Nutrición Hospitalaria

Trabajo Original

Otros

Consumption of avocado oil *(Persea americana)* improves the biochemical profile of rats submitted to long-term androgenic stimulation

El consumo de aceite de aguacate (Persea americana) *mejora el perfil bioquímico de ratas sometidas a estimulación androgénica a largo plazo*

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Abstract

Introduction: indiscriminate use of anabolic steroids is associated with cardiovascular diseases, renal damage, and hepatic toxicity. Contrastingly, nutraceutical foods such as avocados prevent and control several diseases, as they can reduce the effects of oxidative stress.

Objective: this study evaluates the benefits of consuming an avocado oil-based diet to attenuate the systemic damage caused by supraphysiological doses of testosterone, by analyzing the biochemical profile of 28 42-day-old male Wistar rats.

Methods: silicone pellets containing testosterone were surgically implanted, and they received control or avocado oil-based feed. After 20 weeks, all the male rats were anesthetized and their blood samples collected.

Results: although the high hormone concentration had a negative influence on the biochemical profile of these animals, the groups that consumed avocado oil exhibited a reduction in serum triacylglycerols (-21 %; p = 0.0001), VLDL (-20 %; p = 0.0085), LDL (-78 %; p < 0.0001), and total cholesterol (-12 %; p < 0.0001), along with positive changes in their HDL concentrations (+7 %; p = 0.001). The avocado oil groups also manifested a reduction in the total concentration of serum proteins (-24 %; p = 0.0357), albumin (-26 %; p = 0.0015), urea (-14 %; p = 0.04), and creatinine (-33 %; p < 0.0001). The concentration of liver transaminases was found to be higher in the animals included in the induced group (ALT, +66 %; p = 0.0005, and AST, +23 %; p = 0.0021), whereas they remained stable in the avocado oil group.

Conclusion: from the above, it may be concluded that supraphysiological doses of testosterone are related to increased risk factors for cardiovascular, renal, and hepatic diseases, and that the consumption of avocado oil shields the biochemical profile, thus reducing the associated risk factors.

Resumen

Introducción: el uso indiscriminado de esteroides anabólicos se asocia con enfermedades cardiovasculares, daño renal y toxicidad hepática. En cambio, los alimentos nutracéuticos como el aguacate previenen y controlan varias enfermedades, ya que pueden reducir los efectos del estrés oxidativo.

Objetivo: este estudio evalúa los beneficios de consumir una dieta basada en aceite de aguacate para atenuar el daño sistémico causado por dosis suprafisiológicas de testosterona mediante el análisis del perfil bioquímico de 28 ratas Wistar macho de 42 días de edad.

Métodos: se implantaron quirúrgicamente perdigones de silicona que contenían propionato de testosterona y los animales recibieron una alimentación de control o una basada en el aceite de aguacate. Después de 20 semanas se anestesiaron todos los animales y se recogieron sus muestras de sangre.

Resultados: aunque la alta concentración de hormonas tuvo una influencia negativa en el perfil bioquímico de estos animales, los grupos que consumieron aceite de aguacate mostraron una reducción de los triglicéridos séricos (-21 %; p = 0,0001), las VLDL (-20 %; p = 0,0085), las LDL (-78 %; p < 0,0001) y el colesterol total (-12 %; p < 0,0001), con cambios positivos en las LDL (+7 %; p = 0,001). Los grupos alimentados con aceite de aguacate manifestaron una reducción de la concentración total de proteínas séricas (-24 %; p = 0,0357), albúmina (-26 %; p = 0,0015), urea (-14 %; p = 0,04) y creatinina (-33 %; p < 0,0001). Se encontró que la concentración sérica de transaminasas hepáticas era mayor en los animales del grupo inducido (ALT: +66 %; p = 0,0005, y AST: +23 %; p = 0,0021), mientras que en los grupos con aceite de aguacate, los parámetros hepáticos se mantuvieron estables.

Conclusión: de todo ello se puede concluir que las dosis suprafisiológicas de testosterona están relacionadas con un aumento de los factores de riesgo de sufrir enfermedades cardiovasculares, renales y hepáticas, y que el consumo de aceite de aguacate protege el perfil bioquímico, lo que reduce los factores de riesgo asociados.

