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Effects of Testosterone and whey Protein on Prepubertal Rats

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Abstract:- In contemporary society, influenced by media ideals, the pursuit of an aesthetic body extends beyond health boundaries, with adolescents often drawn to the positive effects of anabolic androgenic steroids (AAS) for physical enhancement. However, the dearth of knowledge about the repercussions on the adolescent body necessitates exploration. AAS misuse, prevalent in gyms, poses health risks, particularly with doses surpassing therapeutic levels. Adverse effects include metabolic, endocrine, cardiovascular, hepatic, neural, aesthetic, behavioral, and psychiatric consequences. This study aims to assess the impact of testosterone and protein supplements (Whey Protein) on prepubertal rats, utilizing an experimental model involving 40 male Wistar albino rats divided into four groups. The research adheres to ethical standards, seeking to enhance our understanding of the intricate hormonal dynamics affected by these substances, contributing to the broader discourse on adolescent health and well-being.

Keywords:- *Protein, Physical Exercise, Testosterone, Wistar Rats.*

I. INTRODUCTION

In today's media-influenced society, the quest for the ideal aesthetic goes beyond the limits of health. The search for the perfect body goes beyond the limits of physical and mental health.

The use of anabolic steroids to improve both physical performance and aesthetics is well known among adults and athletes. As a result, adolescents are influenced by the positive effects of these substances from an early age, while ignoring their negative effects. However, little is known about their effects on the adolescent body.

Anabolic androgenic steroids (AAS) are synthetic drugs with similar activity to testosterone that are used for therapeutic, sporting and aesthetic purposes due to their anabolic and androgenic properties (Celotti & Cesi, 1992; Hebert. Haupt, George & Rovere, 1984; Kuhn, 2002). These drugs are often used in gyms and physical training centres without any criteria or control by people wishing to improve their physical and aesthetic performance, posing a health risk to the users (Andersen, Bartlett, Morgan & Brownell, 1995; Fuller, 1993). Doses are usually 10 to 100 times higher than the therapeutic dose and 2/3 of abuse occurs among non-athletes, including adolescents who are constantly influenced by the positive effects (Cunha, Moura, Bernardes, Tanno & Marcondes, 2005; Kam & Yarrow, 2005; Pope Junior & Katz, 1988).

Abuse of EAA has several negative effects on the body, with adverse metabolic, endocrine, cardiovascular, hepatic, neural, aesthetic, behavioural and psychiatric consequences (Kindermann, 2006; Manetta & Silveira, 2000; Redondo, 2007; Rocha, Carmo, Roque, Hashimoto, Rossoni, Frimm, Anéas, Negrão, Krieger & Oliveira, 2007; Tagarakis, Bloch, Hartmann, Hollmann & Addicks, 2000; Takahashi, Tatsugi & Kohno, 2004). Although several beneficial effects of aerobic resistance training on skeletal muscle are known, little is known about their association with the use of exorbitant amounts of testosterone. Most studies relate the use of these resources to strength athletes or those who train at high intensity.All synthetic and semi-synthetic AAS marketed by the pharmaceutical industry and illegal laboratories, which often surpass the legal trade, are derived from testosterone.

Testicular growth, spermatogenesis and steroidogenesis are regulated by the pituitary gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH increases the production of cyclic AMP in the interstitial cells of the testicle, increasing the transformation of cholesterol into estrogens. FSH acts by promoting spermatogenesis and increasing LH activity and testosterone production. The male hormones released by the Leydig cells reach the seminiferous tubules and the circulation, virilizing the individual.

In mammals, testosterone release is pulsatile and regulated by negative feedback. When there is a lack of testosterone, the hypothalamus is stimulated to secrete gonadotropin-releasing hormone, which stimulates the pituitary gland to secrete FSH and LH, increasing testosterone production. The high amount of testosterone suppresses the release of FSH and LH, decreasing the endogenous synthesis of the hormone and spermatogenesis and leading to testicular atrophy.

