Influences of testosterone undecylate on serological parameters of obese and non-obese rats

Influências do undecilato de testosterona em parâmetros sorológicos de ratos obesos e não obesos

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ABSTRACT

This study aimed to assess the impact of testosterone undecylate on renal and hepatic functions by examining serum markers in obese and non-obese rats. The methodology involved treating these animals with testosterone undecylate or a control solution over a period of 28 days and analyzing serum samples for markers such as urea, creatinine, and liver enzymes. The results demonstrated no significant differences in creatinine, AST, ALT, GGT, ALP, and CK levels between the treated and control groups, suggesting that testosterone undecylate does not adversely affect renal or hepatic functions in the conditions tested. The study concludes that testosterone undecylate, within the administered doses, appears safe, presenting a favorable safety profile and good tolerability, making it a viable option for hormonal therapy in clinical and scientific settings.

Keywords: Androgen; Anabolic; Kidney; Liver; Muscle

RESUMO

Este estudo teve como objetivo avaliar o impacto do undecilato de testosterona nas funções renal e hepática, examinando marcadores séricos em ratos obesos e não obesos. A metodologia envolveu o tratamento desses animais com undecilato de testosterona ou uma solução controle durante um período de 28 dias e a análise de amostras de soro para marcadores como ureia, creatinina e enzimas hepáticas. Os resultados demonstraram que não houve diferenças significativas nos níveis de creatinina, AST, ALT, GGT, ALP e CK entre os grupos tratados e controle, sugerindo que o undecilato de testosterona não afeta adversamente as funções renal ou hepática nas condições testadas. O estudo conclui que o undecilato de testosterona, nas doses administradas, parece seguro, apresentando um perfil de segurança favorável e boa tolerabilidade, tornando-o uma opção viável para terapia hormonal em contextos clínicos e científicos.

Palavras-chave: Andrógeno; Anabolizante; Rim; Fígado; Músculo.
INTRODUCTION

Testosterone undecylate is a synthetic androgenic anabolic steroid, derived from testosterone. Testosterone is synthesized in males primarily in the testicles and in small amounts by the adrenal gland's cortical region. It is responsible for the expression of male sexual characteristics and the maintenance of functions dependent on the male hormone. Depending on the target organ, testosterone activity can be androgenic, for example, acting in sperm production, in the prostate, seminal vesicles, and epididymis. It can also exert an anabolic function in muscles, bones, kidneys, liver, and in the production of red blood cells (Evans, 2004; Sahlin et al., 2020; Soares et al., 2022).

It can be noted that testosterone plays an important role in various tissues of the body (Soares et al., 2022). The liver is the primary organ involved in the metabolism of the drug, and studies have linked continuous use to some side effects observed in the hepatic laboratory profile. For example, there is an increase in the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), a decline in the concentration of plasma proteins synthesized in the liver such as albumin, and an increase in total cholesterol and LDL (Low Density Lipoprotein), with a decrease in HDL (High Density Lipoprotein) (Hartgens et al., 2004).

Disturbances in the urinary tract can also be observed, as testosterone is responsible for excreting most of the products obtained in metabolism and controlling the majority of the constituents of body fluids (Guyton and Hall, 2011). Androgen abuse may trigger an increase in creatinine, urea, and uric acid, sodium retention, an increase in nitrogen balance, and an elevation in the enzyme creatine kinase (CK) (Zhao, Moon, and Park, 2013; Sahlin et al., 2015; Campbell et al., 2023).

