Chapter 1 Effect of the consumption of *Stevia rebaudiana* Bertoni as a natural and artificial sweetener on fatigue and oxidative stress of skeletal muscle

Capítulo 1 Efecto del consumo de la *Stevia rebaudiana* Bertoni como edulcorante natural y artificial sobre la fatiga y el estrés oxidante del músculo esquelético

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Abstract

Stevia rebaudiana Bertoni has non-caloric sweetening properties and its use has been linked to therapeutic effects. However, Stevia sp is sold as a substitute for sugar commercially, not only includes steviosides but is also combined with other high-intensity artificial sweeteners, which questions its safety. Our objective was to evaluate the effect of natural and artificial *Stevia rebaudiana* Bertoni on fatigue and oxidative stress of skeletal muscle. Twenty-four male Wistar rats were divided into: (C) rats receiving water, (S); rats receiving 41.2 g/L sucrose solution (SRA); rats receiving solution with commercial sweetener (*Svetia*) 6 g/L; (SRN); rats receiving solution with the dried and powdered leaf of the *Stevia* sp. plant. 4.4 g/L. Eight weeks after the treatment, muscle tension recording, and measurement of oxidative stress markers were performed: levels of reactive oxygen species (ROS) and catalase activity. Additionally, body weight, postprandial glucose, and food intake were recorded throughout the experiment. The SRA caused an increase in body weight and a significant reduction in the resistance time to muscle fatigue and the maximum and total muscle tension force. Treatment with SRN caused significant improvements in the parameters studied ($p < 0.05$). We conclude that natural *S. rebaudiana* is an essential alternative for weight control and the development of antioxidant defense against muscle fatigue but not in synergy with artificial sweeteners.

***Stevia rebaudiana* Bertoni, Muscle fatigue, antioxidant**

Resumen

Stevia rebaudiana Bertoni tiene propiedades edulcorantes no calóricas y su uso se ha relacionado con efectos terapéuticos. Sin embargo, la Stevia sp se vende como sustituto del azúcar comercialmente, no sólo incluye esteviósidos sino que también se combina con otros edulcorantes artificiales de alta intensidad, lo que pone en duda su seguridad. Nuestro objetivo fue evaluar el efecto de la Stevia rebaudiana Bertoni natural y artificial sobre la fatiga y el estrés oxidativo del músculo esquelético. Veinticuatro ratas Wistar macho fueron divididas en: (C) ratas que recibieron agua, (S); ratas que recibieron una solución de sacarosa de 41,2 g/L (SRA); ratas que recibieron una solución con edulcorante comercial (*Svetia*) de 6 g/L; (SRN); ratas que recibieron una solución con la hoja seca y pulverizada de la planta *Stevia* sp. 4,4 g/L. Ocho semanas después del tratamiento, se realizó el registro de la tensión muscular y la medición de los marcadores de estrés oxidativo: niveles de especies reactivas del oxígeno (ROS) y actividad de la catalasa. Además, se registraron el peso corporal, la glucosa postprandial y la ingesta de alimentos durante todo el experimento. El SRA provocó un aumento del peso corporal y una reducción significativa del tiempo de resistencia a la fatiga muscular y de la fuerza de tensión muscular máxima y total. El tratamiento con SRN provocó mejoras significativas en los parámetros estudiados ($p < 0,05$). Concluimos que la *S. rebaudiana* natural es una alternativa esencial para el control del peso y el desarrollo de la defensa antioxidante contra la fatiga muscular, pero no en sinergia con los edulcorantes artificiales.

Stevia rebaudiana Bertoni, Fatiga muscular, Antioxidante

1.1 Introduction

Skeletal muscle myopathy is a common clinical condition and a much less studied complication in chronic diseases; these muscle alterations lead to a reduced ability to withstand fatigue (Fernández et al. 2009).

The consumption of sugars in a balanced way in the daily diet has important properties since it favors the rapid supply of glucose to the brain and muscle, carbohydrates being essential for the development of cognitive functions and physical activity. Cane sugar or sucrose has been the sweetener par excellence for centuries; however, its excessive intake has been linked to different conditions: obesity, diabetes and metabolic syndrome (Gil-Campos et al. 2015).

There are several reports on replacing cane sugar with non-caloric herbal high-intensity sweeteners, and these substances can provide the sweet taste but substitute the caloric effects of carbohydrates and provide antioxidant effects. *Stevia rebaudiana* Bertoni is a native South American shrub, and its leaves are a powerful alternative to caloric sweeteners. In addition to their sweetening properties, Stevia sp. Extracts have been linked to other therapeutic effects, as their phenolic compounds have potential antioxidant properties and their phytochemicals help reduce blood sugar, cholesterol, and blood pressure. However, when Stevia rebaudiana Bertoni is sold as a sugar substitute, many commercial brands include its steviosides in their formula and combine it with other artificial high-intensity sweeteners (EAI). Although the use of these sweeteners is widespread, their safety as food additives remains controversial, cytotoxic effects and metabolic effects have been reported, casting doubt on whether the consumption of these additives is the most appropriate tool for the control of metabolic diseases (Stephens -Camacho et al. 2018). The current results on the use of antioxidants to delay muscle fatigue in humans are contradictory and constitute the main focus of discussion about the role of reactive oxygen species (ROS) in muscle fatigue. However, some authors have reported on the direct antioxidant activity of Stevia rebaudiana Bertoni leaf extracts on oxidative stress markers in different organs. Therefore, studies of this nature are necessary on the role of the antioxidants in natural Stevia rebaudiana Bertoni as their role in synergy with artificial sweeteners in the inactivation of ROS and its effect on muscle fatigue (Fernández et al. 2009; Ruiz -Ruiz et al. 2017).

1.2 General objective

To evaluate the effect of natural and artificial *Stevia rebaudiana* Bertoni on fatigue and oxidative stress of skeletal muscle.

1.3 Specific objectives

- Check the effect of natural and artificial *Stevia rebaudiana* Bertoni on rats' body weight and plasma glucose levels.

- To evaluate the contractile response and resistance to skeletal muscle fatigue by consuming natural and artificial *Stevia rebaudiana* Bertoni in rats.

- To evaluate the effect of natural and artificial *Stevia rebaudiana* Bertoni on oxidative stress and the antioxidant capacity of skeletal muscle in rats.

1.4 Materials and methods

1.4.1 Experimental animals

Twenty-four male rats of the Wistar strain with an initial weight of 300-320 g were used, kept in a room at a temperature of ~ 24 ° C, with a typical 12 h light / dark cycle with free access to food and water.

