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Soft Drink Consumption and Concurrent Training: Effects on Soleus Muscle of Animals in Growth Stage

Consumo de Refrigerante y Entrenamiento Físico: Efectos en el Músculo Sóleo de Animales en Fase de Crecimiento

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SUMMARY: The objective of this study was to investigate the effects of soft drink consumption and the realization of one concurrent training protocol on the Soleus muscle of Wistar rats. In total, 32 male Wistar rats were used, post-weaning, in the biological phase (30 days old). The animals were distributed into four groups: Control (C); Control and Soda (CSD); Concurrent Training (CT); Concurrent Training and Soda (CTSD). The concurrent training protocol was composed of aerobic and resistance exercises. The aerobic training protocol included three sessions per week on non-consecutive days, for 30 minutes of swimming exercises, with an intensity of 80 % of the anaerobic threshold (Lan). The resistance training protocol (strength) included four series of 10 jumps, with overload corresponding to 50 % of the body weight of each animal. Forty-eight hours after the last session, the animals were submitted to a surgical process for removal of Soleus muscle samples and histological slides. All experimental groups showed increases in transverse section area and body mass. The animals that received soft drinks (CSD and CTSD) demonstrated lower ingestion of water and feed. Lastly, it was observed that trained animals ingested smaller amounts of soft drinks compared to control animals (TCR vs CR) (p<0,05). It is possible to conclude that soft drink ingestion did not interfere negatively in muscle adaptation. However, animals that ingested soft drinks showed increased values for body mass and decreased water and feed ingestion.

KEY WORDS: Soft drinks; Physical Education and Training; Skeletal Muscle; Wistar rats; Histology.

INTRODUCTION

The consumption of soft drinks is commonly practiced in much of the world, a trend that has intensified with the habit of consuming processed foods due to their convenience in daily life. However, these foods contain high amounts of sucrose and are associated with low satiety and insufficient energy compensation in subsequent meals (Rivera *et al*., 2004; Nielsen & Popkin, 2005). Therefore, they are linked to cases of overweight and obesity, as well as diabetes and metabolic syndrome (Malik *et al*., 2006; Dhingra *et al*., 2007).

It is well established in the literature that physical training is one of the ways to combat obesity and overweight. The body's responses to training can produce various anatomical and physiological changes, including those that affect muscle tissue. This tissue has the ability to adapt to different training modalities, responding in various ways to each of them (Camargo Filho *et al*., 2006).

Muscle fibers have a high capacity for adaptation. Depending on the stimuli and conditions to which they are subjected, they can promote changes in metabolism and fiber size. Considering that the Soleus is one of the main muscles used in experimental models for studying physiological

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responses to exercise, and from a physiological perspective, physical exercise can be characterized as aerobic or anaerobic, depending on its intensity and duration (Artifon *et al*., 2013).

Thus, the use of these two forms of exercise is referred to as "Concurrent Training" (CT) (Machado *et al*., 2014). Concurrent training combines two types of training with different characteristics, such as endurance and strength exercises, resulting in combined adaptations from both types of training (Hickson, 1980).

Aerobic or endurance exercises are characterized by low to moderate intensity and long duration. Endurance training leads to cardiorespiratory adaptations, increased plasticity of striated skeletal muscle, and fat loss (Castoldi *et al*., 2013; Santos *et al*., 2020).

Anaerobic or strength training, on the other hand, is intense, short in duration, and promotes adaptations in strength, power, and hypertrophy of striated skeletal muscle. The distinguishing characteristic of these two types of training is directly related to the predominant metabolic pathway, which canbe creatine-phosphate or anaerobic glycolysis in strength (anaerobic) exercises, and aerobic glycolysis or oxidative pathways in endurance (aerobic) exercises (Santos *et al*., 2020).

However, there is a lack of studies investigating the effects of soft drink consumption combined with training protocols. Therefore, the specific aim of this study is to analyze the effects of soft drink consumption and physical training on the Soleus muscle of growing animals.