- *Objectives*

To evaluate the influence of the hormone testosterone and protein supplements (Whey Protein) on prepubertal rats.

➢ *Methodology (Material and Methods)*

- *Animals:*

We used 40 male Wistar albino rats of prepubertal age, weighing an average of 20g, from the Center for Experimentation in Animal Models (CEMA) at the University of Marília (UNIMAR). Prior to the experiment, all the animals were acclimatized for three days to the laboratory conditions, divided into four groups of 10 animals, placed in plastic boxes (40x30x17cm) in a room with controlled temperature (20°C - 25°C) and light/dark cycle (12/12 hours), with water and food ad libitum.

➢ *Experimental Model*

The animals were randomly divided into 4 groups of 10 (n=10/group) and named according to the treatment received as:

- Grupo 1 (G1) - Control.
- Grupo 2 (G2) -Treated: the animals were given testosterone (5mg/kg) and a food supplement (Optimum Whey Protein).
- Grupo 3 (G3) - Treated: the animals were given testosterone.
- Grupo 4 (G4) - Treated: the animals were given a food supplement (Whey Protein Optimum).

All the experimental procedures used are in accordance with the Ethical Principles in Animal Experimentation (COBEA) and the research ethics committee has submitted the project for approval.

➢ *Euthanasia*

At the end of the experiment, the animals were euthanized by intraperitoneal administration of an overdose of thiopental.

After euthanasia, blood was collected from the portal vein for later biochemical analysis, the heart, liver, gastrocnemius muscle, kidney and visceral fat were removed for later weighing and the epididymis was removed for histological analysis.

➢ *Statistical Analysis*

The results were expressed as mean ± standard deviation. Analysis of variance (ANOVA) or Mann-Whitney were used to compare the groups, depending on the data analyzed, with a significance level of 5%.

II. RESULTS

With regard to initial weight, we found that the control group (G-1) had an average of 41.4, the testosterone group (G-2) had an average of 38, the testosterone and whey-protein group (G-3) had an average of 43.1 and the whey-protein group (G-4) had an average of 39. As a result, there was no statistically significant difference between the groups on the first day (September 16). With regard to final weight, we observed that the control group (G-1) obtained an average of 185.9, the testosterone group (G-2) obtained an average of 198, the testosterone and whey-protein group (G-3) obtained an average of 184.4 and the whey-protein group (G-4) obtained an average of 175.4. This also made it clear that there were no statistically significant changes on the last day (October 16).

With regard to the total weight of the rats, there was no statistically significant difference between the four groups.