They were randomly divided into 4 groups (n = 6): (C) control, rats receiving unsweetened water; (S) sucrose: rats receiving sucrose solution (41.2 g / L); (SRA) Artificial *Stevia rebaudiana*: rats receiving solution with commercial sweetener of the brand “Svetia” (6 g / L); (SRN) Natural *Stevia rebaudiana*: rats receiving solution with the dried and powdered leaf of the *Stevia rebaudiana* Bertoni plant (4.4 g / L).

For 8 weeks, the rats received the solutions daily and were exchanged for new ones every 24 h; also, constant monitoring and recording was carried out simultaneously (1:00 pm) of the amount of solution and food consumed.

Once a week, the following were measured from the beginning of the treatments to the end: postprandial glycemia (GP) with a commercial glucometer (Accu-Check Performa ©), weight on a conventional scale and waist diameter with a measuring tape.

1.4.2 Animal ethics approval

The animals used were cared for by their nature and the experiment's objectives, following the recommendations of the Official Mexican Standard NOM-062-ZOO-1999 on the technical specifications for the production, care and use of laboratory animals, and approved by the Bioethics and Biosafety Committee of the UMSNH Chemical-Biological Research Institute.

1.4.3 Solutions and sweeteners

Three types of sweeteners of different natures were used. The concentrations of the solutions were chosen according to the substances sweetening power, tested in a preliminary test; based on the previous background, they were perceived with similar intensities of sweetness and without a detectable aftertaste.

- Control (C): Conventional drinking water without any added sweetener.

- Sucrose (S): Sucrose or cane sugar was used from a commercial brand of typical use, which meets the standard characteristics of sugar. 24.5 g were dissolved in 1 L of water.

- *S. rebaudiana* artificial (SRA): artificial sweetener of the “Svetia ©" brand that is used daily as a substitute for sugar, the content of this sweetener per 1 g of product (1 sachet) consisting: sucrose, extract of Stevia (steviol glucosides) 25 mg, isomalt 10 mg and sucralose 6 mg. 6 g (6 sachets) dissolved in 1 L of water were used.

- Natural *S. rebaudiana* (SRN): dried and powdered *Stevia rebaudiana* Bertoni plant leaves in their natural state, 4.4 g were used, this quantity was supplied as an infusion, mixed with hot water for 5 min and was filtered with the help of a funnel and filter paper, to finally made 1 L in its corresponding drinker.

The solutions were administered to the rats and were made available to them with free access in plastic drinkers with metal nozzles with a capacity of 1 L.

1.4.4 Euthanasia and muscle dissection

Once the treatment was finished, the rats were sacrificed by decapitation to dissect the Extensor Digitium Longus (EDL) and the Soleus muscle of the two hind limbs.

The first hind limb's dissected EDL muscle and soleus were preserved in Eppendorf tubes with Krebs-Ringer physiological solution (118 mM NaCl, 4.75 mM KCl, 1.18 mM MgSO4, 24.8 mM NaHCO3, 1.18 mM KH2PO4; pH 7.4) and brought to ultra-freezing at -80 ° C.

The dissected EDL muscle and soleus muscle of the second hind limb was placed and fixed with entomological pins in a Petri dish lined with a transparent resin bottom; this was previously filled with the Krebs-Ringer physiological solution (118 mM NaCl, 4.75 mM KCl, 1.18 mM $MgSO_4$, 24.8 mM $NaHCO_3$, 1.18 mM KH_2PO_4; pH 7.4) plus an addition of 10 mM C6H12O6 and 24.8 mM $NaHCO_3$. The solution was also supplied with carbon gas (95% CO_2 and 5% O_2). Mounted muscles were cleaned using a stereoscopic microscope to remove excess connective tissue and fatty tissue adhered to the muscle to record tension finally.

4.5 Tension recording

In an isometric tension recording chamber, the muscle was mounted through its tendons, one of the tendons was hooked to an optical transducer hook (World Precision Instruments, USA), the second tendon was attached to the bottom of the chamber. Once the muscle was assembled, the fibers were adjusted and tensioned 1.3 times their length at rest. At all times, the muscle was immersed in Krebs-Ringer physiological solution added with glucose and bicarbonate; the tissue received constant carbogen gas. Inside the recording chamber, two platinum-iridium electrodes were placed directly into the solution where the muscle is submerged, but without making direct contact with the muscle, connected to a stimulus isolating unit (Grass. USA), for fatigue induction stimulation, started 10 min after muscle placement.

In turn, the transducer was connected to an amplifier and a digital-analog interface (World Precision Instruments, USA), allowing the tension generated by each muscle to be acquired on a computer, using the MDAC software (Word precision Instruments, USA).

1.4.6 Fatigue protocol

The fatigue protocol was induced in skeletal muscle by repetitive electrical stimulation. Applied in EDL muscle pulses 100 V, 300 ms of duration and a frequency of 50 Hz; and in soleus muscle with 100 V pulses, 300 ms in duration and a frequency of 45 Hz by a stimulus isolating unit and a stimulator (Grass, USA). Tissue was stimulated until the tension decreased approximately 60-70% to the initial tension.

1.4.7 Homogenization

The skeletal muscle samples were thawed to remove the tendons and immediately homogenize in Eppendorf tubes with Krebs Ringer solution with a Dragon Lab D-500 homogenizer, and aliquots were made for the corresponding biochemical tests. The homogenates were deep-frozen at -80 ° C.

1.4.8 Biochemical tests

1.4.8.1 Measurement of reactive oxygen species levels

The production of reactive oxygen species was determined using the fluorescence probe 2 ', 7'-dichlorodihydrofluorescein diacetate (H2DCFDA). Muscle tissue homogenate protein (1 mg/ml) was resuspended in 5 µL of dichlorofluorescein and completed with ROS Buffer (10 mM HEPES, 100 mM KCl, 3 mM $MgCl_2$ and 3 mM KH_2PO_4; pH 7.4) for a 2000 µL total volume, were incubated cold with shaking for 15 min. Baseline fluorescence was recorded after samples were tempered; finally, the samples were shaken cold for 60 min to record the final fluorescence. Changes in fluorescence were measured at excitation/emission wavelengths of 485nm / 520nm on a Shimadzu RF-5301PC spectrofluorometer (Shimadzu, Kyoto, Japan). Data were expressed as fluorescence delta (ΔF) in arbitrary units.

1.4.8.2 Catalase activity

Catalase activity was analyzed by measuring the conversion of hydrogen peroxide to oxygen with a Clark type oxygen electrode connected to a biological oxygen monitor (5300A Biological Oxygen Monitor, YSI, Ohio, USA). 1 mg/ml of protein from the muscle tissue homogenates were resuspended in a 0.1 M phosphate buffer (pH 7.4) at 25 ° C and monitored for 1 min. Then, 6 mM H2O2 was added to the chamber, and the conversion of hydrogen peroxide to oxygen was measured with the oxygen electrode for 3 min.