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INTRODUCTION

The last three decades have witnessed a continuous growth in the use of anabolic steroids by high performance athletes and adolescents, as well as young non-athletes (1-3). Several young people engage in anabolic steroid use before the age of ten (2). Steroid abuse has effects on the physiology of cardiac and vascular tissues (4,5). Several authors have demonstrated the positive association between testosterone use, as an anabolic agent, and the occurrence of cardiovascular disease (CVD) (5,6). In many cases, a random use of anabolic steroids might lead to increased blood pressure, myocardial infarction, renal and hepatic damage, and even death in individuals without a previous history of disease (1,7-11). Certain other dysfunctions also occur, which degrade the user's quality of life.

On the other hand, the consumption of nutraceutical foods may assist in the process of weight loss and body fat reduction, and in the overall improvement of organic functions. Avocado (*Persea americana*) oil has components that act on the body's systems and may reduce and/or delay the severity or expansion of some diseases (12). Avocado oil contains antioxidants and anti-inflammatory elements, including lecithins, phytosterols (particularly beta-sitosterol), monounsaturated fats, lutein, and vitamins A and E (13). These components are capable of altering lipid and cholesterol metabolism (14), decreasing the risk of cardiovascular and renal diseases (15,16), and modulating the metabolism of testosterone (17). Appropriate nutrition, which includes this type of food and is associated with physical exercise, forms the prophylaxis or even an additional part of the therapy for some diseases, especially CVD.

Thus, based on the existing knowledge of the effects of testosterone and the benefits of avocado oil as a nutraceutical, we aimed to evaluate the biochemical effects of avocado oil consumption in animals that were submitted to prolonged androgenic stimulation with supraphysiological doses of testosterone.

MATERIALS AND METHODS

ANIMALS

The use of animals was approved by the Animal Use Ethics Committee under the CEUA-765/2016 protocol. Materials from 28 adult male Wistar rats aged 42-50 days were used. The rodents in this study were subdivided into 4 groups of 7 rodents each, and arranged as follows: control group (CG)—animals that received a control casein-based feed; avocado oil group (AOG)—animals that received an avocado oil-based feed; induced group (IG)—animals that received testosterone and received a control casein-based feed; and induced avocado oil group (IAOG)—animals that received testosterone and an avocado oil-based feed. The rodents were housed in individual plastic cages on a 12:12-h light/dark cycle and at a constant temperature of 22 ± 1 °C. They had free access to water, and received the experimental feeds *ad libitum*. Body weight and feed intake were monitored weekly.

INDUCTION

Induction of testosterone was performed by using silicone pellets (Dow Corning, cat. no. 508-009 Silastic™ Tube, 1.98 mm I.D. × 3.18 mm O.D., 5 cm long) filled with testosterone propionate (1 mg) and sealed with a surgical adhesive (18). This method was preferred to daily intramuscular injection because in addition to reducing the handling of the animals, it also ensured that the animals were not subjected to excessive stress, which could affect the results of the work. The rodents were anesthetized intraperitoneally with xylazine (10 mg/kg) and ketamine (75 mg/kg), and the pellets were implanted in their dorsal scapular region with an incision of approximately 10 mm. For skin closure a cyanoacrylate-based surgical adhesive was used. The pellets were replaced every four weeks for 20 weeks. As testosterone was released, the amount of hormone within the pellet decreased, necessitating thus replacement every four weeks for the maintenance of androgenic stimulus.