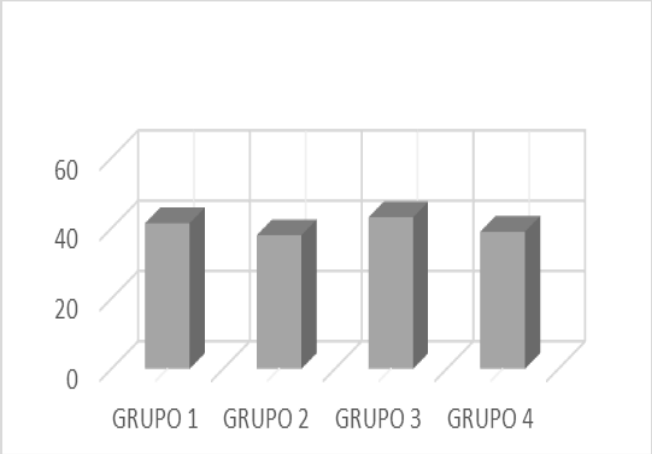

Fig 1 Starting Weight 16/set/2015

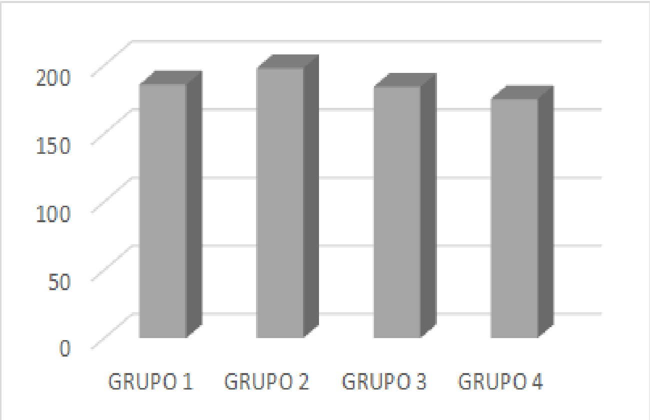

Fig 2 Final Weight 16/OUT/15

With regard to heart weight, we observed that the control group (G-1) obtained an average of 0.7782, the testosterone group (G-2) obtained an average of 1.033, the testosterone and Whey Protein group (G-3) obtained an average of 0.8345 and the Whey Protein group (G-4) obtained an average of 1.088. As a result, there was no statistically significant difference between the groups.

With regard to liver weight, we observed that the control group (G-1) obtained an average of 9.025, the testosterone group (G-2) obtained an average of 11.424, the testosterone and Whey Protein group (G-3) obtained an average of 8.5572 and the Whey Protein group (G-4) obtained an average of 8.890. As a result, there was no statistically significant difference between the groups. With regard to liver weight, there was no statistically significant difference between the four groups, since the p-value was 0.0797.

With regard to testicular weight, we observed that the control group (G-1) obtained an average of 1.055, the testosterone group (G-2) obtained an average of 0.82, the testosterone and Whey Protein group (G-3) obtained an average of 0.8628 and the Whey Protein group (G-4) obtained an average of 1.3306. As a result, there was no statistically significant difference between the groups.

With regard to the ALTLP enzyme, control group 1 (G-1) showed a statistically significant difference in relation to groups 2 and 3, while group 4 showed no difference. Group 2 showed a statistically significant difference in relation to group 3 and there was no difference in relation to group 4. Finally, there was a difference between groups 3 and 4. Group 3 was the only group to show a significant decrease in the enzyme compared to the other groups, while group 4 showed the greatest increase.

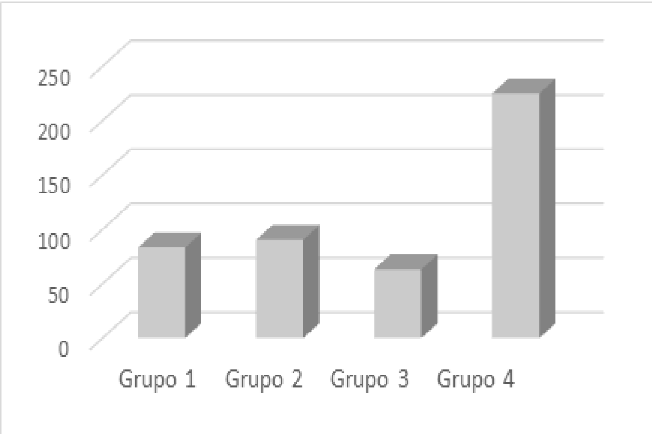

Table 3 Alanine Transaminase (ALT)

With regard to the ASTL enzyme (C), we observed that the control group (G-1) obtained an average of 167.37; the testosterone group (G-2) obtained an average of 163.4; the testosterone and whey protein group (G-3) obtained an average of 246.69; and the whey protein group (G-4) obtained an average of 562.77. As a result, there was no statistically significant difference between the groups.

Group 4 (Whey-Protein) was the group that obtained a significant increase in the enzyme compared to the other groups.