1.4.9 Statistical analysis

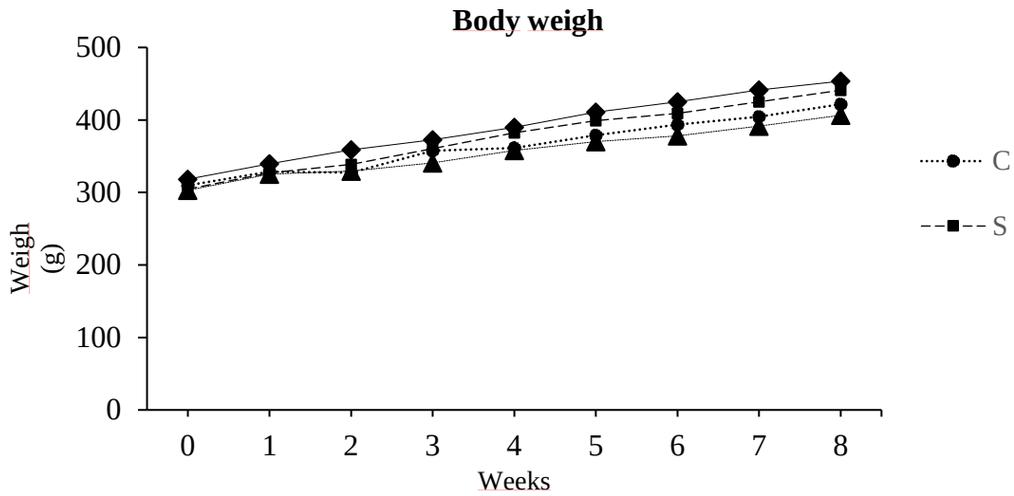
A 1-way ANOVA analyzed the results obtained with Tukey's posthoc test. Statistically significant differences were defined as $P < 0.05$.

1.5 Results

1.5.1 Effect of sucrose sweeteners, artificial and natural *Stevia rebaudiana* on body weight and postprandial blood glucose

At the end of the experiment, body weight was higher in the SRA group (453.5 ± 57.91 g) than in the C (421.7 ± 51.17), S (441 ± 17.24) and SRN (406.5 ± 39.90) groups; however, this increase it was not significant. On the other hand, the group of rats administered with sucrose also showed a more significant increase in body weight than groups C and SRN; however, the results are not significant. While the SRN group showed less weight gain than the control group, this was not significant either.

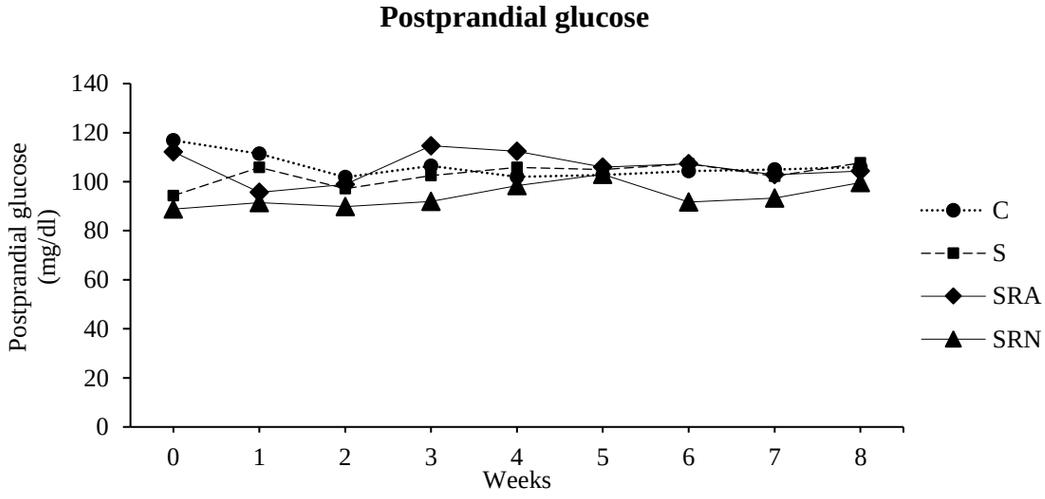
Graphic 1.1 Bodyweight. Throughout 8 weeks of treatment with the oral administration of sweetener solutions



C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = *Stevia rebaudiana* natura. C (421.7 ± 51.17), SRA (453.5 ± 57.91 g), S (441 ± 17.24) and SRN (406.5 ± 39.90), l. n = 6; Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

Regarding the postprandial blood glucose level, no significant differences were observed throughout the treatment in any group. A decreasing trend was observed after the fifth week in the SRN group (91.67 ± 11.23) than in the other groups studied C (104.3 ± 8.52), S (107.3 ± 18.35) and SRA (107.3 ± 5.08); however, the differences were not significant and these values and all those reported during the 8 weeks are within the normal ranges of glucose in the blood in a healthy individual.

Graphic 1.2 Postprandial glucose. Throughout the 8 weeks of treatment with the oral administration of sweetening solutions

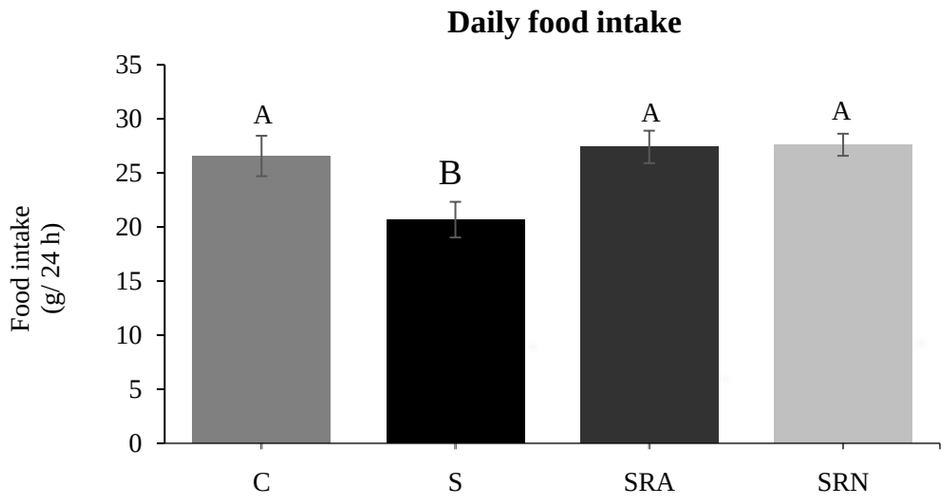


C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*. n = 6; Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

Effect of sucrose sweeteners, artificial and natural Stevia rebaudiana on food intake

Food consumption was significantly lower in group S (20.68 ± 1.65) than in the other groups studied C (26.57 ± 1.87), SRA (27.41 ± 1.50) and SRN (27.61 ± 1.02). The groups administered with the two types of *Stevia* sp. (SRA and SRA) presented a daily food intake similar to group C.