From this perspective, the present study aimed to evaluate the effects of testosterone undecylate on the kidneys and liver by assessing serum markers of renal function (urea and creatinine) and hepatic function (alanine aminotransferase - ALT, aspartate aminotransferase - AST, gamma-glutamyltransferase - GGT, total proteins, albumin, globulin, alkaline phosphatase - ALP, and creatine kinase - CK) in obese and non-obese rats.
MATERIALS AND METHODS

The experiment was conducted in accordance with the standards of the Brazilian College for Animal Experimentation (COBEA), following approval by the Animal Experimentation Ethics Committee (CEEA) of the University of Uberaba. Frozen serum samples from 96 Rattus norvegicus were used from the project titled 'Influence of Testosterone Undecylate on Bone Repair in Normal and Obese Rats,' a study previously conducted and approved by the UNIUBE CEEA with protocol number 001/2020.

The animals were equally divided into four experimental groups: 1) non-obese animals (control group), 2) non-obese animals treated with testosterone undecylate 8 mg/kg, every 15 days (testosterone undecylate group), 3) obese animals (obese control group), and 4) obese animals treated with testosterone undecylate 8 mg/kg, every 15 days (obese testosterone undecylate group).

All assessments were conducted using serum collected at 3, 7, 19, and 28 days of treatment, with samples taken from six animals from each group at each evaluation time.

For the induction of obesity in the animals, a high-calorie diet was used, consisting of 40% condensed milk, 11% refined sugar, 40% feed (Purina-Labina, São Paulo, SP, Brazil), and 9% water (LACERDA et al., 2015). The control diet consisted of a conventional diet, with a micronutrient composition of 54.7% carbohydrates, 5.7% fat, 13.2% protein, 22.2% water, and 3.4% fiber. The control diet contains 1.4% calcium and 1.0% phosphorus, while the high-calorie diet contains 1.0% calcium and 0.6% phosphorus. Regarding the composition of macronutrients and minerals, both diets met the detailed requirements set by the National Research Council.

Regarding the analyses conducted, it is noted that the design used was completely randomized. Parametric data were subjected to analysis of variance (ANOVA) and to the Kolmogorov-Smirnov and Shapiro-Wilk normality tests. Data that followed a Gaussian distribution were subjected to the unpaired Student's t-test and the means compared using the Student-Newman-Keuls test, with a confidence level of 95% adopted. Data that did not follow a Gaussian distribution were subjected to the Kruskal-Wallis test; medians were compared using the Dunn test, adopting a confidence level of 95%. Morphological changes in the hemogram cells were presented descriptively.
RESULTS

Effects of testosterone undecylate on the kidneys

Urea

Serum urea levels were significantly higher (p<0.05) in non-obese rats that received testosterone undecylate at day 28. Urea levels were not significantly altered by testosterone undecylate in the obese animal group. Comparing non-obese and obese groups that received testosterone undecylate, higher serum urea levels (p<0.05) were observed in non-obese animals on day 28. No significant differences were observed in the urea levels of obese and non-obese control rats (Figure 1, Table 1).

![Serum urea](image)

Figure 1 - Means and standard deviations of plasma urea concentration in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days. *Indicates significant difference by the Student-Newman-Keuls test (p<0.05).

Table 1 - Means and standard deviations of plasma urea concentration in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>D3</th>
<th>D7</th>
<th>D19</th>
<th>D28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>47,50 (14,12)</td>
<td>36,20 (8,11)</td>
<td>41,71 (12,41)</td>
<td>38,57 (13,00)</td>
</tr>
<tr>
<td>Undecylate</td>
<td>49,25 (3,86)</td>
<td>37,86 (7,27)</td>
<td>46,43 (6,60)</td>
<td>60,14 (4,98)</td>
</tr>
<tr>
<td>Control obese</td>
<td>52,83 (8,23)</td>
<td>41,50 (2,23)</td>
<td>44,80 (21,50)</td>
<td>49,00 (12,25)</td>
</tr>
<tr>
<td>Undecylate obese</td>
<td>30,75 (9,18)</td>
<td>42,43 (14,76)</td>
<td>39,00 (10,84)</td>
<td>35,29 (16,47)</td>
</tr>
</tbody>
</table>
Creatinine

No significant differences were observed in serum creatinine levels among animals in both the obese and non-obese rat groups. The use of testosterone undecylate also showed no influence on serum creatinine levels in both groups (non-obese and obese) (Figure 2, Table 2).