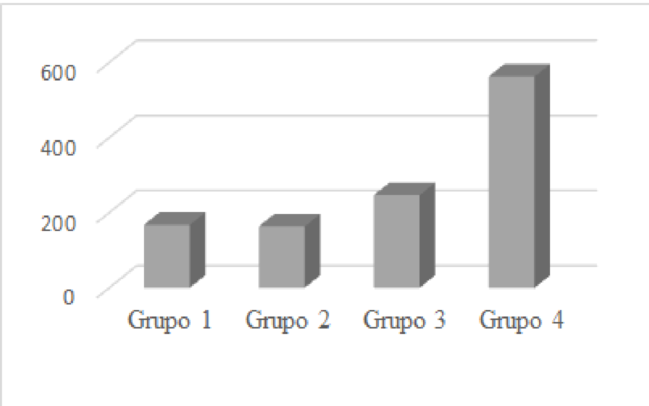

Fig 4 Aspartate Aminotransferase (AST)

With regard to CHO2I (C), control group 1 (G-1) showed no statistically significant difference from groups 2, 3 and 4. Group 2 showed a statistically significant difference in relation to groups 3 and 4. Finally, there was no difference between groups 3 and 4.

With regard to GLUC3 (C), control group 1 (G-1) showed no statistically significant difference from groups 2 and 4, while group 3 showed a difference. Group 2 showed a statistically significant difference in relation to group 3 and no difference in relation to group 4. Finally, there was no difference between groups 3 and 4. Group 2 was the group that obtained a significant increase in GLUC3 (C) compared to the other groups. Groups 3 and 4 had a significant decrease.

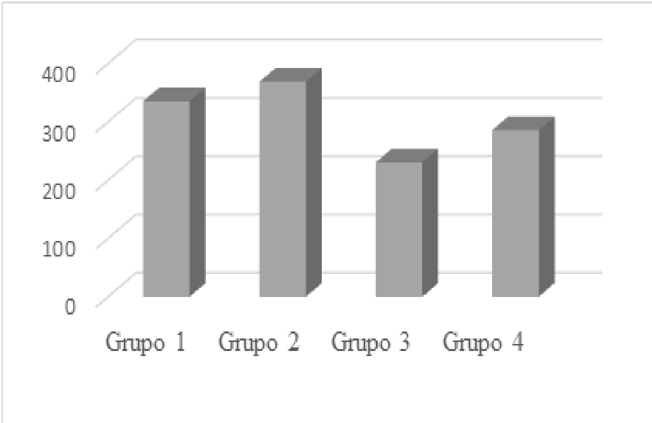

Fig 5 GLUC3 (C)

With regard to HDLC3 (C), control group 1 (G-1) showed no statistically significant difference from group 2, while there was a difference between groups 3 and 4. Group 2 showed a statistically significant difference in relation to groups 3 and 4. Finally, there was no difference between groups 3 and 4.

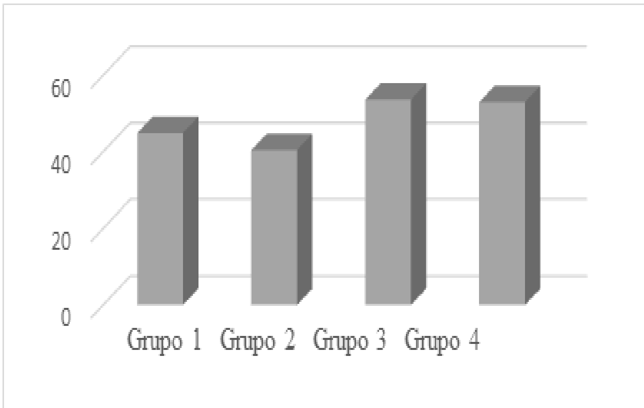

Fig 6 HDLC3 (C)

With regard to TRIGL (C), control group 1 (G-1) showed no statistically significant difference from groups 3 and 4, while group 2 showed a difference. Group 2 showed a statistically significant difference in relation to groups 3 and 4. Finally, there was no difference between groups 3 and 4. Group 2 was the group that had a significant increase in TRIGL (C) compared to the other groups.