Graphic 1.3 Food intake. Throughout 8 weeks of treatment with the oral administration of sweetener solutions

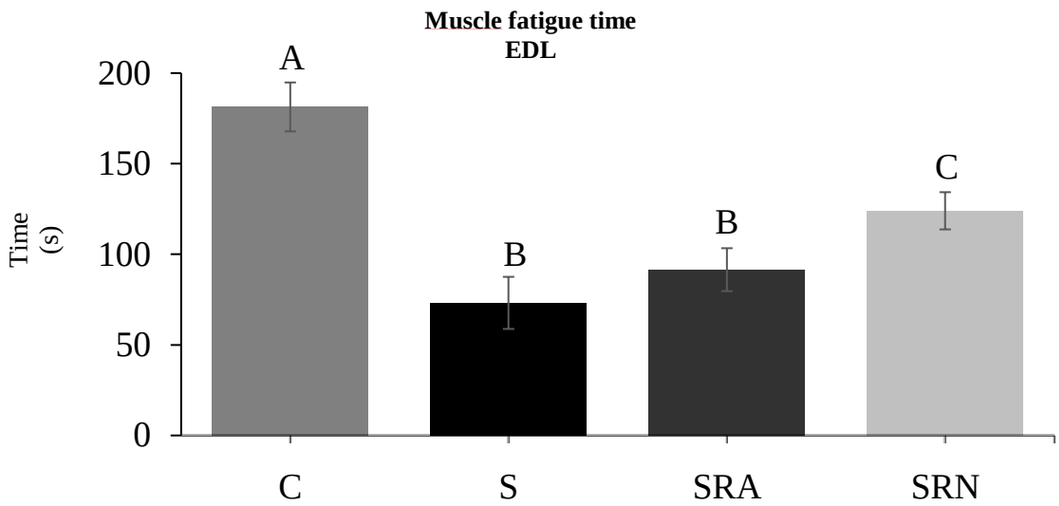


C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test)

Effect of sucrose sweeteners, artificial and natural Stevia rebaudiana on skeletal muscle fatigue resistance time

Regarding the time of resistance to fatigue in the long digitorum extensor muscle (EDL), all the groups administered with the sweeteners S (73.17 ± 14.38), SRA (91.50 ± 11.84) and SRN (124.5 ± 10.27) presented a significant reduction in the time to resist muscle fatigue compared to group C (181.3 ± 13.5). Furthermore, the S and SRA groups showed a more significant reduction in muscle fatigue than the SRN group.

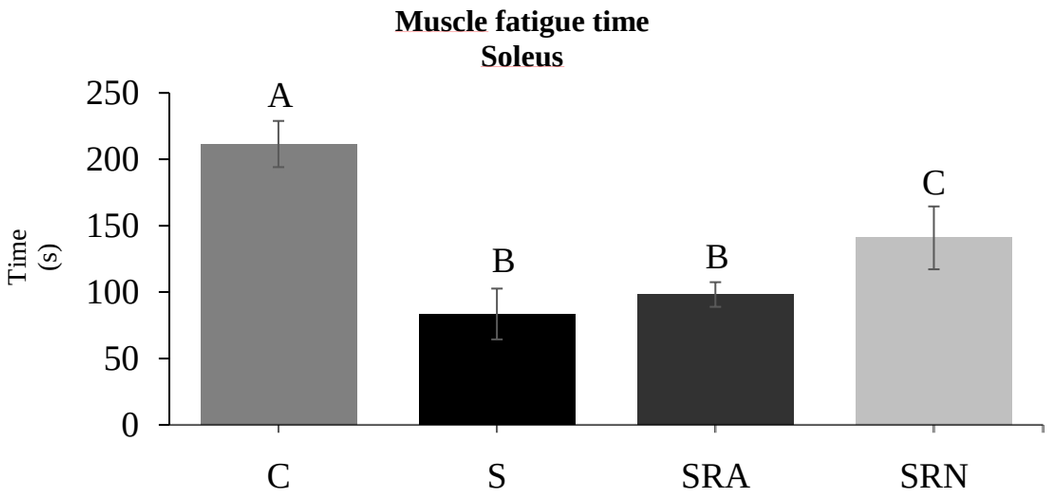
Graphic 1.4 Fatigue resistance time in EDL muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions



C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

On the other hand, the soleus muscle presented a greater resistance to fatigue in all the groups than the treated groups of the EDL muscle; this is probably due to its higher proportion of slow-twitch type I fibers. The administered sweeteners caused a significant reduction in the time of resistance to fatigue in all groups S (83.50 ± 19.13), SRA (98.17 ± 9.28) and SRN (140.8 ± 23.68) to control C (211.5 ± 17.40), being significantly lower in the S and SRA groups than in the SRN group.

Graphic 1.5 Fatigue resistance time in the soleus muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions



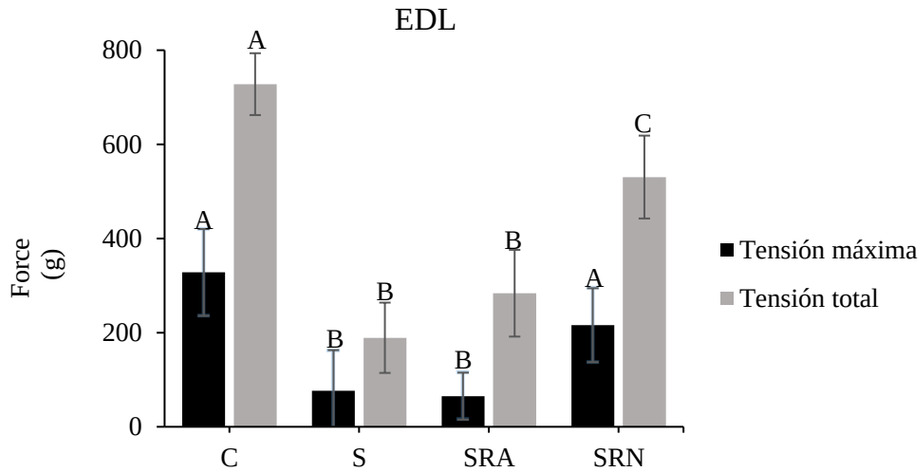
C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test)

Effect of sucrose sweeteners, artificial and natural Stevia rebaudiana on maximum tension and total skeletal muscle tension

The EDL muscle presented a significant decrease in muscle contraction force over maximum tension in the groups treated with S (76.64 ± 86.23), SRA (65.13 ± 49.94) and SRN (215.9 ± 78.58) compared to group C (327.9 ± 92.21), with the largest significant decrease observed in the S group; also the maximum tension force was significantly greater in the SRN group than in the S and SRA groups.

