso abusivo de hormonas esteroides administradas crónicamente puede ocasionar cambios en el perfil lipídico, lo que lleva a un aumento de LDL y niveles reducidos de HDL. El promedio (53,44%) de la composición de lípidos de la pulpa de aguacate está compuesto por ácido oleico (que es un fitosterol), y el estudio del efecto hipolipemiant de estas sustancias se ha celebrado para la prevención y el control de la dislipemia.

**Objetivo:** evaluar el potencial de reducción de lípidos del aceite de aguacate en ratones Wistar machos adultos sometidos a hiperestimulación androgénica prolongada.

**Material y métodos:** veintiocho ratas se dividieron en 4 grupos de 7 animales: Grupo Control (GC); Grupo de Aceite de Aguacate (GOA), alimentado a base de aceite de aguacate; Grupo Inducido (GI) y el grupo alimentado con base de aceite de aguacate inducida por la dieta (GIOA). La inducción fue hecha mediante perdigones de silicona subcutáneos, implantados por cirugía, llenos de 1 ml de propionato de testosterona, que fueron cambiados cada 4 semanas.

**Resultados:** VLDL (GIOA:  $28,14 \pm 4,45$ ; GI:  $36,83 \pm 5,56$  mg/ml); triglicéridos (GIOA:  $140.07 \pm 22.66$ , GI  $187: 2 \pm 27$  mg/ml); HDL (GIOA:  $40,67 \pm 1,2$ ; GI:  $35,09 \pm 0,8$ ; GOA:  $32,31 \pm 2,61$  eGC:  $32,36 \pm 4,93$  mg/ml); testosterona (GIOA:  $1,42 \pm 0,46$ ; GI:  $2,14 \pm 0,88$ ; GOA:  $2,97 \pm 1,34$  eGC:  $1,86 \pm 0,79$  ng/ml).

**Conclusión:** El aceite de aguacate ha tenido un efecto regulador directo sobre el perfil lipídico, actuando eficazmente en los animales sometidos a estimulación de andrógenos durante períodos prolongados.

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Palabras clave: *Hormonas esteroides. Dislipidemia. Fitosteroles. Potencia hipolipemiant.*

## Introduction

Within the last decades, androgenic anabolic steroids (AAS) had their deployment increasingly frequent among athletes who aim at improving their physical performance<sup>1</sup>, or by people who seek aesthetical perfectioning<sup>2,3</sup>. The chronic use of AAS may cause alterations in the lipidic profile of abusers, with an increase in the levels of low density lipoproteins (LDL) and a decrease of high density lipoproteins (HDL)<sup>4</sup>. The therapeutic use of AAS has been reported for at least five decades, having its indication associated to cases of hypogonadism and hormone replacement. The synthetic steroid hormones, as well as the endogenous type also have anabolic and androgenic activity<sup>5</sup>.

The frequent consumption of steroid hormones by high performance athletes causes alterations in the lipidic profile of these individuals, through the increase in the concentration of LDL and decrease in the concentration of HDL cholesterol, thus elevating the risk of atherosclerosis by deposition of cholesterol plates on the walls of blood vessels, associated to an increase of platelet aggregation and probable endothelial malfunction. As a consequence of dislipidemia associated to the alteration of blood clotting and endothelial malfunction, there is an elevation of the risk of coronary spasm<sup>6</sup>.

The main androgenic hormone which has an anabolic role is testosterone. Such hormone is originated from cholesterol and about 95% of it is secreted by the Leydig cells, in the testicles and 5% by the adrenal cortex<sup>7</sup>. With aging, the level of testosterone declines gradually and its lack may cause a substantial reduction in life quality<sup>8</sup>. Exogenous testosterone may be administered by many pharmacologic ways, such as injections, adhesives, topic gel, pills or implants<sup>9,10</sup>.

Phytosteroids are fatty acids of vegetable origin vegetal with a chemical structure which is analogous to cholesterol. Sitosterol, campesterol and stigmasterol are steroids of vegetable origin which can be found in fruit, cereal, soy, and vegetable oils. Oleic acid (omega 9), which is a sitosterol, is the main component of avocado oil. The study of these substances and their hypolipemiant effect has been performed, aiming at prevention and control of dislipidemias<sup>11</sup>. In average, 53.44% of the lipidic composition of the avocado pulp is composed of oleic acid, and may perform an important function in the treatment of dislipidemias<sup>12</sup>.