![Serum creatinine graph](image)

Figure 2 - Means and standard deviations of plasma creatinine concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days. **Indicates significant difference by the Student-Newman-Keuls test (p<0.05).

Table 2 - Means and standard deviations of plasma creatinine concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>D3</th>
<th>D7</th>
<th>D19</th>
<th>D28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,10 (0.63)</td>
<td>0,86 (0.36)</td>
<td>1,14 (0.42)</td>
<td>0,99 (0.36)</td>
</tr>
<tr>
<td>Undecylate</td>
<td>0,90 (0.31)</td>
<td>1,16 (0.54)</td>
<td>0,91 (0.29)</td>
<td>1,26 (0.53)</td>
</tr>
<tr>
<td>Control obese</td>
<td>0,77 (0.10)</td>
<td>1,07 (0.83)</td>
<td>1,31 (1,35)</td>
<td>1,72 (0,77)</td>
</tr>
<tr>
<td>Undecylate obese</td>
<td>1,00 (0.33)</td>
<td>1,49 (0.82)</td>
<td>1,25 (0.68)</td>
<td>1,12 (0.30)</td>
</tr>
</tbody>
</table>

Effects of testosterone undecylate on the liver

Aspartate Aminotransferase (AST)

No significant differences were observed in the serum levels of aspartate aminotransferase (AST) among animals in the groups of obese and non-obese rats.
Furthermore, no influence of testosterone undecylate on serum AST levels was observed in both groups (non-obese and obese) (Figure 3, Table 3).

![Serum aspartate aminotransferase (AST)](image)

**Figure 3** - Means and standard deviations of plasma aspartate aminotransferase concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days. *Indicates significant difference by the Student-Newman-Keuls test (p<0.05).

**Table 3** - Means and standard deviations of plasma aspartate aminotransferase concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>D3</th>
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<th>D28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34,33 (9,25)</td>
<td>37,20 (7,40)</td>
<td>49,57 (25,84)</td>
<td>37,71 (10,63)</td>
</tr>
<tr>
<td>Undecylate</td>
<td>52,00 (29,28)</td>
<td>30,86 (12,75)</td>
<td>39,00 (9,47)</td>
<td>43,57 (14,39)</td>
</tr>
<tr>
<td>Control obese</td>
<td>55,83 (7,83)</td>
<td>27,00 (7,17)</td>
<td>29,20 (6,50)</td>
<td>39,00 (26,56)</td>
</tr>
<tr>
<td>Undecylate obese</td>
<td>32,25 (9,98)</td>
<td>28,86 (14,65)</td>
<td>37,80 (4,21)</td>
<td>39,71 (6,53)</td>
</tr>
</tbody>
</table>

**Alanine Aminotransferase (ALT)**

No significant differences were observed in serum alanine aminotransferase (ALT) levels among animals in both the obese and non-obese rat groups. Additionally, no influence of testosterone undecylate on serum ALT levels was observed in either group (non-obese and obese) (Figure 4, Table 4).
Figure 4 - Means and standard deviations of plasma alanine aminotransferase concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days. *Indicates significant difference by the SNK test (p<0.05).

Table 4 - Means and standard deviations of plasma alanine aminotransferase concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>D3</th>
<th>D7</th>
<th>D19</th>
<th>D28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12,83 (3,60)</td>
<td>15,00 (5,15)</td>
<td>19,71 (6,05)</td>
<td>20,71 (7,65)</td>
</tr>
<tr>
<td>Undecylate</td>
<td>16,50 (2,65)</td>
<td>19,14 (7,31)</td>
<td>19,14 (3,44)</td>
<td>18,86 (12,46)</td>
</tr>
<tr>
<td>Control obese</td>
<td>14,67 (4,76)</td>
<td>10,50 (2,65)</td>
<td>11,20 (1,64)</td>
<td>11,17 (4,35)</td>
</tr>
<tr>
<td>Undecylate obese</td>
<td>11,00 (1,83)</td>
<td>18,29 (13,21)</td>
<td>19,20 (11,84)</td>
<td>15,00 (7,98)</td>
</tr>
</tbody>
</table>