MATERIAL AND METHOD

Animals: In this study, 32 male Wistar rats, in the post-weaning biological phase (30 days old), were used. They were housed in groups of five animals per polyethylene box, with a controlled ambient temperature (22±2 °C) and a 12-hour light/dark cycle, with free access to water and food (laboratory rat chow). The research was conducted in accordance with ethical guidelines for animal experimentation, following approval from the Ethics Committee on Animal Use (CEUA-3359).

Experimental Groups: The animals were divided into four groups (n=32): control (C) [n=8], control with soft drink (CSD) [n=8], concurrent training (CT) [n=8], and concurrent training with soft drink (CTSD) [n=8].

Group CT and CTSD. The animals were initially subjected to an adaptation period to the water environment and equipment for 10-20 minutes/day, three days a week, for one week, with progressively increased overload and duration, following the method proposed by Manchado *et al*. (2006). This adaptation period reduces stress caused by the water environment and physiological changes resulting from physical training (Chimin *et al*., 2009).

The training model consisted of two distinct exercise sessions, requiring different energy sources during execution: one predominantly aerobic (swimming) and the other predominantly anaerobic (jumps).

The aerobic training protocol consisted of three non-consecutive weekly sessions, with 30 min of swimming exercise at an intensity of 80 % of the anaerobic threshold (AT), determined from the study by Manchado *et al*. (2006). A tank with 25 cm diameter PVC tubes and 100 cm height, with 70 cm water depth, was used for the aerobic part (swimming) (Castoldi *et al*., 2013; Ozaki *et al*., 2014).

For the anaerobic (strength) training protocol, four sets of 10 jumps were performed, with a load corresponding to 50 % of each animal's body weight. A PVC tube with a diameter of 25 cm and a height of 80 cm, with a depth of 38 cm, was used for this purpose. The overload was fixed using a specially designed vest attached to the thoracic region for this type of exercise (Castoldi *et al*., 2013).

The CTSD group differed due to the *ad libitum* consumption of soft drinks, introduced 30 days after birth and maintained for 120 days (90 days of hyperglycemia induction + 30 days of training), a period corresponding to the end of the experiment.

Description of Groups C and CSD.

Group C: The animals remained free in their cages, with unrestricted access to water and food.

Group CSD: The animals remained free in their cages, with unrestricted access to water and food; however, soft drinks were made available starting 30 days after birth and maintained for 120 days (90 days of hyperglycemia induction + 30 days of training), corresponding to the end of the experiment.

Prescription of aerobic training intensity. To prescribe the aerobic training, a value corresponding to a percentage of body weight was used (Manchado *et al*., 2006). Thus, the threshold intensity was set at 5 % of body weight, and 80 % of this value was used for aerobic training intensity.

Hyperglycemia induction. The animals were induced to hyperglycemia by ad libitum consumption of soft drinks (guaraná type). The soft drink consisted of carbonated water, fructose syrup, caramel coloring, phosphoric acid, natural flavorings, caffeine, low sodium content, and a caloric value of 0.41 calories per gram (0.41 cal/g).

Tissue sample collection. Forty-eight hours after the last exercise session, the animals underwent a surgical procedure. Samples were collected following the methodology described by Águila *et al*. (1997). The animals were anesthetized with a combination of two anesthetics, ketamine hydrochloride (70 mg/kg) and xylazine hydrochloride (15 mg/kg), injected intraperitoneally as proposed by Seraphim *et al*. (2001).

Histological Processing of Striated Skeletal Muscle. Soleus muscle samples were immersed in n-hexane and cooled in liquid nitrogen using the method for freezing non-fixed tissues, and subsequentlystored in an ultra-low temperature freezer (-80 ºC). Sections of 5 µm were produced in a cryostat microtome at -20 ºC, collected on slides, and then stained with hematoxylin-eosin (HE) for a general analysis of the structure (Dal Pai, 1995).