Considering the risks of the use of high doses of androgens by athletes who want to improve their physical performance or by people who seek aesthetical perfectioning and their possible influence in dislipidemic processes, we intend to assess, in this work, the hypolipemiant potential of the avocado oil on adult male Wistar rats submitted to prolonged androgenic hyperstimulation

## Materials and methods

### *Experimental Outlining*

The research project was approved by the Committee of Ethics in the Use of Animals of Universidade Federal Fluminense (CEUA-UFF) under number 382. 28 young adult male Wistar rats (42-50 days) were selected from the do bioterium of the Laboratory Animal Center (NAL) of Universidade Federal Fluminense (UFF), which were placed in plastic cages, in a constant cycle of 12 hours of light and 12 hours of darkness under a temperature of  $22 \pm 1^\circ\text{C}$ , maintained in the experimental bioterium of the Morphology Department of the Biomedical Institute/UFF. The rats were divided in 4 groups of 7 animals: the control group (CG); Avocado Oil Group (AOG) fed with a staple based on Avocado Oil; Induced Group (IG); and the Induced Group fed with a food based on Avocado Oil (AOIG). The inducing was performed through surgery to subcutaneously implant silicon pellets (Dow Chemicals), cut in 5 cm and stuffed with 1 ml of testosterone propionate (0,1 ml de Androgenol<sup>®</sup>) which were replaced at every 4 weeks as previously described<sup>13</sup>. The surgical procedure was performed by the Morphology Department of the Biomedical Institute/UFF.

### *Experimental Feeding*

The experimental staples prepared were isocaloric and added with vitamin and mineral compounds, according to the recommendations of the *American Institute of Nutrition (AIN-93M)*<sup>14</sup> during the experimental period. The staple offered to the avocado oil groups had a 7% concentration of avocado oil, while the staple offered to the control group had 7% of soy oil. All animals were weighed at the beginning of the biologic essay and, from that moment on, twice a week during the whole experiment. The weighings were performed on a digital scale, Gehaka brand, with a precision of 0,05 g (Table I).

### *Euthanasia of animals and gathering of biologic material*

At the end of the experimental period at the bioterium, the animals were euthanised. The animals were anaesthetised with 75 mg/kg of cetamine + 10 mg/kg de xilazine, mixed in the same syringe, and the dose calculated was intraperitoneally administered. After realizing anaesthetic condition through the absence of pedal reflex, animals were submitted to bleeding by intracardiac puncture from which 10 ml of blood were obtained. After bleeding, an additional dose of the anaesthetic was administered, which led to death of the animal.

**Table I**  
Composition of each 100 g of staples employed in the essay during the maintenance stage (protein 14% AIN-G)

Nutrients	soy (g)	avocado (g)
Casein1	14	14
Starch3	58,95	58,95
Refined Sugar4	10	10
Mineral Compound AIN 93M1	3,5	3,5
Vitamin Compound 1	1	1
Soy oil 5	7	
Avocado oil 7		7
Celulose6	5	5
Coline Bitartarate de 1	0,25	0,25
Cistine1	0,3	0,3
Tert-Butil hidroquinone	0,0014	0,0014
Total	100,001	100,001

The blood samples collected were deposited in essay flasks for serology with a separating gel, centrifuged at 3000 rpm for 15 minutes. The serum obtained was stored at -20°C for dosaging of hormonal and biochemical concentrations.

#### Hormonal dosaging

The dosaging of the serum testosterone concentration was performed by the radioimmunoessay method (RIE), using a diagnostic commercial set, in solid state from Beckman Coulter (Immunotech®). The exams were performed in the hormone laboratory of the Brazilian Diagnósis and Veterinary Especialities Institute (PROVET/São Paulo, Brasil), using o WIZARD2 equipment from Perkin Elmer. All parameters of quality of the essay were performed according to instructions from the international scientific community.

#### Biochemical Analysis

A dosaging of the concentration of total and partial cholesterol and triglicerides was performed by means of the dry biochemical methodology (VITROS 250® Johnson e Johnson).

#### Statistical Analysis

The data were presented under the average and standard deviation form. So as to test the normal distribution of values, the Kolmogorov-Smirnov test was deployed. For data analysis, the ANOVA univariad

test was deployed, associated to the Tukey-Kramer multiple comparison test. The significance in all tests was established to the level of  $p < 0,05$ . The statistical analyses were performed by the Graph Pad Prism version 5.0, 2007 (San Diego, CA, USA) program.