EXPERIMENTAL FEEDS

The experimental feeds were isocaloric, and had a vitamin and mineral mix added to them in accordance with the recommendations of the American Institute of Nutrition (AIN-93M) (19). In this study, the lipid concentration values were adjusted from 4 % (AIN93M) to 7 % in order to enhance the effects of avocado oil while maintaining the balance in the control casein-based diet. For caloric compensation, the carbohydrate percentage was adjusted. The feed offered to the avocado oil (AOG) and induced avocado oil (IAOG) groups had a 7 % concentration of avocado oil, while that offered to the control (CG) and induced (IG) groups had 7 % of soybean oil. In the avocado oil groups, soybean oil was replaced with avocado oil to maintain the same lipid concentration in all experimental feeds. This oil was extracted using a cold press to maintain its natural properties. The fatty composition of avocado oil is listed in table I. The ingredients of the experimental feeds (Table II) were weighed and homogenized with boiling water for starch gelatinization using an industrial mixer (Hobart®, São Paulo, SP, Brazil). The resultant dough, after identification, was transformed into pellets and dried in a ventilated incubator (Fabbe-Primar® n° 171, São Paulo, SP, Brazil) at 60 ºC for 24 h, and stored under refrigeration until use.

BIOCHEMICAL PROFILE

At the end of the experimental period at the bioterium, the animals were euthanized. The rodents were anesthetized with 75 mg/kg of ketamine plus 10 mg/kg of xylazine, and the calculated dose was intraperitoneally administered. After the anesthetic condition was established through the absence of pedal reflex, the rats were then subjected to bleeding by intracardiac puncture, from which 10 mL of blood were obtained. After bleeding, an additional dose of anesthesia was administered, which led to the death

CONSUMPTION OF AVOCADO OIL *(PERSEA AMERICANA)* IMPROVES THE BIOCHEMICAL PROFILE OF RATS SUBMITTED TO LONG-TERM ANDROGENIC STIMULATION

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Table I. Fatty acid composition and total lipids in avocado oil

Lipids	g/100 g of feed	%/100 g of avocado oil
Total lipids	14 g	
Saturated fatty acids (SFA)	1 g	
Monounsaturated fatty acids (MUFA)	10 g	
Polyunsaturated fatty acids (PUFA)	3 g	
C16:0 Palmitic acid		9-19.5 %
C16:1 Palmitoleic acid		2-9 %
C18:0 Stearic acid		< 2 %
C18:1 Oleic acid (omega 9)		42-81 %
C18:2 Linoleic acid (omega 6)		6-18.5 %
C18:3 Linolenic acid (omega 3)		< 2 %

Table II. Composition of every 100 g of feed used in the study

Nutrients	g/100 g	
	Control	Avocado oil
Casein[a]	14.00	14.00
Starch[b]	58.95	58.95
Refined sugar[c]	10.00	10.00
Mineral mix[a]	3.50	3.50
Vitamin mix[a]	1.00	1.00
Soybean oil[d]	7.00	0.00
Avocado oil[e]	0.00	7.00
Cellulose[f]	5.00	5.00
Choline bitartarate[a]	0.25	0.25
Cystine[a]	0.30	0.30
Tert-Butylhydroquinone[a]	0.0014	0.0014

The ingredients used in the preparation of the diet were supplied by: a: M. Cassab Industry and Commerce Limited (São Paulo, SP, Brazil); b: Maizena Unilever Bestfoods Brazil Limited (Mogi Guaçu, SP, Brazil); c: União (Rio de Janeiro, RJ, Brazil); d: Liza Cargill Agriculture Limited (Mairinque SP, Brazil); e: TBF foods - Copra food industry limited (Maceió, AL, Brazil); f: MicrocelBlanver Limited (Cotia, SP, Brazil).

of the rodents. Blood samples were held for about two hours at room temperature for clot retraction. Thereafter, they were centrifuged at 958.5 g for five minutes to obtain the serum, and stored for 24 h at -20 °C. Then, biochemical analyses of urea, creatinine, albumin, total proteins, cholesterol, triacylglycerols, low-density lipoproteins (LDL), high-density lipoproteins (HDL), very low-density lipoproteins (VLDL), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were performed using LabTest colorimetric kits (LabMax, Belo Horizonte, Brazil). Serum testosterone and estradiol were measured by radioimmunoassay (RIA) using a commercial, solid-state Beckman Coulter kit (Immunotech®). The tests were performed in the laboratory of the Brazilian Institute of Diagnosis and Veterinary Specialties (PROVET/São Paulo, Brazil) using a Perkin Elmer (RIA) WIZARD2 equipment. All parameters of assay quality were checked in accordance with the instructions available from the international scientific community. All the results of the control group were compared to the reference values found in the literature for this animal model (7,20).