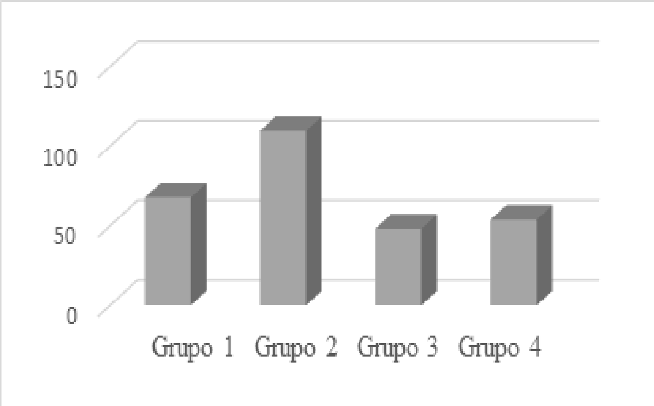

Fig 7 Triglycerides (TRIG)

With regard to VLDL, control group 1 (G-1) showed no statistically significant difference from group 4, while there was a difference between groups 2 and 3. Group 2 showed a statistically significant difference in relation to groups 3 and 4. Finally, there was no difference between groups 3 and 4. Group 2 was the group that had a significant increase in VLDL compared to the other groups.

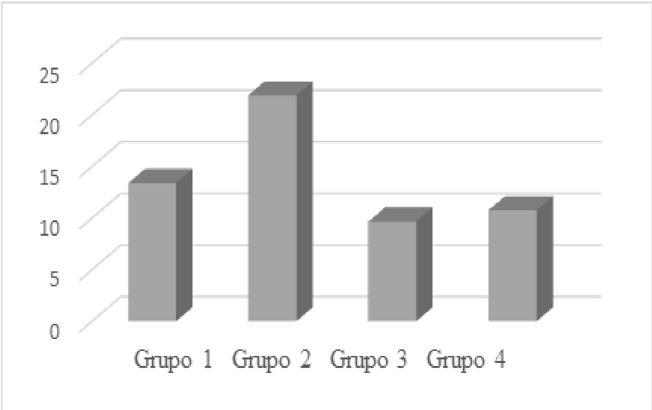

Fig 8 VLDL

With regard to LDL, control group 1 (G-1) showed no statistically significant difference from group 4, while there was a difference between groups 2 and 3. Group 2 showed a statistically significant difference in relation to groups 3 and 4. Finally, there was a difference between groups 3 and 4. Group 3 had a significant increase in LDL compared to the other groups. While groups 2 and 4 had a significant decrease compared to the other groups.

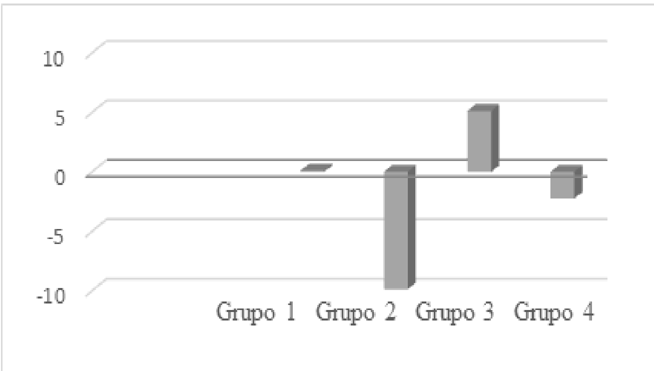

Fig 9 LDL-Cholesterol

With regard to testosterone, control group 1 (G-1) showed no statistically significant difference from group 4, while groups 2 and 3 showed a difference. Group 2 showed a statistically significant difference in relation to group 4 and there was no difference in relation to group 3. Finally, there was no difference between groups 3 and 4. Groups 2 and 3 had a significant increase in testosterone compared to the other groups.