Regarding the total tension, the groups S (189.1 ± 74.69) and SRA (283.8 ± 92.13), SRN (530.5 ± 88.01) presented a significant decrease in the total contraction force to the C (727.91 ± 65.71), being the action of SRN more favorable for the maintenance of muscle contraction compared to the sweeteners of S and SRA.

Graphic 1.6 Maximum tension and total tension of the EDL muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions

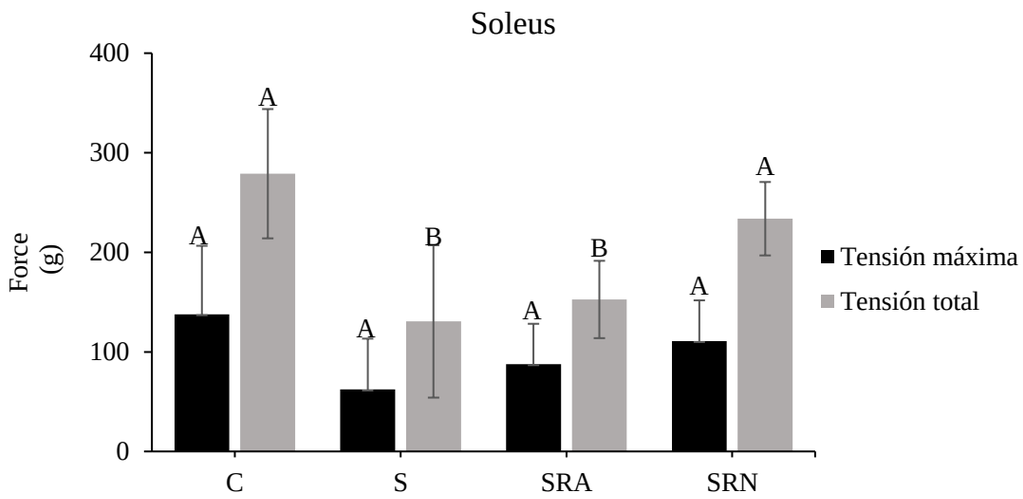


C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

While in the soleus muscle, no significant differences were found in the maximum tension force between groups C (137.8 ± 68.73), S (62.23 ± 51.07), SRA (87.66 ± 40.54) and SRN (110.8 ± 40.90).

Regarding the total tension, the sweeteners administered S (130.6 ± 76.54), SRA (152.6 ± 38.85) and SRN (233.7 ± 36.89) affected the total contraction force due to a significant decrease compared to C (279.0 ± 64.92). The S and SRA groups presented the highest decrease, while the SRN the lowest decrease.

Graphic 1.7 Maximum tension and total tension of the soleus muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions

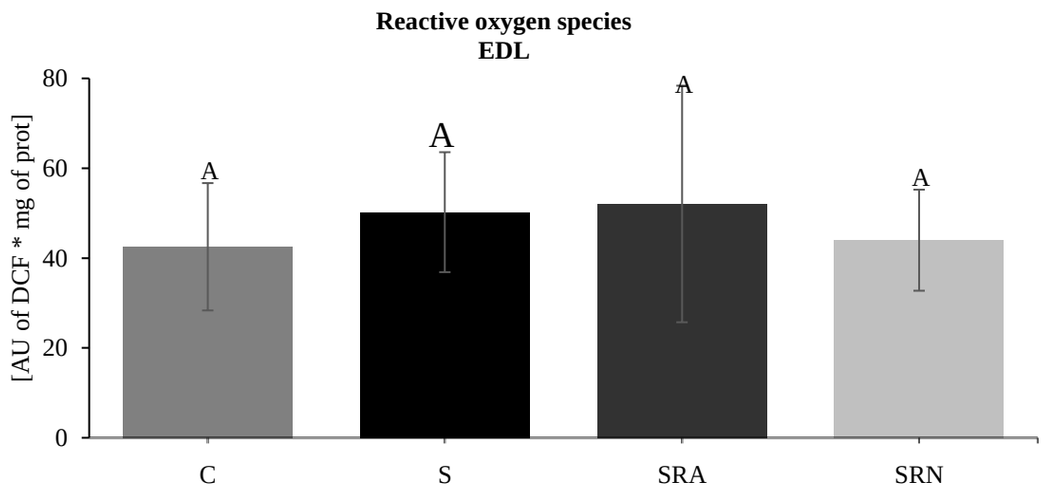


C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

Effect of sucrose sweeteners, artificial and natural Stevia rebaudiana on the level of reactive oxygen species of skeletal muscle

No significant differences were observed in ROS levels between groups C (42.53 ± 14.17), S (50.22 ± 13.35), SRA (52.05 ± 26.33) and SRN (43.99 ± 11.25) in the EDL muscle. An increasing trend was observed in groups S and SRA; however, these results are not conclusive.

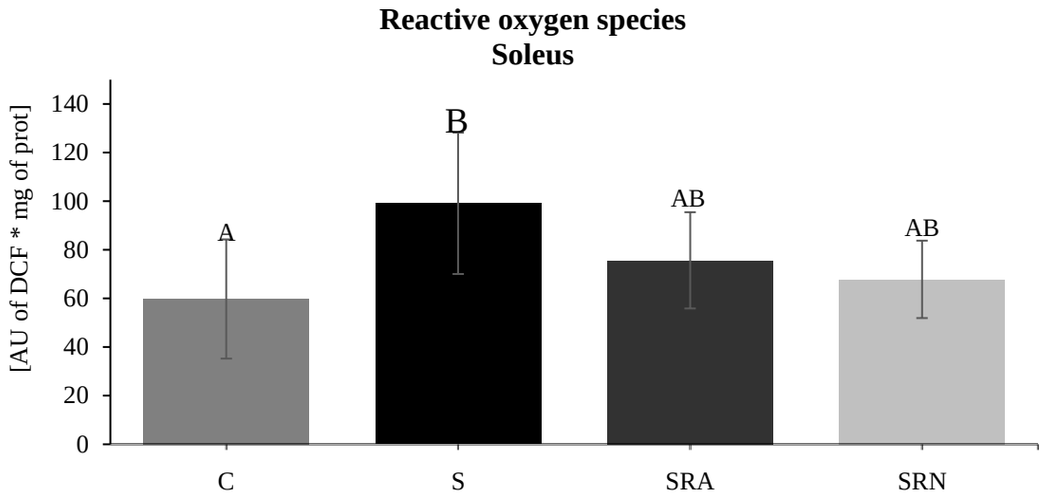
Graphic 1.8 Levels of reactive oxygen species in EDL muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions



C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test)

In the soleus muscle, ROS levels were significantly higher in group S (99.13 ± 29.08) than in group C (59.78 ± 24.52). Furthermore, the groups treated with Stevia sp. (SRA (75.68 ± 19.8) and SRN (67.84 ± 15.92) showed reductions in ROS levels compared to group S but not group C, these were not significant.