**Alkaline Phosphatase (ALP)**

No significant differences were observed in the serum levels of alkaline phosphatase (ALP) among animals in both the obese and non-obese rat groups. Furthermore, no influence of testosterone undecylate on serum ALP levels was observed in either group (non-obese and obese) (Figure 5, Table 5).
Figure 5 - Means and standard deviations of plasma alkaline phosphatase concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days. *Indicates significant difference by the SNK test (p<0.05).

Table 5 - Means and standard deviations of plasma alkaline phosphatase concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>D3</th>
<th>D7</th>
<th>D19</th>
<th>D28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>52,50 (23,78)</td>
<td>67,60 (24,93)</td>
<td>69,57 (35,22)</td>
<td>55,14 (25,30)</td>
</tr>
<tr>
<td>Undecylate</td>
<td>62,75 (16,01)</td>
<td>72,00 (21,73)</td>
<td>51,00 (21,57)</td>
<td>66,57 (11,25)</td>
</tr>
<tr>
<td>Control obese</td>
<td>77,33 (17,83)</td>
<td>68,00 (17,26)</td>
<td>44,40 (17,95)</td>
<td>54,00 (24,21)</td>
</tr>
<tr>
<td>Undecylate obese</td>
<td>33,00 (9,83)</td>
<td>36,43 (8,06)</td>
<td>48,80 (12,74)</td>
<td>50,71 (11,10)</td>
</tr>
</tbody>
</table>

Gamma-Glutamyl Transferase (GGT)

No significant differences were observed in the serum levels of gamma-glutamyl transferase (GGT) among animals in both the obese and non-obese rat groups. Additionally, no influence of testosterone undecylate on serum GGT levels was observed in either group (non-obese and obese) (Figure 6, Table 6).
Figure 6 - Means and standard deviations of plasma gamma-glutamyl transferase concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days. *Indicates significant difference by the SNK test (p<0.05).

Table 6 - Means and standard deviations of plasma gamma-glutamyl transferase concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>D3</th>
<th>D7</th>
<th>D19</th>
<th>D28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,67 (0,82)</td>
<td>2,00 (1,23)</td>
<td>4,29 (2,14)</td>
<td>1,86 (1,07)</td>
</tr>
<tr>
<td>Undecylate</td>
<td>2,75 (0,96)</td>
<td>3,86 (2,34)</td>
<td>2,29 (1,11)</td>
<td>4,86 (3,76)</td>
</tr>
<tr>
<td>Control obese</td>
<td>3,50 (3,21)</td>
<td>2,75 (0,96)</td>
<td>5,80 (6,34)</td>
<td>2,67 (1,51)</td>
</tr>
<tr>
<td>Undecylate obese</td>
<td>9,25 (10,63)</td>
<td>5,57 (6,93)</td>
<td>4,00 (1,41)</td>
<td>5,29 (7,09)</td>
</tr>
</tbody>
</table>

**Total Proteins**

No significant differences were observed in serum total protein (TP) levels among animals in both the obese and non-obese rat groups. TP levels were not significantly altered by testosterone undecylate in the group of obese animals. When comparing the non-obese and obese groups that received testosterone undecylate, higher levels (p<0.05) of serum TP were found in the obese animals on day 28. Significant differences were observed in TP levels between the control obese and non-obese rats on day 28 (Figure 7, Table 7).
Figure 7 - Means and standard deviations of plasma total protein concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days. *Indicates significant difference by the SNK test (p<0.05).