STATISTICAL ANALYSIS

The data were presented under the average and standard deviation form. To test the normal distribution of values, the Kolmogorov-Smirnov test was deployed, and for data analysis an ANOVA univariate test, in association with the Tukey-Kramer multiple comparison test, was carried out. For all tests, significance was established at the level of $p < 0.05$. The statistical analyses were performed using the Graph Pad Prism, version 5.0, 2007 program (San Diego, CA, USA).

RESULTS

At the beginning of the experiment, all animals had a mean body weight of 260 ± 10 g, with no statistical differences between the groups ($p = 0.2333$). At experiment days 40 ($p < 0.0001$) and 60 ($p < 0.0165$), the animals in the IG exhibited a higher body weight when compared to those in the CG. At the end of the experiment, all the animals presented similar values of body weight ($p > 0.2448$). These data are presented in table III.

Table III. Mean values of rat body weight and food intake at 40, 80, and 120 days of experiment onset

Parameter	CG	IG	AOG*	IAOG*	p-value
Body weight at 40 days	299.7 ± 21.8 [a]	363.0 ± 13.6 [b]	319.6 ± 29.7 [a]	345.7 ± 13.0 [a]	< 0.0001
Body weight at 80 days	368.4 ± 30.2 [a]	407.8 ± 12.8 [b]	387.1 ± 21.0 [a]	389.5 ± 14.8 [a]	< 0.0165
Body weight at 120 days	440.6 ± 20.2 [a]	444.4 ± 15.2 [a]	445.0 ± 9.4 [a]	429.6 ± 16.5 [a]	> 0.2448
Food intake at 40 days	21.9 ± 1.7 [a]	24.5 ± 1.4 [a]	21.8 ± 2.1 [a]	23.1 ± 1.9 [a]	< 0.0377
Food intake at 80 days	27.7 ± 3.3 [a]	29.7 ± 0.9 [a]	27.5 ± 2.7 [a]	28.7 ± 0.7 [a]	< 0.2675
Food intake at 120 days	31.6 ± 1.7 [a]	33.8 ± 1.2 [a]	31.7 ± 2.3 [a]	32.7 ± 1 [a]	< 0.0694

*Experimental groups. The letters a, b and c represent significant differences between groups. CG: control group; IG: induced group; AOG: avocado oil group; IAOG: induced avocado oil group.

Table IV. Biochemical profile of the experimental groups

Parameters	Groups				p-value
	Control group (CG)	Induced group (IG)	Avocado oil group (AOG)*	Induced avocado oil group (IAOG)*	
Testosterone (ng/dL)	314.3 ± 74.2 [a]	701.3 ± 145.4 [b]	186.4 ± 79.1 [a]	120.2 ± 29.1 [c]	< 0.0001
Estradiol	44.8 ± 35.4 [a]	32.9 ± 35.2 [a]	218.3 ± 117.2 [b]	17.4 ± 15.4 [a]	< 0.0001
Total proteins (g/dL)	6.4 ± 0.4 [a]	7.0 ± 0.1 [a]	5.1 ± 0.6 [b]	5.2 ± 0.8 [b]	0.0357
Albumin (g/dL)	2.9 ± 0.4 [a]	2.9 ± 0.2 [a]	2.3 ± 0.2 [b]	2.2 ± 0.3 [b]	0.0015
Urea (mg/dL)	30.5 ± 3.5 [a]	34.2 ± 2.2 [b]	28.4 ± 3.6 [a]	29.4 ± 3.0 [a]	0.0455
Creatinine (mg/dL)	0.5 ± 0.0 [a]	0.6 ± 0.0 [a]	0.4 ± 0.0 [b]	0.4 ± 0.0 [b]	< 0.0001
ALT (UI/L)	15.7 ± 4.8 [a]	25.1 ± 6.2 [b]	16.1 ± 1.8 [a]	20.1 ± 2.4 [a]	0.0005
AST (UI/L)	47.2 ± 5.5 [a]	58.3 ± 7.9 [b]	46.4 ± 2.7 [a]	55.4 ± 7.2 [a]	0.0021
Triacylgcerols (mg/dL)	106.8 ± 16.5 [a]	161.4 ± 23.4 [b]	92.2 ± 25.6 [a]	127.1 ± 20.0 [a]	0.0001
Cholesterol (mg/dL)	48.7 ± 9.9 [a]	65.3 ± 12.7 [b]	31.2 ± 6.3 [c]	57.7 ± 6.9 [a]	< 0.0001
HDL (mg/dL)	22.3 ± 3.83 [a]	26.7 ± 5.42 [b]	19.2 ± 4.6 [a]	28.5 ± 5.44 [b]	0.0010
LDL (mg/dL)	4.4 ± 3.5 [a]	21.9 ± 8.8 [b]	4.6 ± 2.3 [a]	4.6 ± 4.0 [a]	< 0.0001
VLDL (mg/dL)	30.7 ± 5.8 [a]	35.1 ± 5.2 [b]	24.2 ± 4.0 [a]	28.1 ± 4.45 [a]	0.0085