Graphic 1.9 Levels of reactive oxygen species in the soleus muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions

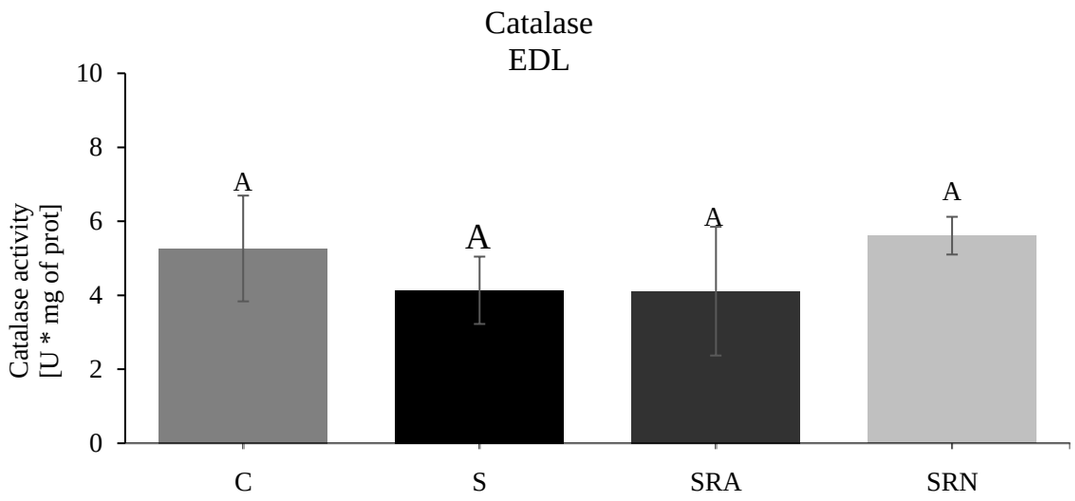


C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana;* n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

Effect of sucrose sweeteners, artificial and natural Stevia rebaudiana on the activity of the antioxidant enzyme catalase (CA) of skeletal muscle

In this study, we demonstrated that catalase activity in EDL muscle showed a decreasing trend in groups S (4.13 ± 0.91) and SRA (4.11 ± 1.74) compared to group C (5.26 ± 1.43) and SRN (5.61 ± 0.51); however, the results are not significant.

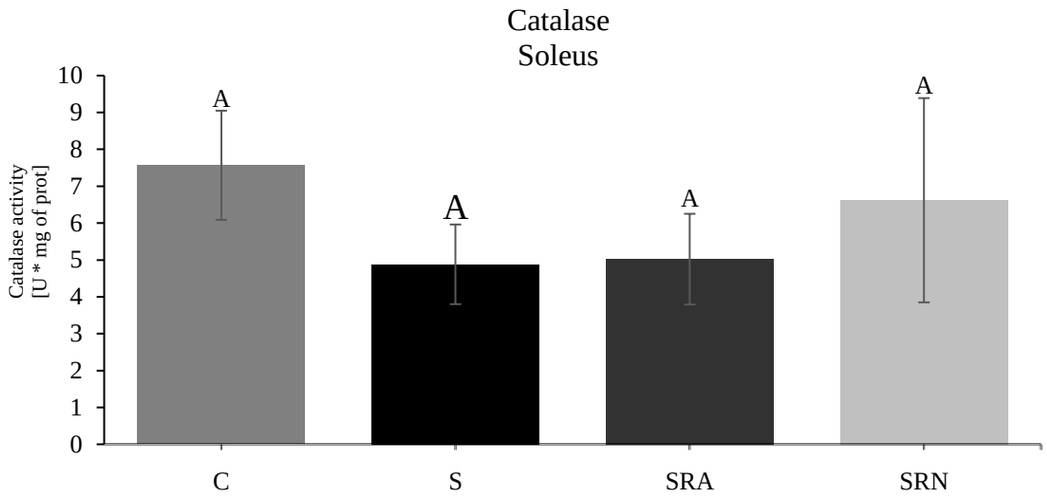
Graphic 1.10 Catalase levels in EDL muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions



C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

The catalase activity in the soleus muscle was lower in the groups administered with the sweeteners S (4.88 ± 1.08), SRA (5.02 ± 1.23) and SRN (6.62 ± 2.77), than in group C (7.56 ± 1.48), the activity in the SRN group being more improved, however, these results are not significant.

Graphic 1.11 Catalase levels in the soleus muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions



C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

1.6 Discussion

The growing concern about the increase in chronic diseases has led to a reduction in the consumption of simple sugars and an increase in the intake of artificial and natural non-caloric sweeteners, such as *Stevia rebaudiana* Bertoni, which is an excellent alternative, it is characterized by providing an intensely sweet flavor and have a zero caloric intake. However, the safety of these sweeteners as food additives remains unclear.

As previous studies have shown, the present study showed that the intake of Stevia sp. (SRN) at a dose of 4.4 g / L does not cause alterations in body weight and can be a compromising alternative to cane sugar or sucrose, however, steviol glycosides in combination with high intensity artificial sweeteners such as sucralose and Isomaltose (SRA) at a dose of 6 g / L, seems not to have the same effects on body weight, showing a trend of increase in body weight more significant than groups C and S, this may be due to the synergy not positive that steviol glycosides could be made in combination with the two artificial sweeteners, in addition, this is consistent with some antecedents that have reported that the intake of non-caloric sweeteners causes a positive body weight compensation due to physiological effects other than those produced due to the caloric consequences, a higher consumption of food after its ingestion, metabolic disorders and the stimulation of taste due to greater consumption of products sweets (Stephens-Camacho et al. 2018).

Furthermore, the supply of S, SRA and SRN sweeteners did not affect blood glucose levels, so Stevia sp did not show hypoglycemic effects as previously reported by Barriocanal et al. 2008, at least not within the tested concentration range and applied time. It is known that the antihyperglycemic, insulinotropic and glucagonostatic effects reported for the glycosides of Stevia sp. are highly dependent on high plasma glucose levels. Previous studies have reported that Stevia sp. have hypoglycemic effects in experimental animal models of DM, increasing the expression of GLUT4 in skeletal muscle, which increases glucose uptake and explains its hypoglycemic effects in DM; perhaps that is why in this study, no significant difference was observed due to that organism without any pathology were used (Gonzáles, 2013; Mouillot et al. 2020).