#### Results

In our study, we found differences statistically significant in the Induced Group fed with a food based on Avocado Oil (AOIG), which had the seric level of cholesterol at 16.91% bigger than the control group(CG); the Induced Group (IG);with an increase of 14.82% in relation to the Avocado Oil Group (AOG) and 20.26% bigger than the control group (CG).

In relation to LDL, the Induced Group fed with a food based on Avocado Oil (AOIG), presented an increase of 54.25% in comparison to the the Induced Group (IG), 63% bigger than the Avocado Oil Group Avocado Oil Group (AOG) This group Avocado Oil Group (AOG) also had the LDL level 58.30% smaller than the level of the control group (CG).

AOIG presented values of VLDL 23.59% smaller than the level of the Induced Group (IG); in AOG VLDL was 23.82% bigger than the CG.

In the case of HDL, AOIG presented an increase of 11.28% in comparison to the the Induced Group (IG), 20.55% bigger than the (GOA) e 20.43% bigger than the.

The Induced Group fed with a staple based on Avocado Oil (AOIG) had the trigliceride seric level 25.17% smaller than the IG; the GI seric level was 32.75% bigger than the CG.

When the seric levels of testosterone were assessed, it was observed that the levels of AOIG were 52.18% smaller than the Avocado Oil Group (AOG).

The other groups did not present significant differences.

The numeric data are expressed in table II.

#### Discussion

There are controversies in studies made in relation to the alterations in the levels of total cholesterol after administration of testosterone. Injections of testosterone seem not to present any effect on this variable after 21 days, when administered to high performance athletes, but when the use surpasses 42 days, a reduction in total cholesterol may occur<sup>15</sup>.

In our study, the administration of testosterone Propionate during 90 days resulted in an increase of seric levels of total cholesterol. This fact may be confirmed, because the IG had its levels of total cholesterol bigger than the CG; There was no significant difference between the induced groups(IG and AOIG),but a beneficial effect of Avocado Oil over total cholesterol is evidenced because even after administration of testosterone propionate for 90 days, the groups fed with a

food based on Avocado Oil did not present significant differences in levels of total cholesterol, contrary to what happened with the groups fed with control food, in which there was significant increase. Similar results in an experimental study with human patients with dislipidemia<sup>16</sup>, in which there was reduction in total cholesterol of patients with dislipidemia who introduced 200 g of Avocado Oil in their diet for 30 days.

Seric levels of LDL cholesterol also increased (low density lipoprotein) also increased after 90 days of administration of testosterone, which is in accordance in studies with Rhesus monkeys<sup>17</sup>, and in humans<sup>18</sup>, but in disaccordance to<sup>19</sup>. This fact evidenced by the increase in levels of these lipoproteins in AOIG, when compared to AOG. Yet, the levels of LDL cholesterol in AOIG was bigger than in the CG, which demonstrates that Avocado Oil did not improve LDL levels in the group induced with testosterone. But in the non testosterone induced groups, Avocado Oil significantly reduced levels of LDL cholesterol, which may be because of an increase of expression of the LDL receptor in the liver provoked by the action of phytosteroids present in Avocado Oil<sup>20</sup>. In a study with rabbits<sup>21</sup>, revealed that the watery extract from the avocado seed diminished the levels of total cholesterol, LDL e triglicerides.

The analysis of levels of VLDL cholesterol (very low density lipoprotein) showed us that the administration of testosterone propionate for 90 days increases levels of VLDL cholesterol, because in this study such levels in the IG were significantly higher than in the CG. The influence of testosterone on levels of VLDL was observed<sup>22</sup>, because after orquiectomy in young rats, reductions in the levels of testosterone, VLDL and triglicerides was noticed. Another relevant feature in our study was that Avocado Oil could improve the lipidic profile of the group which suffered induction by testosterone, diminishing levels of VLDL. The consumption of Avocado Oil seems to revert the effect of testosterone on VLDL.

Seric levels of HDL among the control group (CG); Avocado Oil Group (AOG) fed with a food based on Avocado Oil; Induced Group (IG); and the Induced

Group fed with a food based on Avocado Oil (AOIG) did not present any significant difference. The administration of testosterone for 90 days seems not to have induced any reduction in levels of HDL in IG, in accordance to studies<sup>9,23</sup>. But our studies are not in accordance to studies<sup>19,24</sup>, which suggest that activity by Hepatic enzyme (HL) and by Lipoprotein Lypase (LPL), which catabolize HDL have their tenham suas activities increased by testosterone. In the Induced Group fed with a food based on Avocado Oil (AOIG), there was a significant increase in levels of HDL, in relation to the other groups. improved the lipidic profile of the group which suffered testosterone induction, as it did increase the HDL in this group.

