USE OF CORTICOSTEROIDS ASSOCIATED WITH EXERCISES AND INTAKE OF ω3: EFFECTS IN BONE, MUSCLE AND IN PLASMA LIPIDS

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ABSTRACT
Glucocorticoids (GC) are one of the most effective anti-inflammatory drugs for the treatment of several chronic inflammatory processes and immune diseases. Despite the many positive effects, they are also associated with several side effects such as osteoporosis, and muscle mass loss. On the other hand, the use of ω3 and the practice of exercises may produce health benefits on these parameters. Based on these facts, the aim of this work was to evaluate the effects of the use of GC, intake of omega 3 and practice of physical activity. Sixty male Wistar rats were selected for this study according to the following groups: G1: control group; G2: GC group; G3: GC and practice of exercises; G4: GC, practice of exercises and intake of ω3; G5: GC and use of ω3 and G6: ω3 group. The results of our study showed that the use of GC increased loss in the body weight and in muscular mass but did not interfere in the cholesterol total, triglycerides and HDL-c levels. The association of GC with exercises produce similar effects to those found in animals treated only with GC. When associating GC, exercising and Omega 3, we observe improvement in HDL-c levels but no differences were found in the other parameters. The use of GC and Omega 3 resulted in effects similar to those found in the group that received only GC.

KEYWORDS: Glucocorticoids, omega 3, exercise, bone, muscle mass.

INTRODUCTION
Glucocorticoids (GC), glucocorticosteroids, corticosteroids or steroids are one of the most effective and used anti-inflammatory drugs for the treatment of several chronic inflammatory processes and immune diseases. These molecules have the ability of diffusing the cell membrane and reaching receptors in the cytoplasm. After binding, there is activation of several anti-inflammatory genes that encode proinflammatory cytokines or chemokines, and adhesion molecules inflammatory enzymes and receptors. Furthermore, GC decrease inflammatory protein secretion as Tumor Necrosis Factor-α (TNF-α) and proinflammatory Interleukins (IL). [1-3]

Despite the many positive effects that these drugs can promote on the body, they are also associated to numerous side effects upon long-term use and high doses such as osteoporosis, fast twitch muscle fiber atrophy and development of myopathy, hidden infections, hyperglycemia, glycosuria, sodium retention with edema or hypertension, hypokalemia, peptic ulcer, and other conditions. [4-6]

Other compounds may also produce anti-inflammatory effects as the polyunsaturated fatty acids ω3 (eicosapentaenoic acid and docosapentaenoic acid). They can help protecting against numerous inflammatory or oxidative diseases such as cardiovascular disease, arthritis, allergies and other inflammatory conditions. One possible mechanism of action for ω3 is the inhibition of pro-inflammatory eicosanoid production. [9-11]

The practice of moderate exercises also may reduce pro-inflammation cytokine production. Besides, exercises may reduce risk factors for obesity, diabetes, hypertension, and may improve pulmonary pathologies and other conditions leading to the reduction of oxidative and inflammatory processes. [12-13]

As the use of GC is very common and prescribed worldwide for several clinical conditions but many studies show that they induce several metabolic disorders. On the other hand, the use of ω3 and the practice of exercises may produce health benefits. Based on these facts, the aim of this work was to evaluate the
effects of the use of GC, intake of omega 3 and practice of physical activity in Wistar rats.

METHODS

Ethical principles

This research obtained the approval of the Animal Research Ethics Committee of the University of Marilia (UNIMAR) with registration number 71/2014. The animals were cared after the Canadian “Guide for the Care and Use of Experimental Animals” that follows principles for the care of laboratory animals.

Animal model

Sixty male Wistar rats weighing approximately 210-230g were selected. They were kept in the vivarium at UNIMAR (University of Marília – Marília – São Paulo - Brazil) under a dark/light cycle of 12 hours, room temperature of 22 ± 2°C, and relative air humidity of 60 ± 5%. After a period of seven days of acclimation to the laboratory conditions, animals were randomly divided into 6 experimental groups and the experimental protocol lasted 75 days according to the following phases.

Phase 1 (30 days)

The control group (G1) received only water throughout the experiment and the groups G2 to G5 received GC ad libitum for 30 days.

Phase 2 (45 days)

After phase 1, these groups were treated according to the outline below for 45 days:

G1: Control group that received only water;
G2: Group treated with GC ad libitum;
G3: Group received GC ad libitum and practiced physical activity;
G4: Group received GC ad libitum, practiced exercise and received ω3;
G5: Group received GC ad libitum and ω3;
G6: Group that received ω3 (this group received only ω3 and was inserted in the experimental protocol only in phase 2).