Also, in this study, it was shown that treatment with sucrose (S) at a dose of 41.2 g / L showed a significant reduction in the food consumption of rats; this may be due to the effects of sucrose on the systems of brain reward, it can bind to brain receptors that promote the release of dopamine, a neurotransmitter closely related to the generation of pleasurable sensations, which may have suppressed the rats' appetite in the weeks they received the sucrose solution.

While the consumption of *Stevia* sp (SRA and SRN) does not seem to modify appetite or food intake in rats, this contradicts a study by Farhat et al. 2019, who observed that Stevia sp might decrease the sensation of appetite, but this study was conducted with subjective tests. Furthermore, little is known about the differences in the activation of peripheral and central taste pathways, the central food and reward systems between sugar and non-caloric sweeteners. These results support the hypothesis that the consumption of sweet flavors in the absence of calories produces significantly different effects than the consumption of sweet flavors associated with calories (Durán et al. 2013; Mouillot et al. 2020).

The importance of studying skeletal muscle as a potential link between consuming the non-caloric artificial sweetener and Stevia sp is due to the relationship of food as a source to meet immediate energy needs, the reserve of nutrients and energy that the cells of the different organs and tissues use. Play an important regulatory role in metabolism; an altered energy pathway and substrate cause a higher incidence of the prevalence of muscle fatigue in chronic diseases. In this study, the sweeteners administered (S, SRA and SRN) caused a significant reduction in the fatigue resistance time in both fast and slow muscle, which could indicate that excessive intake of sugars regardless of their nature, whether natural or artificial, contribution or non-contribution of calories, causes a decrease in the time of fatigue resistance; however, a significant improvement was also shown within the deleterious effects of excess sweeteners. Administration of the SRN to the other two could be reflected in the antioxidant effects reported for the leaves of Stevia sp.

In addition, regarding the muscle contraction force, it has been described that the extracts of Stevia sp. They can aid skeletal muscle functionality by increasing muscle mass, strength and also improving mitochondrial function. In this research, it is shown that the concentrations of the sweeteners (S, SRA and SRN) applied caused in the EDL muscle a significant decrease in the force of muscle contraction, both in the maximum tension and in the total tension, which indicates that The different sweeteners in the study do not differ at least in causing this increase in muscle strength. However, it is also highlighted that the least harmful effect occurs in the SRN sweetener, which could once again indicate the critical role of leaf consumption whole of Stevia rebaudiana Bertoni due to its antioxidant phenolic properties found in it and not in industrially extracted glycosides.

Regarding the soleus muscle that presents type 1 slow-twitch fibers, no significant effect was found on the maximum tension in the treatment with sweeteners; however, on the total tension, the concentrations of S and SRA caused a significant decrease in the force of contraction, while the concentration of SRN significantly increased contractile dysfunctions almost the same as the control. The improved synthesis of proteins could explain these effects in the muscle fibers by the natural phytochemicals with the biological activity of the leaves of Stevia sp. (El-Mesallamy et al. 2017; El-Mesallamy et al. 2018).

The antioxidant activity of *S. rebaudiana* Bertoni is well established; however, the effect of these antioxidant compounds in association with high-intensity artificial sweeteners are not known. The sweetening compounds did not exhibit significant antioxidant activity in the EDL muscle since they could not decrease ROS levels to counteract the increase in oxidative stress induced by fatigue; there is an increase in the S and SRA groups concerning the control it is not significant.

In soleus muscle, the caloric intake of sucrose seems to affect ROS levels, which is consistent with reported data on the damage caused by excessive consumption of free sugars in the diet to different organs and systems (Cabezas-Zabala et al. 2016). As for Stevia sp, it does not produce a significant antioxidant defense, as it does not decrease ROS levels both in the treatment of RAS and in SRN. The SRA shows an increasing trend; however, it is not significant. This lack of antioxidant activity contrasts with the data reported by other authors, probably because the compounds used in this group are commercial sweeteners that contain steviol glycosides without appreciable amounts of polyphenols, naturally present in the leaves of Stevia sp., Probably responsible for antioxidant activity. However, the SRN group does not show significant protection against fatigue in both muscles; this could be because its effectiveness in other studies is due to its central effects in improving insulin secretion only in hyperglycemic individuals (Salvador-Reyes et al. 2014). These findings suggest that high-intensity sweeteners, especially SRA in a high dose, could have a deleterious effect on skeletal muscle through increases in the levels of reactive oxygen species of the soleus muscle (Rizzo et al. 2013; Ruíz-Ojeda et al. 2019).

In addition, it is known that muscle fatigue depletes the antioxidant defense system, thus promoting the generation of free radicals, which is why the antioxidant enzyme catalase (CAT) acts to protect cells against oxidative stress by degrading hydrogen peroxide in water and oxygen. , its activity varies depending on the fabric (Chance and Maehly, 1955). The catalase values presented in this study for skeletal muscle are in line with others previously reported (Paltian et al. 2019). In this research, we show that the glycosides of Stevia sp. in synergy with sucralose and isomaltose (SRA) caused a reduction in CAT activity together with group S in the soleus muscle and the EDL muscle. SRN seems to have an antioxidant effect due to its phenolic and flavonoid compounds; it should be noted that it does not present a significant increase in this activity, probably because the reported effects were in systems deteriorated by metabolic diseases (Chen et al. 2005). These results agree with other studies that have not demonstrated the antioxidant activity of Stevia sp. in healthy individuals.

The findings of this study, combined with evidence from other research on the sweetening effects of Stevia sp, suggest a possible beneficial role in the treatment of chronic diseases on muscle fatigue and the maintenance of health, but not of steviol glycosides in combination with artificial high-intensity sweeteners.

1.7 Conclusion

In conclusion, the leaves of Stevia sp. natural are an essential alternative for weight control and the development of antioxidant defense against muscle fatigue and its deleterious effects on skeletal muscle functionality compared to their extracts industrially extracted and marketed in synergy with other artificial sweeteners. More studies are needed to obtain more conclusive results because alternatives to sugar in the diet to avoid chronic diseases could increase the risk of these diseases.

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1.9 References

Academy of Nutrition and Dietetics; Fitch, C., K. S. Keim. 2012. Position of the Academy of Nutrition and Dietetics: use of nutritive and nonnutritive sweeteners. J Acad Nutr Diet. 112(5):739-58.

American Dietetic Association (ADA). 2004. Position of the American Dietetic Association: use of nutritive and nonnutritive sweeteners. J Am Diet Assoc. 104(6):1013.