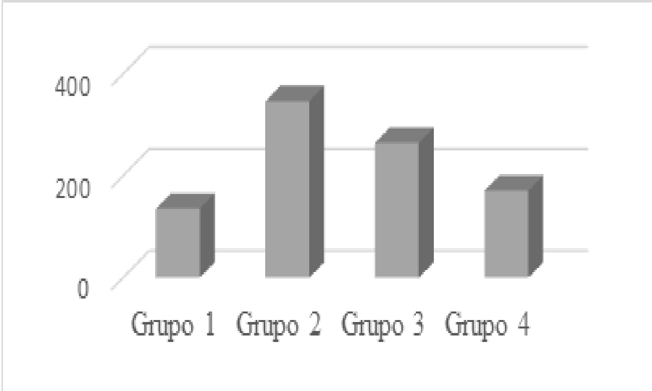

Fig 10 Testosterone

III. DISCUSSION

In relation to the total weight of the rats, there was no statistically significant difference between the four groups. Just as in the study "Influence of whey proteins on liver enzymes, lipid profile and bone formation in hypercholesterolemic rats", by the authors Fabiano Kenji HARAGUCHI1 Maria Lucia PEDROSA, Heberth de PAULA Rinaldo Cardoso dos SANTOS Marcelo Eustáquio SILVA, where the weight gains of the rats were compared to observe changes in relation to the various diets. With regard to the "PS" group: rats fed a diet made up of whey proteins instead of casein, we can see that the body weight of the rats in grams did not undergo a significant change in average compared to the control group (C), which was fed a standard diet. In relation to the use of testosterone, it was also found that the groups had no statistically significant differences in body weight before steroid treatment began. Thus, it can be seen that all the groups had similar body weights. As in the article "EFFECTS OF TESTOSTERONE, ESTRADIOL OR PROGESTERONE ON BODY DECOMPOSITION IN CASTRATE RATS", where it was found that the groups had similar body mass gain, even with the use of testosterone.

In terms of heart weight, there was no statistically significant difference between the groups, but there was a difference between the means of the groups. As a method of observation, the hearts of all the groups were weighed and so it can be seen that in the group that only used testosterone (G2), there is a difference in the average compared to the control group (G1), due to the fact that testosterone leads to a case of muscle hypertrophy, as in the work "Effects of anabolic steroids on the muscle of rats that have undergone aerobic training", by the author Gilson Sampaio Pinheiro Filho.

With regard to liver weight, there was no statistically significant difference between the four groups, since the p-value was 0.0797. As a method of observation, the liver weights of all the rats in their given groups were measured as a sample to observe lesions in the target organ caused by the use of testosterone or the whey-protein rich diet. As in the article "Influence of whey proteins on liver enzymes, lipid profile and bone formation in hypercholesterolemic rats", by the authors Fabiano Kenji HARAGUCHI, Maria Lucia PEDROSA, Heberth de PAULA, Rinaldo Cardoso dos SANTOS, Marcelo Eustáquio SILVA, where livers were weighed to observe changes in relation to the use of proteins. With regard to the "PS" group: rats fed a diet composed of whey proteins as a substitute for casein, we can see that the weight of the liver in grams did not undergo a significant change in average compared to the control group (C) with a standard diet.

As a method of observation, the testicles of the rats in all the groups were weighed and no statistically significant differences were observed between the groups. However, in relation to the weight averages, it can be seen that in the groups that used testosterone (G2 and G3), there was a difference in the average in relation to the control group and the group that used whey-protein. Thus, as in the study "Anabolic androgenic steroids and their relationship with sports practice", by the authors Tatiana Sousa Cunha, Nádia Sousa Cunha, Maria José Costa Sampaio Moura, Fernanda Klein Marcondes; we can conclude that the use of steroids leads to atrophy of the testicular tissue, which can lead to infertility and impotence.