Animals treated with corticosteroids received a solution prepared with 5 mg of prednisone in 500 mL of water that was given ad libitum. Groups treated with omega 3 received 3mg/Kg/day by gavage route and the physical activity performed was swimming (three times a week for one hour). All the animals received rat fed ad libitum.

Collection of blood samples and determination of the biochemical profile, Atherogenic Index (AI).

After treating animals for 75 days, animals were anesthetized with Thiopental (sodium pentobarbital) until complete sedation, after which blood samples were collected to determine total cholesterol, triglycerides, and HDL-c. Atherogenic Index (AI) was calculated after Schulpis, Karikas[14] and also used by Munshi, Joshi, Rane[15]; AI = (Total cholesterol – HDL-c)/HDL-c.

Collection of bones and muscular tissue.

After complete sedation and death of the animals we collected the femur and the gastrocnemius muscle. Bone and muscle were weighted in a balance with precision scale.

Statistical analysis

The variables were presented as means ± standard deviation. The data were analyzed by One-way Analysis of Variance (ANOVA). p values of <0.05 were considered significant.

RESULTS

In Table 1 we may see the results for biochemical and anthropometric profile of the animals. All animals that received corticosteroids lost body weight and also presented decrease in the muscle weight (G2 to G5). No significant differences were found for the weight of the bone and in levels of triglycerides and total cholesterol. Animals from G4 (association of corticosteroids, exercises and ω3) increased the levels of HDL-c. All the animals presented the same body weight at the beginning of the treatment (data not shown).

Table 1: Biochemical and anthropometric parameters of G1 to G6 at the end of the the research.

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGC</td>
<td>77±39.31</td>
<td>106.2±105.2</td>
<td>106.8±62.14</td>
<td>86.2±38.01</td>
<td>117.0±58.8</td>
<td>64.3±39.2</td>
</tr>
<tr>
<td>TC</td>
<td>59.7±3.8</td>
<td>72.2±16.4</td>
<td>65.0±10.9</td>
<td>73.1±12.7</td>
<td>63.1±7.5</td>
<td>63.7±10.1</td>
</tr>
<tr>
<td>HDL-c</td>
<td>27.3±2.3</td>
<td>28.6±3.4</td>
<td>31.1±5.2</td>
<td>34.8±5.7*</td>
<td>30.0±2.7</td>
<td>28.9±2.8</td>
</tr>
<tr>
<td>Weight**</td>
<td>251.1±20.9</td>
<td>174.7±23.4*</td>
<td>207.2±23.5*</td>
<td>187.6±17.9*</td>
<td>199.2±25.6*</td>
<td>239.3±19.6</td>
</tr>
<tr>
<td>Muscle</td>
<td>2.20.47</td>
<td>1.5±0.31*</td>
<td>1.8±0.28*</td>
<td>1.6±0.26*</td>
<td>1.6±0.32*</td>
<td>1.9±0.21</td>
</tr>
<tr>
<td>Bone</td>
<td>0.88±0.13</td>
<td>0.81±0.11</td>
<td>0.86±0.06</td>
<td>0.80±0.07</td>
<td>0.78±0.16</td>
<td>0.79±0.10</td>
</tr>
</tbody>
</table>

(*) indicate a significant difference between the treatments at a level of 5%. TGC: triglycerides; CT: total cholesterol; HDL-c: High Density Lipoprotein; (**): body weight at the end of phase 2. G1: control group; G2: GC group; G3: GC + exercises; G4: GC + exercises + ω3; G5: GC + ω3 and G6: ω3 group.

Results for the Atherogenic Index (AI) are found in Table 2. The higher index is found in the group G2 that was treated only with corticosteroids. The lowest values are found in the groups of animals performing physical activity.
**Table 2: Atherogenic Index (AI) in groups G1-G6.**

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI</td>
<td>1.19</td>
<td>1.28</td>
<td>1.09</td>
<td>1.08</td>
<td>1.15</td>
<td>1.15</td>
</tr>
</tbody>
</table>

G1: control group; G2: GC group; G3: GC + exercises; G4: GC + exercises + ω3; G5: GC + ω3 and G6: ω3 group.

**DISCUSSION**

The practice of exercises or the combination of exercising and intaking of ω3 did not avoid the protein catabolism induced by corticosteroids in this experimental model (groups G3 to G5). We may suggest that the loss of the body weight that occurred in these groups is due to the loss of muscle mass observed in these animals.