According to<sup>25</sup> during, trigliceride levels may not suffer alterations when HDL and LDL levels decrease or increase. Yet, in our study, in the assessment of trigliceride levels, the Induced Group fed with a control food presented higher levels than the control group, demonstrating that the treatment with testosterone for 90 days was enough to increase trigliceride levels. AOIG had a significant reduction in trigliceride levels, when compared to the Induced Group fed with a control food, demonstrating that Avocado Oil acted reverting the action of testosterone on triglicerides.

Enforcing the regulating effect of Avocado Oil on lipidic profiles<sup>16</sup>; suggested that a diet containing daily 200 g of Avocado consumed during 30 days in a role, followed by human patients with mixed dislipidemia was sufficient to reduce Cholesterol (9.2%) and Trigliceride (10.3%) levels, and to increase HDL (6.3%) levels. although they have different methodologies, the results from this study are in accordance to our results, in which Avocado Oil use for 90 days seems to have acted beneficially on the lipidic profile of rats submitted to prolonged androgenic stimulum, altering Cholesterol, VLDL, HDL and Trigliceride levels.

The rats which suffered hormonal induction and had lower seric testosterone levels than the ones fed with Avocado Oil and did not. The other groups did not present significant differences in testosterone levels. Avocado Oil seems to have presented an antian-drogenic effect, by reducing testosterone levels in the

**Table II**  
*Hormonal Biochemical Analysis*

<i>Parâmetros</i>	<i>AOIG</i>	<i>IG</i>	<i>AOG</i>	<i>CG</i>	<i>Value P</i>
Colesterol (mg/ml)	73.42±7.97ab	76.50±3.01a	65.16±4.66bc	61.00±4.63c	0.0004
Ldl (mg/ml)	4.00±1.23a	1.83±1.25bc	1.48±0.37c	3.55±1.37ab	0.0015
Vldl (mg/ml)	28.14±4.45c	36.83±5.56ab	35.50±4.03abc	25.52±6.59c	0.0057
Hdl(mg/ml)	40.67±1.26a	35.90±0.80b	32.31±2.61bc	32.36±4.93bc	0.0001
Triglicerídes (mg/ml)	140.07±22.66c	187.20±27.3ab	160.00±27.66bc	125.88±38.24c	0.0101
Testosterone (ng/ml)	1.40±0.46c	2.14±0.88bc	2.90±1.34ab	1.86±0.79bc	0.0439

Control Group (CG); Avocado Oil Group (AOG) fed with a food based on Avocado Oil; Induced Group (IG); Induced Group fed with a staple based on Avocado Oil (AOIG). Average values within a line different letters are significantly different (one-way ANOVA,  $P < 0.05$ ).



IG, in relation to the group fed equally but which did not suffer hormonal induction. This effect may have been potentialized by our methodology and by reasons linked to endocrine balance. In mammals, secretion of testosterone is made in a pulsing way, and is controlled by negative feedback, i.e., excess of testosterone suppresses secretion of LH and FSH, decreasing endogenous production of testosterone<sup>26</sup>. In our study, administration of testosterone was by subcutaneous implant of silicon pellets containing the, with continuous and uniform hormone liberation, and replaced at every 21 days. Euthanasia and collection of samples corresponded to a day of replacement of pellets and thus exogenous testosterone was probably already in low levels, and the endogenous was already suppressed. Similar antiandrogenic effects were observed<sup>27</sup>, in which rats treated with soy presented a significant reduction in testosterone and FSH levels<sup>13</sup>, also noted a significant decrease in seric testosterone levels in rats treated with linseed flour<sup>28</sup>, related decrease in seric testosterone levels in rats treated with canola oil for 84 days.

Beta - sitosterol present in Avocado Oil is an inhibitor of the conversion of testosterone in its active metabolite, dihydrotestosterone (DHT)<sup>29</sup>, as well as finasteride. The use of finasteride causes alterations in testosterone FSH and LH levels, with significant reduction of the last two<sup>30</sup>, and thus similar alterations may have occurred in the animals of the induced group AIOG, in which reduction of LH and FSH levels, in synergism with exogenous administration of testosterone may have caused a decrease in seric testosterone levels at 6 the time of biologic sampling.

This work suggests that Avocado Oil exerted a direct regulating effect on the lipidic profile, efficiently acting on animals submitted to androgenic stimulation over a prolonged period.

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