Barriocanal, L. A. , M. Palacios,G. Benitez, S. Benitez, J. Jimenez,N. Jimenez y V. Rojas. 2008. Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans. A pilot study of repeated exposures in some normotensive and hypotensive individuals and in Type Type 1 and Type 2 diabetics. Regulatory Toxicology and Pharmacology 51. 37–41

Baynes, J. W. y M. H. Dominiczak. 2011. Bioquímica médica. Tejidos especializados y su función. 3a. ed. Barcelona, España: Elsevier España. S.L

Cabezas-Zabala, C. C., B. C. Hernández-Torres y M. Vargas-Zárate. 2016. Azúcares adicionados a los alimentos: efectos en la salud y regulación mundial. Revisión de la literatura. Sugars Added in Food: Health Effects and Global Regulation. Rev. Fac. Med. Vol. 64 No. 2: 319-29.

Cárdenas-Rodríguez N. y J. Pedraza-Chaverri. 2006. Especies reactivas de oxígeno y sistemas antioxidantes: aspectos básicos. Educación Química 17(2).

Chance, B. y A. C. Maehly. 1955. Assay of catalases and peroxidases. En: Colowick SP, Kaplan NO, eds. Methods in enzymology. New York: Academic; 764-5.

Chen, T. H., S. C. Chen, P. Chan, Y. L. Chu, H. Y. Yang y J. T. Cheng. 2005. Mechanism of the Hypoglycemic Effect of Stevioside, a Glycoside of Stevia rebaudiana. Planta Medica, 71(2), 108–113. DOI: 10.1055/s-2005-837775

Durán, S., K. Cordón y M. Rodríguez. 2013. Edulcorantes no nutritivos, riesgos, apetito y ganancia de peso. Rev Chil Nutr. 40(3):309-14. DOI: 10.4067/S0717-75182013000300014

El-Mesallamy, A.M.D., A. M. Hussein, S. A. Mahmoud y K. M. Elazab. 2017. Modulation of Metabolic and Contractile Dysfunctions of Soleus Muscle in Type 1 Diabetic Rats by Stevia Rebaudiana Bert. Extracts and Some of Its Derivatives: Comparative Experimental Study. International Journal of Scientific & Engineering Research Volume 8, Issue 9, 1543 ISSN 2229-5518odd.

El-Mesallamy, A.M.D., S. A Mahmoud, K. M Elazab, S. A Hussein, M. y A. M Hussein. 2018. Attenuation of metabolic dysfunctions in the skeletal muscles of type 1 diabetic rats by Stevia rebaudiana extracts, via AMPK upregulation and antioxidant activities. Acta Sci. Pol. Technol. Aliment., 17(3), 289–297. DOI: 10.17306/J.AFS.0567

European Food Information Council (EUFIC). 2013. What is an Acceptable Daily Intake (ADI)? https://www.eufic.org/en/understanding-science/article/qas-on-acceptable-daily-intakesadis (access March 2020).

Farhat, G., V. Berset y L. Moore. 2019. Effects of Stevia Extract on Postprandial Glucose Response, Satiety and Energy Intake: A Three-Arm Crossover Trial. Nutrients. 11 (12), 3036; DOI: https://doi.org/10.3390/nu11123036

FDA. Has Stevia been approved by FDA to be used as a sweetener? U.S Food & Drug Administration. Office of the Commissioner; 2018. Disponible en https://www.fda.gov/aboutfda/transparency/basics/ucm194320.htm

Fernández, J.M., M.E. Silva-Grigolettob e I. Túnez-Fiñanac. 2009. Estrés oxidativo inducido por el ejercicio. Revista Andaluza de Medicina del Deporte;2(1):19-34.

Gil-Campos, M., M. A. San José-González, J.J. Díaz-Martín. 2015. Uso de azúcares y edulcorantes en la alimentación del niño. Recomendaciones del Comité de Nutrición de la Asociación Española de Pediatría. An Pediatr (Barc). 83:353.e1-7.

International Sugar Organization (ISO). 2012. Edulcorantes alternativos en un contexto de altos precios del azúcar. MECAS (12)04. Disponible en http://www. isosugar.org/Members %20documents/2012/MECAS(12)04 %20- %20Alternative %20Sweeteners %20in %20a %20Higher %20Sugar %20Price %20Environment %20- %20Spanish.pdf

Lima, Y. C., M. A. Kurauti, G. F Alves, J. Ferezini, S. Piovan, A. Malta, F. L. Alves de Almeida, R. M. Gomes, P. C. Freitas, P. Gimenez, S. C. Costa y C. E. Mareze. 2019. Whey protein sweetened with Stevia rebaudiana Bertoni (Bert.) increases mitochondrial biogenesis markers in the skeletal muscle of resistance-trained rats. Lima et al. Nutrition & Metabolism 16:65 https://doi.org/10.1186/s12986-019-0391-2

Mouillot, T., A. Parise, C. Greco, S. Barthet, M. C. Brindisi, L. Penicaud, C. Leloup, L. Brondel y C. Jacquin-Piques. 2020. Differential Cerebral Gustatory Responses to Sucrose, Aspartame, and Stevia Using Gustatory Evoked Potentials in Humans. Nutrients, 12(2), 322. DOI:10.3390/nu12020322

OMS (Organización Mundial de la Salud), 2015. Global Health Observatory Data Repository. Disponible en http://apps.who.int/gho/data/node.main.1?lang=en

Paltian, J. J., C.R. da Fonseca, M. P. Pinz, C. Luchese y E. Wilhelm. 2019. Post-mortem interval estimative through determination of catalase and Δ-aminolevulinate dehydratase activities in hepatic, renal, skeletal muscle and cerebral tissues of Swiss mice, Biomarkers, 24:5, 478-483, DOI: 10.1080/1354750X.2019.1619837

Rizzo, B., L. Zambonin, C. Angeloni, E. Leoncini, F. Vieceli, D. Sega, C. Prata, D. Fiorentini y S. Hrelia. 2013. Steviol glycosides modulate glucose transport in different cell types. Oxidative Medicine and Cellular Longevity Volume 2013, Article ID 348169.

Ruiz-Ruiz, J. C., Y. B. Moguel-Ordoñez y M. R. Segura-Campos. 2017. Biological activity of Stevia rebaudiana Bertoni and their relationship to health. Critical reviews in food science and nutrition. DOI: 10.1080/10408398.2015.1072083.

Salvador-Reyes R., M. Sotelo-Herrera y L. Paucar-Menacho. 2014. Study of Stevia (Stevia rebaudiana Bertoni) as a natural sweetener and its use in benefit of the health. Scientia Agropecuaria 5. 157 – 163.

Stephens-Camacho, N. A., S. Valdez-Hurtado, G. Lastra-Zavala y L. I. Félix-Ibarra. 2018. Consumo de edulcorantes no nutritivos: efectos a nivel celular y metabólico. Perspectivas en nutrición humana. Vol. 20, N° 2, p. 185-202. DOI: 10.17533/udea.penh.v20n2a06.