Many authors have found reduction in muscle mass after the use of GC both in human and animal models. The chronic use of these drugs is related with the reduction in muscle mass, and loss of strength once they increase the production of myostatin that work as a negative regulator of skeletal muscle growth. The modifications in the muscle mass occur mainly in fast twitch muscle fibers with modifications in the turnover rate of muscle proteins. Subsequent alterations reach myosin and actin that are the main contractile proteins. There is a diminished production of these proteins and a reduction in myosin-heavy chain with disarray of myosin myofilaments and increased proteolytic activity. The reduction of myosin, nebulin and titin proteins content conducts to the reduction of muscle elasticity, increased tone and stiffness. The use of GC in large doses may reduce testosterone and insulin levels resulting in negative interference in synthesis of proteins contributing to the protein catabolism. The catabolic GC effects on skeletal muscle also depend on the functional activity of muscle.\(^{[16-23]}\)

Many situations may lead to the decrease in bone mass and density but the use of glucocorticoid (GC) is directly involved. The use of these drugs for a short period (3 months) do not adversely affect the bone mineral density. At physiological concentrations, cortisol induces the production of collagen by osteoblasts, nevertheless, at higher amounts it may lead to delayed maturation of these cells, interferes in the production of alkaline phosphatase, collagen, osteocalcin, and brings interference in the bone matrix mineralization. This process modifies the developing of actin filaments, leading to changes in the shape of the cells and apoptosis. These drugs also induces the production of an enzyme related to the degeneration of the bone matrix as well they stimulate the production of insulin-like growth factors that may modulate the response of osteoblasts to PTH, 1,25(OH)2D3 and cytokines interfering in the metabolism of vitamin D (and reduction of its receptors), decrease the intestinal calcium absorption and increase loss of this mineral in the urine. All these consequences culminates in bone remodeling cycle mainly in the first 6–12 months of treatment. The most affected bones are those with trabecular structure, frequently the vertebral bodies.\(^{[24-28]}\) Our results did not show modifications in the weight of the femur, probably because the doses of the GC in the period of the experiment was not enough to promote alterations in the bone mass.

Lin et al.\(^{[29]}\) studied the effects of methylprednisolone sodium succinate in rabbits and observed reduction in the bone mineral density of the femur and reduction in the body weight after 4 and 8 weeks. Other authors found similar results in different models.\(^{[30-33]}\)

In our study, we did not observe significant differences in the total cholesterol and triglycerides levels in all groups. HDL-c levels were significantly higher only in the group that received the association of GC, ω3 and practiced physical activity, indicating that only physical activity or intake of ω3 is not enough to increase this lipoprotein. Lin et al.\(^{[29]}\) also evaluated the levels of total cholesterol and triglycerides after using methylprednisolone and found no significant differences in these parameters (authors did not evaluate the HDL-c levels). Both exercises and the use of ω3, such as those found in fish oil, have been implicated with modulation of apolipoprotein B, decrease of plasma lipids and increase of HDL-c, improvement in blood pressure, systemic vascular resistance, myocardial efficiency, arterial wall dysfunction, and several other effects. They may also exhibit antioxidant properties leading to beneficial effects on endothelial function, macrophage and monocyte function, and inflammation, thus they are related to the prevention of development and progression of atherothrombotic issues.\(^{[34-38]}\)

It is very well established in the literature that physical practice also plays numerous beneficial roles in the body, as well as in homeostasis of plasmatic lipids, body weight and improvement of musculature.\(^{[39-40]}\) In our study, the exercise did not improve total cholesterol and triglycerides levels and increased HDL-c only when associated with the intake of ω3. It also did not prevent the weight loss associated with the use of GC. Many authors suggest that physical activity can prevent or reduce the damage caused by GC of damage to the bones and muscles of patients with diseases that require the use of these drugs. The practice of exercises may reduce the inflammation and may per se have additional effects on the pharmacological treatment of the inflammation.\(^{[41-45]}\)

The practice of exercises in our study showed better results for the Atherogenic Index. The higher the AC, the higher is the risk of oxidative stress and inflammation. G2 showed the higher value for this index, indicating
that GC increase risk of diseases associated to inflammation and oxidative conditions. On the other hand, the practice of exercises reduced this status. This index is also associated with the size of the pro-antitherogenic lipoprotein particles and is known to predict a cardiovascular risk.\textsuperscript{13-46}

**CONCLUSION**

The results of our study showed that the use of GC increased loss in the body weight and in muscular mass but did not interfere in the total cholesterol, triglycerides and HDL-c levels. The association of GC with exercising produce similar effects to those found in animals treated only with GC. When associating GC, exercising and Omega 3, we observe improvement in HDL-c levels but no differences were found in the other parameters. The use of GC and Omega 3 resulted in effects similar to those found in the group that received only GC.

**AUTHOR DISCLOSURE STATEMENTS**

No competing financial interests exist. There is no conflict of interest for any author.

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