Table 7 - Means and standard deviations of plasma total protein concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>D3</th>
<th>D7</th>
<th>D19</th>
<th>D28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.70 (0.47)</td>
<td>7.76 (0.73)</td>
<td>8.90 (0.66)</td>
<td>7.23 (0.34)</td>
</tr>
<tr>
<td>Undecylate</td>
<td>7.45 (0.37)</td>
<td>8.29 (1.18)</td>
<td>7.24 (0.83)</td>
<td>7.63 (1.12)</td>
</tr>
<tr>
<td>Control obese</td>
<td>6.70 (0.65)</td>
<td>9.28 (0.70)</td>
<td>8.62 (1.68)</td>
<td>9.68 (1.14)</td>
</tr>
<tr>
<td>Undecylate obese</td>
<td>8.65 (0.51)</td>
<td>8.13 (0.94)</td>
<td>8.96 (1.17)</td>
<td>9.63 (1.19)</td>
</tr>
</tbody>
</table>

Albumin

No significant differences were observed in serum albumin levels among animals in both the obese and non-obese rat groups. Albumin levels were not significantly altered by testosterone undecylate in the group of obese animals. A significant difference in albumin levels was observed between the control obese and non-obese rats on day 28 (Figure 8, Table 8).
Figure 8 - Means and standard deviations of plasma albumin concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days. *Indicates significant difference by the SNK test (p<0.05).

Table 8 - Means and standard deviations of plasma albumin concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>D3</th>
<th>D7</th>
<th>D19</th>
<th>D28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.38 (0.32)</td>
<td>3.49 (0.18)</td>
<td>3.97 (0.20)</td>
<td>3.60 (0.37)</td>
</tr>
<tr>
<td>Undecylate</td>
<td>3.28 (0.09)</td>
<td>3.76 (0.69)</td>
<td>3.51 (0.28)</td>
<td>3.74 (0.39)</td>
</tr>
<tr>
<td>Control obese</td>
<td>3.29 (0.16)</td>
<td>4.18 (0.43)</td>
<td>3.80 (0.71)</td>
<td>4.35 (0.43)</td>
</tr>
<tr>
<td>Undecylate obese</td>
<td>3.73 (0.40)</td>
<td>3.76 (0.40)</td>
<td>3.90 (0.40)</td>
<td>4.11 (0.33)</td>
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</tbody>
</table>

**Globulin**

No significant differences were observed in serum globulin levels among animals in the non-obese rat groups. Globulin levels were significantly altered by testosterone undecylate in the group of obese animals on day 3. When comparing the non-obese and obese groups that received testosterone undecylate, higher levels (p<0.05) of serum globulin were found in the obese animals on day 28. A significant difference in globulin levels was observed between the control obese and non-obese rats on day 28 (Figure 9, Table 9).
Figure 9 - Means and standard deviations of plasma globulin concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days. *Indicates significant difference by the SNK test (p<0.05).

Table 9 - Means and standard deviations of plasma globulin concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>D3</th>
<th>D7</th>
<th>D19</th>
<th>D28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.32 (0.50)</td>
<td>4.28 (0.61)</td>
<td>3.03 (0.57)</td>
<td>3.63 (0.32)</td>
</tr>
<tr>
<td>Undecylate</td>
<td>3.32 (0.49)</td>
<td>4.53 (0.58)</td>
<td>3.73 (0.79)</td>
<td>3.89 (0.77)</td>
</tr>
<tr>
<td>Control obese</td>
<td>4.73 (0.46)</td>
<td>5.10 (0.51)</td>
<td>4.82 (1.21)</td>
<td>5.33 (0.84)</td>
</tr>
<tr>
<td>Undecylate obese</td>
<td>4.93 (0.31)</td>
<td>4.37 (0.76)</td>
<td>5.06 (0.82)</td>
<td>5.51 (0.98)</td>
</tr>
</tbody>
</table>

**Effects of testosterone undecylate on muscle**

**Creatine Kinase (CK)**

No significant differences were observed in serum creatine kinase (CK) levels among animals in both the obese and non-obese rat groups. Furthermore, no influence of testosterone undecylate on serum CK levels was observed in either group (non-obese and obese) (Figure 10, Table 10).
Figure 10 - Means and standard deviations of plasma creatine kinase concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days. *Indicates significant difference by the SNK test (p<0.05).