Experimental groups. Letters a, b and c represent significant differences between groups.

Serum testosterone concentration increased by 123 % in the IG when compared to the values observed in the CG (p < 0.0001). The AOG presented serum testosterone values close to the values in the CG, whereas the mice in the IAOG presented slightly lower serum testosterone levels. These results were statistically significant when compared to the control group. The serum estradiol concentration increased 4-fold (p < 0.0001) in the AOG when compared to the other groups.

A reduction in serum total protein (p = 0.0357), accompanied by a significant reduction in serum albumin (p = 0.0015), could be observed in the avocado oil-fed groups. The groups that received the avocado oil-based diet also showed a reduction in serum urea levels (10 % less than the CG and 17 % less than the IG; p = 0.0455) and creatinine levels (20 % less than the CG and 33.3 % less than the IG; p < 0.0001). The serum concentration of liver transaminases, ALT (p = 0.0005) and AST (p = 0.0021), were found to be higher in the induced group when compared to the other groups. The IAOG presented values of serum ALT and AST levels close to those of the control mice.

There was a significant increase (p = 0.0001) in the serum triacylglycerol value (TG) of the IG (161.4 ± 23.04) when compared to the CG (106.8 ± 16.5). The concentration of serum TG decreased by about 21 % upon the administration of the avocado oil-based diet to the induced animals. Increased serum cholesterol values were observed in the IG. When compared with the CG and IG, serum cholesterol decreased by about 36 % in the AOG and 12 % in the IAOG, respectively (p < 0.0001). Serum HDL-cholesterol levels remained stable in the groups that received avocado oil, with a slight increase of this parameter the IAOG (p = 0.0010). The IG manifested an increase in HDL and other cholesterol fractions resulting in a proportional increase of total cholesterol. Serum

LDL in the IG was found to be five times higher than that in the AOG, IAOG, and CG (p < 0.0001). Serum VLDL values were also increased in the IG when compared with to the other groups (p = 0.0085). All these numeric values are showed in table IV.

DISCUSSION

Serum testosterone concentration in the control group was similar to the reference values for Wistar rats (20). Increased testosterone concentration in the IG showed that the induction was satisfactory. The AOG and IAOG presented low serum testosterone values, but the IAOG showed a significant reduction when compared with the CG. These data confirm the literature by demonstrating that beta-sitosterol, which is present in avocado oil, has anti-androgenic effects by modulating the 5-alpha-reductase enzyme (17). Avocado oil is rich in these phytosterols, and the concentration of these components in this oil are higher than in other vegetable oils (21). An *in vitro* study (22) showed that the administration of beta-sitosterol may potentially lead to estrogenic activity. The consumption of avocado oil can be considered an alternative to hormone replacement therapies for climacteric women (23). Similarly, it was observed that the addition of the oil to the AOG's diet reproduced this estrogenic effect.