In order to assess possible liver, kidney and muscle damage, the serum enzymes AST and ALT were measured, and there was a significant variation in the blood values of AST and ALT in prepubertal rats on the whey-protein diet (G-4) compared to the other groups. Based on the research "COMPARISON OF RENAL AND HEPATIC PROFILES IN MUSCULATION PRACTITIONERS WHO USE NUTRITIONAL SUPPLEMENTS IN ACADEMIES" by the authors "Bruno Prates Silva1 , Gilvan Cardoso G. Aguiar1 , Kaique Oliveira Fernandes1 , Rodrigo Marques Carneiro1 , Valquíria Mendes Barreto1 , Tarcísio Viana Cardoso2 , Alcimeire Lima Silva" reports that compared to the group of non-users of nutritional supplements, the users of these supplements showed a significant variation in the blood values of AST (24.5U/L and 33.4U/L) and ALT (19.4U/L and 26.9U/L). It should be noted that three samples fell outside the reference value for AST, which varies between (12U/L to 46U/L), and another sample for ALT, (03U/L to 50U/L). The enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are enzymes that are found in large quantities in the cytoplasm of hepatocytes. When liver cells are damaged or destroyed, there is an increase in these enzymes in the circulation. When there is mild damage, the predominant form in the serum is cytoplasmic, raising ALT, while in severe damage there is release of the mitochondrial enzyme, with an increase in AST. This difference has helped in the diagnosis and prognosis of liver diseases (MOTTA, 2009). Changes in the activity of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are important markers of disease or tissue damage, specifically in the liver.

With regard to total cholesterol, group 2 showed a statistically significant difference compared to groups 3 and 4. Finally, there was no difference between groups 3 and 4. Group G-2 showed a significant reduction in total cholesterol levels after taking testosterone. This can also be seen in the study "Evaluation of the influence of hormone therapy with oestradiol and testosterone subdermal implants on bone mineral density and lipid profile", which found a significant reduction in total cholesterol.

With regard to glucose, group 2 was the group that obtained a significant increase in GLUC3 (C) compared to the other groups. According to the laboratory tests, group G-2 had a significant increase in glucose, which was also found in the study "Cardiovascular Effects of Testosterone" by the authors Otavio C. E. Gebara, Núbia W. Vieira, Jayson W. Meyer, Ana Luisa G. Calich, Eun J. Tai, Humberto Pierri, Mauricio Wajngarten, José M. Aldrighi, which showed that glucose increased under the influence of testosterone.

With regard to HDL-cholesterol, group 2 showed a statistically significant difference compared to groups 3 and 4. Finally, there was no difference between groups 3 and 4. In relation to group G-2, which used 1ml of testosterone in each rat, there was a significant increase in HDL-cholesterol. In a cross-sectional study "A dose-response study of testosterone on sexual dysfunction and features of the metabolic syndrome using testosterone gel and parenteral testosterone undecanoate" by the authors Saad F, Gooren LJ, Haider A, Yassin A, in adult men, it was shown that increasing testosterone levels influences the increase in HDL-cholesterol.

With regard to triglycerides, group 2 was the group that had a significant increase in TRIGL (C) compared to the other groups. Group G-2 also showed a significant increase in triglyceride levels. This can also be seen in the study "Influence of anabolic androgenic steroids on aspects of chylomicron metabolism" by the author Aleksandra Tiemi Morikawa, which found that there is a direct relationship between the use of testosterone and an increase in triglycerides.

With regard to VLDL, group 2 was the group that had a significant increase in VLDL compared to the other groups. According to laboratory tests, group G-2 had a significant increase in VLDL. The study "Testosterone-induced changes in the plasma lipoprotein profile are modulated by the expression of cholesteryl ester transfer protein: studies in transgenic and control mice" by the author Andrea Camargo Casquero showed that the use of testosterone led to a significant increase in VLDL.

With regard to LDL-cholesterol, group 3 had a significant increase in LDL compared to the other groups. Groups 2 and 4 had a significant decrease compared to the other groups. Group G-3, which used both testosterone (1ml) and Whey Protein, had a significant increase in LDL-

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cholesterol. The study "Influence of anabolic androgenic steroids on aspects of chylomicron metabolism" by the author Aleksandra Tiemi Morikawa found that there is a direct relationship between testosterone and an increase in LDL-cholesterol levels.

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