Muscle tissue analysis

Optical microscopy. The sections were subjected to staining and histochemical reactions and observed under normal light, with photomicrographs taken using a Nikon® H550S microscope. For image analysis, an Infinity 1 digital camera was used. Interactive measurements for determining the muscle fiber area were made using the software Auxio Vision Rel 4.8 - Carl Zeiss® and NIS-Elements D3.0 - SP7 - Nikon®. A total of 100 muscle fibers were observed in each slide, following the protocol established by Dal Pai (1995).
Statistical analysis. After data collection, the following tests were performed: Shapiro-Wilk test for normality; ANOVA with repeated measures and Bonferroni post-hoc test for the variable "body mass"; Kruskal-Wallis with Dunn post-hoc test for cross-sectional area, food, and water intake; and Mann-Whitney for soft drink consumption. All procedures assumed a 5 % error (p<0.05) and were performed using SPSS 22.0 software.

RESULTS

After analyzing body mass, it was observed that all groups of animals increased their body mass. Additionally, the CTSD group showed the highest value for this variable after the nine-week period (Fig. 1).

Regarding food consumption, it was found that the animals that received soft drinks (CSD and CTSD) showed lower food and water intake. Finally, it was observed that the trained animals consumed less soft drink compared to the control animals (CTSD vs. CSD) (p<0.05) (Fig. 2).

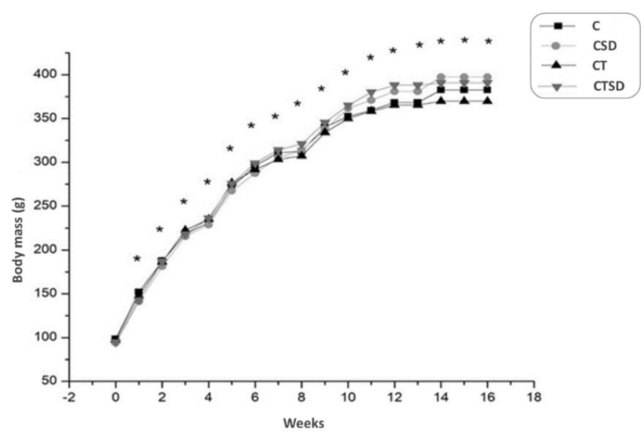

Fig. 1. Body mass evolution during the experiment. (*): Difference between the different weeks of the experiment. Analysis of Variance - ANOVA with repeated measures and Bonferroni post-hoc test. Significance level of 5 % (p<0.05).

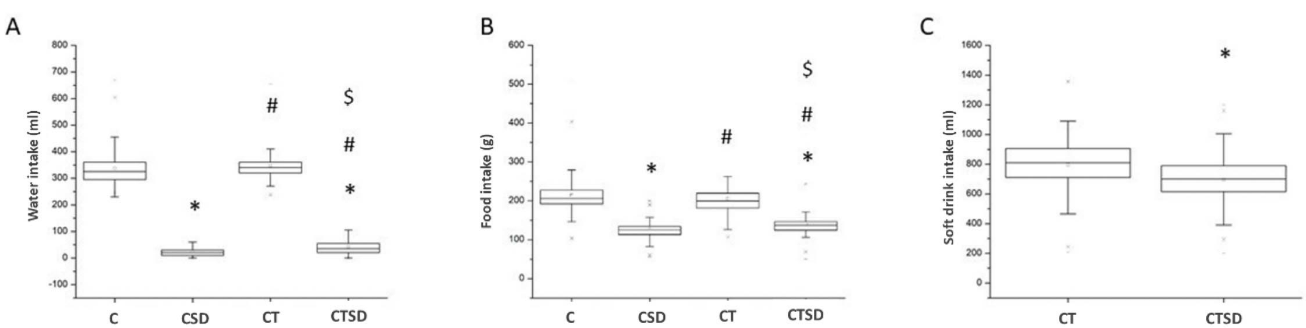

Fig. 2. Food and water consumption during the experiment. C: Control; CSD: Control and soda consumption; CT: Concurrent Training; CTSD: Concurrent Training and soda consumption (A, B): $: Statistically significant difference compared to the control group. (#): Statistically significant difference compared to the CSD group. Kruskal-Wallis test with Dunn's post-test. (C): *: Statistically significant difference between CTSD and CSD. Mann-Whitney test with 5 % significance (p<0.05).