With the use of anabolic steroids, an increased concentration of serum triacylglycerols in the presence of androgenic supraphysiological stimulus is expected. This effect can also be observed in humans despite the presence of physical exercise (24). The administration of steroids, even for a short term, produces unfavorable effects not only on lipases but also on apolipoproteins related to HDL (25). It also raises the low-density fractions

of cholesterol (7,24). Together, these factors significantly increase the risk of cardiovascular diseases. However, on the one hand, if testosterone can increase triacylglycerols, cholesterol, LDL, and VLDL, this study showed that, in contrast, avocado oil consumption improves the lipid profile, even with androgenic stimulus. It is already known that the presence of oleic acid (omega-9, a major fatty acid that is a component of avocado oil), linolenic acid, and linoleic acid (omega-3 and omega-6, respectively) decreases the susceptibility of LDL to oxidation. They can also act to reduce endothelial injury, thereby helping the prevention of atherosclerosis (26). Furthermore, other antioxidants present in avocado oil are highly capable of reducing not only cholesterol and fraction concentration, but also their oxidation (14,26). This became evident when the triacylglycerol values were reduced in the avocado oil groups by about 21 % in relation to the control group, except for the decreased concentration of total cholesterol, LDL and VLDL. However, the HDL fraction in the avocado oil-fed groups showed a positive increase. In addition to the beneficial effect on the lipid profile, avocado oil consumption reduces glucose tolerance and insulin resistance, further decreasing the risk of developing metabolic diseases and CVD (27).

The total protein and seric albuminin values of the CG were within the reference values (20). When compared with the CG, the serum protein concentrations in the IG slightly increased, whereas in the avocado oil groups seric proteins decreased. One of the hypotheses for increased protein in the IG would be the inflammatory potential of testosterone. It has already been shown that testosterone administration promotes inflammatory response as well as the release of cytokines, a fact that would explain the observed results (28,29). Contrastingly, it has been observed that consumption of avocado oil leads to improvement in various inflammatory parameters, including total proteins and globulins (26,30). Protein reduction in the AOG and IAOG groups could be indicative of the anti-inflammatory potential of avocado, which would reduce the number of inflammatory mediators in the body, thus reducing the total protein in both the control and induced animals.

In addition to their inflammatory potential, high concentrations of testosterone might be harmful to renal tissue (4,9,31,32). The values observed for seric albumin, urea, and creatinine in the animals of the IG confirm this hypothesis. In contrast, the results observed in the oil groups showed that consumption of the avocado oil-based diet has a protective effect on the kidney, even for the groups that received testosterone. It has been shown that beta-sitosterol isolated from plants, including avocado, is beneficial for kidney function, improves blood pressure (33), and may reverse the damage caused by diseases such as diabetes (34), renal dysfunction, or nephrotoxicity (16,35).

Hepatic tissue can be damaged in the presence of high levels of plasma testosterone. Long-term exposure to this hormone can lead to irreversible hepatic injury and liver cancer (7,11,36). Increased hepatic enzyme concentrations in induced animals indicated that testosterone caused tissue damage, which is potentially harmful. It is a fact reported in the literature that an avocado oil-based diet induces a decrease in liver parameters, including

ALT and AST, even in the presence of high glucose levels (30), or in induced advanced liver injury by carbon tetrachloride (37). The administration of an avocado oil-based diet was also beneficial in this study, as it significantly reduced indicators of hepatic damage in the IAOG animals. The use of anabolic agents increases the risk of developing hepatic neoplasms with a serious prognosis for the user (36,38). The consumption of avocado oil, as observed in its effects, can protect the liver and even be an ally in the therapy against hepatic neoplasms and other chemically induced cancers (39).

It can be cited that a limitation of this study is that the mechanisms of action of avocado oil's isolated components were not analyzed. Because the *in natura* product has been used, each of the molecules present in its composition acted on the systems together, which made it difficult to analyze the isolated actions of each component.

Therefore, it may be concluded that the consumption of avocado oil attenuates the systemic damage caused by supraphysiological concentrations of androgens, and also improves renal and hepatic parameters, lipid metabolism, and hormonal and anti-inflammatory parameters, thus indicating its usefulness in the treatment of diseases caused by prolonged androgenic stimulation—mainly cardiovascular, hepatic, and renal. Its consumption by healthy individuals is also safe and recommended.

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