Table 10 - Means and standard deviations of plasma creatine kinase concentrations in non-obese and obese rats treated or not with testosterone undecylate (8 mg/kg, every 15 days) evaluated at 3, 7, 19, and 28 days.

<table>
<thead>
<tr>
<th>Groups</th>
<th>D3</th>
<th>D7</th>
<th>D19</th>
<th>D28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.67 (25.32)</td>
<td>153.60 (47.75)</td>
<td>195.0 (148.00)</td>
<td>53.57 (33.29)</td>
</tr>
<tr>
<td>Undecylate</td>
<td>90.25 (92.25)</td>
<td>88.94 (54.95)</td>
<td>64.43 (35.81)</td>
<td>100.70 (55.69)</td>
</tr>
<tr>
<td>Control obese</td>
<td>121.20 (38.46)</td>
<td>121.00 (31.44)</td>
<td>92.00 (105.00)</td>
<td>181.0 (108.20)</td>
</tr>
<tr>
<td>Undecylate obese</td>
<td>125.30 (63.78)</td>
<td>50.86 (33.41)</td>
<td>121.20 (42.65)</td>
<td>148.00 (68.78)</td>
</tr>
</tbody>
</table>

DISCUSSION

The analysis of the results from this study suggests that testosterone undecylate does not impose significant changes in renal, hepatic, and muscular functions. The data collected show that there are no significant differences in the serum levels of creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and creatine kinase (CK) between animals treated with testosterone undecylate and those untreated, regardless of their obesity status. These findings are significant as they indicate that the administration of testosterone undecylate at the studied dosages can be considered safe in terms of renal, hepatic, and muscular function.
Testosterone is essential for the proper development of male reproductive tissues and the maintenance of male sexual characteristics. Its reduction is linked to various negative outcomes, including significant morbidities and reduced quality of life (Zhao, Moon, and Park, 2013; Francomano, Lenzi, and Aversa, 2014). In this context, our study suggests that testosterone undecylate is safe regarding the preservation of specific organ functions, not causing significant changes in renal, hepatic, and muscular functions. However, the comprehensive role of androgens in health implies that the assessment of the safety and efficacy of hormonal treatments, such as testosterone undecylate, should be continuous, especially when administered over the long term or in different dosages. Therefore, it is essential to consider the broader endocrine and metabolic effects of these therapies.

During this study, an increase in urea levels was noted in normal (non-obese) animals after 28 days of treatment with testosterone undecylate. However, this increase does not seem to indicate kidney damage, as creatinine levels, a more reliable marker of renal function, did not show a significant elevation in this group. This finding suggests that other factors may be contributing to the observed increase in urea. The serum increase in urea can be attributed to various causes beyond kidney injury. Among these, dehydration is a possibility, as it can concentrate urea in the blood due to reduced circulating volume. Another plausible explanation is high protein intake, which increases the production of urea as a byproduct of protein metabolism. Additionally, urinary obstructions that prevent proper urea excretion can also result in elevated levels. Increased catabolism, often observed in states of stress or illness, can similarly cause an elevation in urea production. Lastly, the use of certain medications, such as corticosteroids and some antibiotics, known to impact metabolism and renal excretion, could also be responsible for alterations in urea levels (Salazar, 2014).