Lastly, all experimental groups showed an increase in cross-sectional area ($p<0.05$). Furthermore, although there were no significant differences between them, the trained animals (CT and CTSD) showed slightly higher average values compared to the untrained group (CSD) (Figs. 3A,B).

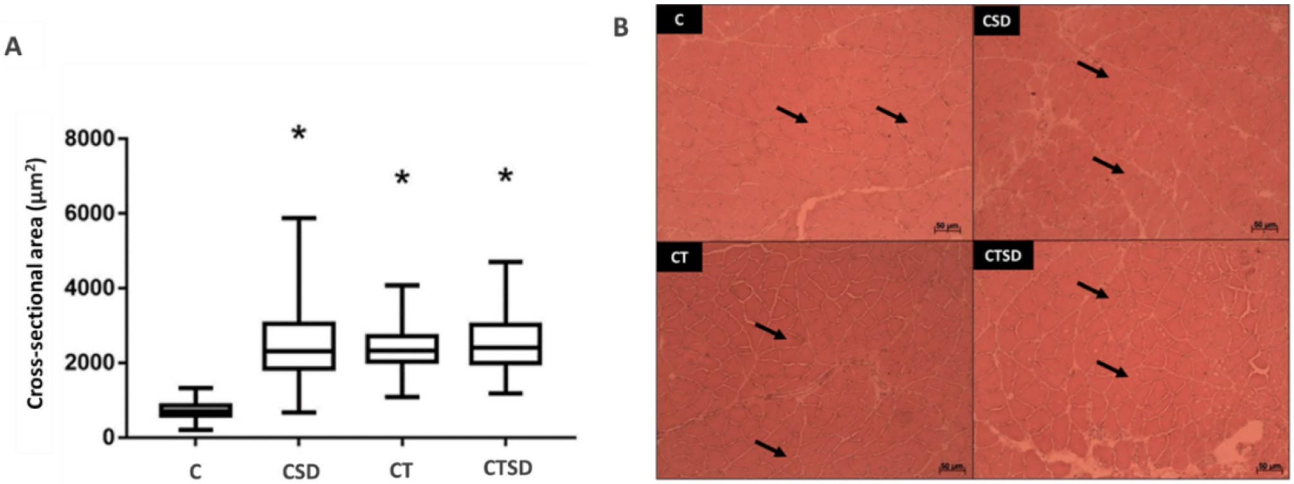

Fig. 3. A: Cross-sectional area in the different animal groups. B: Morphology of the Soleus muscle. Staining with Hematoxylin and Eosin at 10x magnification. Arrows indicate muscle fibers. C: Control; CSD: Control and soda consumption; CT: Concurrent Training; CTSD: Concurrent Training and soda consumption.

DISCUSSION

The objective of the present study was to analyze the changes resulting from soda consumption and concurrent muscle training on the Soleus muscle in rats. It was observed that all experimental groups showed an increase in cross-sectional area ($p<0.05$). Additionally, the groups that underwent the physical training protocol demonstrated a slight increase in the mean values of this variable.

Moreover, it was noted that all groups showed an increase in body mass over the 16 weeks, with no statistically significant differences when comparing the groups ($p>0.05$), but with differences observed across the study time points (weeks) ($p<0.05$). It was also observed that the animals consuming soda had lower intake of both feed and water.
Studies demonstrate that physical training is capable of generating a series of adaptations in skeletal muscle tissue. Research conducted on rodents has shown that, despite different specificities, training induces muscle hypertrophy and consequently increases cross-sectional area (Aleixo *et al*., 2021; Marcelo *et al*., 2021; Ozaki *et al*., 2022).