The stability of hepatic markers (AST, ALT, ALP, and GGT) observed in our study indicates that therapy with testosterone undecylate does not exert adverse effects on the liver, in both normal and obese animals. According to Adachi and Nakada (1999), testosterone is a significant regulator of various metabolic functions in organs such as the liver, adipose tissue, muscles, coronary arteries, and the heart. However, it is crucial to consider that the effects of testosterone undecylate or undecanoate may vary depending on the dosage, duration of treatment, and individual characteristics of the subjects.

To ensure hepatic safety, it is recommended to regularly perform blood tests to monitor liver function during the use of these androgens, in order to identify any potential
adverse effects on the liver (Gibbons et al., 2024). Additional research, such as the study by Harle, Basaria, and Dobs, (2005) on the use of testosterone undecanoate, supports this approach, revealing that no significant changes in liver function were detected, and biochemical tests remained stable throughout the treatment. These findings are reinforced by further studies that observed no notable impacts on liver function as a result of androgen treatment (Harle, Basaria, and Dobs, 2005; Moon et al., 2010; Campbell et al., 2023).

Within the context of this study, an increase in serum total protein levels at 28 days was noted in both obese animals treated with testosterone undecylate and those untreated. This change was specifically linked to an increase in albumin and globulin in normal (non-obese) animals, while only an increase in globulin was observed in obese animals. Intriguingly, obese animals exhibited higher levels of albumin compared to non-obese animals, suggesting that liver function was not adversely affected by induced obesity.

The relationship between obesity and elevated albumin levels is corroborated by other studies, such as that of Mehmetoglu et al. (2012), which propose that obesity may be associated with an increase in albumin due to ischemic mechanisms. This phenomenon indicates a possible increase in oxidative stress and inflammation, factors that could predispose obese individuals to more frequent ischemic events. The implication of these findings is that while obesity may induce changes in the protein profile, the physiological consequences of these changes are complex and may reflect both compensatory adaptations and metabolic vulnerabilities.

Although our study did not demonstrate an increase in albumin levels in groups treated with testosterone undecylate, previous investigations, such as that conducted by Sahlin et al. (2015), reveal that treatment with various androgens, including boldenone undecylate, can provoke an increase in albumin concentration and total proteins. This increase is attributed to the interaction of androgens with androgen receptors, stimulating RNA transcription and subsequent protein synthesis. This dynamic suggests an adaptive response of the body to treatment with anabolic-androgenic steroids, which may result in an increase in protein synthesis not only in muscles but also in the circulatory system. Such findings are corroborated by other studies, including those conducted by Adachi and Nakada (1999), Behre et al. (1999) and Edelstein and Basaria (2010), which confirm the ability of anabolic-androgenic steroids to enhance protein synthesis. These investigations highlight the complexity of the metabolic effects of androgens and emphasize the need
for meticulous evaluation of their impacts, particularly in relation to liver function and hormonal regulation.

The absence of variations in albumin levels observed in our study does not negate the results found in the literature, but highlights the complexity and variability of individual responses to androgen treatment. The interpretation of these results must consider the metabolic changes induced by androgen administration, as well as the influences of preexisting metabolic conditions, such as obesity, which can in themselves modify the protein profile and affect liver function.

The finding of elevated globulin levels in obese animals at 28 days in the current study, regardless of whether they received testosterone undecylate, suggests a complex interaction between obesity and the immune response. This finding may reflect the more pronounced inflammatory state in obese animals, which is known to induce changes in immunoglobulins. This hypothesis is supported by previous studies linking obesity to a pro-inflammatory state and alterations in immune function. Bassols et al. (2014) identified elevated levels of IgG and IgA in overweight children, establishing a correlation of these increases with a less favorable metabolic profile and indicating an interaction between adaptive immunity and insulin resistance in obese children. Alsufyani (2016) reinforces this association by revealing that obesity is linked to increased levels of IgA, suggesting a potential impact on immune function, while levels of IgG and IgM appear to be unaffected by weight gain.

The current study identified a significant alteration in globulin levels, noting an increase in these proteins in obese animals treated with testosterone undecylate on the third day of evaluation. This finding contrasts with the results of previous studies, such as the one conducted by Yu et al. (2003), which indicated a reduction in immunological and hormonal parameters in mice after prolonged injections of testosterone undecanoate. Specifically, Yu et al. (2003) observed a decrease in the levels of immunoglobulins IgA, IgG, and IgM, as well as a suppression in their production by peripheral blood mononuclear cells, possibly mediated by a reduction in IL-6 production by monocytes. These previous studies also demonstrated in vitro that testosterone can inhibit the production of IgM and IgG by human peripheral blood mononuclear cells (PBMCs). The suggested mechanism involves the inhibition of the cytokine IL-6, which is crucial for immune activation. Additionally, Yu et al.'s data indicate variations in IgG levels in different parts of the reproductive system following the administration of testosterone.
undecanoate, with suggestions that changes in IgA levels may be associated with the expression of the secretory component in epithelial cells.

The discrepancy between these results and ours may be attributed to differences in experimental conditions, such as the dosing protocol and the timing of evaluating immune responses. Such variables are fundamental in understanding the complexity of the immune response under the influence of androgens and underline the need for a more in-depth analysis of the effects of testosterone and its derivatives on the immune system.

In the present investigation, no significant changes were observed in the muscle marker creatine kinase (CK), indicating that treatment with testosterone undecylate did not have substantial impacts on muscle tissue under the established experimental conditions. However, the literature provides evidence that testosterone undecylate can enhance muscle mass, strength, and athletic performance in humans, provided it is administered in recommended doses and under medical supervision (Kicman, 2008; Lippi, Franchini & Banfi, 2011). The apparent discrepancy between these results may be attributed to methodological differences, including the populations studied and the dosing regimens used.

The limitations of the current study include the use of male rats, which may restrict the generalization of the results to humans due to physiological and metabolic differences between species. Therefore, there is a highlighted need for additional studies in humans to validate the observations made in animal models.

Furthermore, the observation period limited to four weeks may not capture the long-term effects of testosterone undecylate. Studies with longer durations are essential for a thorough evaluation of the prolonged effects and potential risks associated with continuous use of this androgen. A more detailed analysis of renal and hepatic functions could also contribute to a more comprehensive understanding of the systemic effects of treatment with testosterone undecylate. Therefore, the conclusions drawn should be considered within the context of the specific methodological limitations and the parameters used in the experimental models, underscoring the importance of future studies that may address these issues.

As this study progresses to the next phase, histopathological analysis and immunohistochemical staining of organs will become crucial to deepen our understanding of the interactions of testosterone undecylate with tissues. Exploring histological changes in these tissues may reveal valuable details about the mechanisms of action of testosterone undecylate. Additionally, immunological histopathology, particularly in tissues
associated with the immune system such as the spleen and lymph nodes, will be essential. These investigations will clarify the effects of testosterone undecylate/undecanoate on the immune system at the cellular and tissue levels, providing insights into the immune responses modulated by this hormonal therapy. These future perspectives are fundamental to fully understand both the benefits and potential risks associated with prolonged use of androgens.

CONCLUSION

Under the conditions of this study, it is concluded that testosterone undecylate does not significantly alter renal function nor cause hepatic injury, as no increases were observed in the levels of creatinine, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, and creatine kinase. The results indicate that testosterone undecylate is safe at the employed dose, exhibiting a favorable safety profile and good tolerability. Due to these attributes, testosterone undecylate emerges as a valuable option in hormonal therapy, offering a reliable and effective means of administering testosterone for various clinical and scientific purposes. Furthermore, future investigations, both in vitro and in vivo, may broaden the understanding of the effects of this androgen in diverse areas such as Immunology, Histopathology, Reproductive Biology, Pharmacology, and Clinical Endocrinology, significantly contributing to the advancement of these fields.

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