Furthermore, the nutritional aspect needs to be highlighted, as it is one of the essential factors for increasing physical capacity. In the case of concurrent training, this factor becomes even more important, as this training method utilizes protocols involving both aerobic and resistance exercises (Hickson, 1980; Paulo *et al*., 2005; Castoldi *et al*., 2013).

According to Perez-Schindler *et al*. (2015), the nutritional aspect is one of the factors that is not well understood regarding the use of concurrent training protocols. Additionally, the authors emphasize the use of amino acids before, during, and after training. In this regard, although soda is rich in carbohydrates, it may contribute to increased muscle mass, as the use or co-utilization with amino acids may provide greater gains than when consumed in isolation (Burke *et al*., 2011; Perez-Schindler *et al*., 2015).

Although no significant difference was found in body mass when comparing groups, a difference was observed across different training periods. Furthermore, the groups of animals that consumed soda showed higher values for this variable, although without statistical significance ($p>0.05$).

Studies show that dietary habits, with diets high in carbohydrates and fats, increase the chances of developing obesity and type II diabetes (Santos *et al*., 2015; Castoldi *et al*., 2017). Additionally, although no statistical difference was demonstrated ($p>0.05$), the animals that underwent training showed lower values for body mass.

Finally, the present study observed that animals that consumed soda demonstrated lower water and feed intake. This finding corroborates previous studies in which, across various experimental studies with animals, an increase in body weight and a reduction in solid food intake were evidenced during exposure to caloric sodas. Moreover, despite the reduction in solid food intake, an increase in total energy intake was observed, primarily due to a greater intake of soda, which contributes to increased energy consumption (Goularte *et al*., 2012).

Thus, the present study contributes to the literature by investigating the effects of soda consumption alongside a training protocol on the muscle tissue of Wistar rats. However, some limitations should be considered, such as the duration of soda exposure and the type of training conducted. Future studies aimed at investigating the effects of different periods of dietary induction or various training methods may help clarify the results presented in the current study.

CONCLUSION

It can be concluded that soda did not negatively interfere with the muscle adaptation related to muscle hypertrophy. However, animals that consumed soda showed higher values for body mass and lower intake of water and feed.

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RESUMEN: El objetivo de este estudio fue investigar los efectos de la ingestión de refrigerante (bebidas gaseosas) y realización de un protocolo de entrenamiento concurrente en el musculo sóleo de ratas Wistar. Fueron utilizadas 32 ratas de la raza Wistar, en la fase biológica de post-destete (30 días). Los animales fueron distribuidos en cuatro grupos: Control (C); Control y Refrigerante (CR); Entrenamiento Concurrente (EC) y Entrenamiento Concurrente y Refrigerante (ECR). El protocolo del entrenamiento fue compuesto por ejercicios aeróbicos y de resistencia. El protocolo de entrenamiento aeróbico fue integrado por tres sesiones semanales en días no consecutivos, comprendiendo 30 minutos de ejercicio de natación, con intensidad de 80 % del umbral anaeróbico (Lan). Para el protocolo del entrenamiento con resistencia (fuerza), se utilizaron cuatro series de 10 saltos, con sobrecarga correspondiente a 50 % del peso corporal de cada animal. Después de 48 horas de la última sesión del entrenamiento, los animales fueron sometidos a un proceso quirúrgico para la remoción de las muestras del musculo sóleo y procesamiento histológico. Todos los grupos experimentales demostraron un aumento en el área de sección transversa y masa corporal. Además, los animales que recibieron refrigerante (CR y ECR) demostraron menor consumo de la ración y agua. Por último, se determinó que los animales entrenados ingirieron menor cantidad de refrigerante en relación a los animales de control (ECR y CR) ($p<0,05$). Es posible concluir que el refrigerante no interfirió negativamente en la adaptación muscular. Sin embargo, los animales que ingirieron refrigerante, demostraron valores más elevados en masa corporal y menor ingestión de agua y ración.

PALABRAS CLAVE: Bebidas Gaseosas; Educación y Entrenamiento Físico; Músculo Esquelético; Ratas Wistar; Histología.

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