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Thermal Treatment of Commercial Sweetener Solutions Modulates the Metabolic Responses in C57BL/6 Mice during a 24-Week-Long Exposition

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Abstract: The purpose of this paper was to evaluate the effect of thermal treatment (TT: 121 ± 2 °C, 15 min) on the composition of commercial sweeteners diluted in water (10 °Brix). Additionally, we evaluated the impact of this TT on metabolic responses in C57BL/6 mice during a 24-week treatment. The sweeteners included in this study were sucrose (SC), glucose-63 (GLU63), agave syrup (AS), sucralose (SUC), and steviol glycosides (STG). HPLC analysis showed changes in the concentration of simple sugars of GLU63 and AS after TT. Importantly, in all sweeteners, TT modulated metabolic responses in mice. The mice drinking thermally treated sweetener solution showed an increase of 10–13% ($p < 0.05$) in food intake (AS, SUC, and STG), beverage intake (2–21%; SC and GLU63), weight gain (38%; SUC), energy (10–13%; AS, SUC, and STG), glucose levels (11–17%; SC and STG), GLP-1 (30%; SC) and insulin (88%; AS) release, and the generation of protein carbonyl (SC) and malondialdehyde (all sweeteners tested) compared to mice drinking solution without TT. In conclusion, TT of sweetener solutions accentuates the metabolic responses of healthy mice, which can be related to overweight and its comorbidities.

Keywords: caloric sweeteners; non-caloric sweeteners; orexigenic and anorexigenic peptides; oxidative damage

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1. Introduction

Ultra-processed foods are affordable and highly appetizing due to their high calorie, sugar, fat, salt, and low fiber content [1]. The manufacture of ultra-processed foods employs thermal treatments with temperatures higher than 100 °C and various additives, including caloric and non-caloric sweeteners [2]. Caloric sweeteners are metabolized within the cell cytoplasm and are highly associated with overweight and obesity [3]. Thus, as an alternative, non-caloric sweeteners have been proposed. These sweeteners provide the same sweetness as sucrose and use metabolic pathways different from those of caloric sweeteners and thus do not produce calories [4]. However, non-caloric sweeteners can adversely affect glucose homeostasis and eating behavior in humans and animal models [5]. Non-caloric sweeteners bind to the T1r3 taste receptors, stimulating insulin [6] and incretin release (GLP-1 and GIP) [7] and increasing the levels of propionate lipogenesis precursors [8].

Moreover, the severe thermal treatment to which sweeteners are subjected in the food industry manufacturing processes can result in the decomposition and release of toxic substances [9,10]. Several studies suggest that the products obtained from the decomposition of some sweeteners after being subjected to temperatures higher than 180 °C are glyoxal/methylglyoxal, furfural, 5-methylfurfural, chloropropanol, and dioxins, among others. These products are closely related to diabetogenesis, mutagenesis, and the formation of advanced glycation end products (AGEs) [9,11–13]. However, knowing the changes in the sweetener's composition after thermal treatments is not enough; further studies are required to understand the health effects of these changes. Therefore, this study aimed to evaluate the effect of thermal treatment (121 ± 2 °C, 15 min) on the chemical composition of commercial sweeteners diluted in water (10 °Brix) and its impact on the metabolic response (biochemical parameters, anorexigenic and orexigenic peptides, and oxidative stress) of C57BL/6 mice during the 24-week-long treatments. We hypothesized that thermal treatment above 100 °C will provoke changes in the sweetener's composition. Furthermore, these changes will negatively impact metabolic responses in C57BL/6 mice during a 24-week-long exposure.

2. Materials and Methods

2.1. Commercial Sweeteners and Preparation of Sweetened Solutions

The sweeteners used in this study were purchased from local suppliers: sucrose with a purity grade higher than 98%, glucose-63 (syrup composed of 100% glucose which is obtained by acid-enzymatic hydrolysis of corn starch), agave syrup (92.8% fructose, 0.2% glucose, 0.1% sucrose, and 6.7% fructans), sucralose with a purity grade of 98–102%, and steviol glycosides with a concentration of rebaudioside A of 95–100% were obtained. Sweeteners were dissolved in water at a concentration of 10 °Brix. Sweetened solutions were subjected to thermal treatment (121 ± 2 °C for 15 min). Finally, solutions were allowed to cool to room temperature for further use in in vivo studies.

2.2. Impact of the Thermal Treatment on Sweetener Composition

2.2.1. Caloric Sweeteners

Free sugars were measured using sucrose, glucose, and fructose as standards. Previously diluted and filtered samples were injected into acrodisc filters (PTFE Phenex, 25 mm filter diameter, 0.20 µm pore size) and analyzed by HPLC Surveyor LC Plus, Thermo Scientific, (Waltham, MA, USA). The analytical column used was Phenomenex Rezex RPM-carbohydrates Na^{+2} (8%) of 300 × 7.8 mm. The mobile phase was Milli-Q grade water with a 0.4 mL/min flow. The column and detector temperatures were maintained at 80 °C and 37 °C, respectively. All samples were analyzed in triplicate.

2.2.2. Non-Caloric Sweeteners

For sucralose, the chromatographic separation was carried out using a CORTECS UPLC C18 column with a particle size of 1.6 µm and an ID of 100 × 3.0 mm (Milford, MA, USA). The mobile phases used in this analysis were A-Water (80%) and B-Methanol (20%), with an isocratic flow of 0.2 mL/min and an injection volume of 2 µL. The analysis was carried out in Single Ion Recording (SIR) mode, tracking the molecular ion of sucralose (419 m/z) with a cone voltage of 45 V and by multiple reaction monitoring (MRM) and positive polarity by UPLC Acquity H Class, Acquity, (Sheboygan, WI, USA)/MS Xevo TQ-S micro, Water, (Milford, MA, USA).

For steviol glycosides, the chromatographic separation was carried out using a CORTECS UPLC C18 column with a particle size of 1.6 µm and an ID of 100 × 3.0 mm (Milford, MA, USA). The mobile phases were A: HCOOH 0.1% and B: acetonitrile: HCOOH 0.1%, which were injected in a proportion of 70% A, an isocratic flow of 0.2 mL/min, and an injection volume of 2 µL. The analysis was performed in SIR mode, tracking the molecular ion of rebaudioside A (965.60 m/z) and rebaudioside C (949.60 m/z) with a cone voltage of 45 V

and negative polarity by UPLC Acquity H Class, (Waters, Milford, MA, USA)/MS Xevo TQ-S micro, (Waters, Milford, MA, USA).

2.3. In Vivo Study

Six-week-old male C57BL/6 mice (n = 72) were randomly assigned to six experimental groups (n = 6). The experimental groups were CTL: purified water (control), SC: sucrose diluted in drinking water (10 °Brix), GLU63: glucose-63 diluted in drinking water (10 °Brix), AS: agave syrup diluted in drinking water (10 °Brix), SUC: sucralose diluted in drinking water (10 °Brix), STG: steviol glycosides diluted in drinking water (10 °Brix). The experiment lasted 24 weeks. All animals were confined and kept in cages (12/12 light and dark cycles). Before the experiment, mice were subjected to an acclimatation period of one week. Furthermore, during the 24-week experiment, food and drink intake and weight gain were monitored weekly. Following the official Mexican standard NOM-062-ZOO-1999 [14], mice were fed with PicoLab Rodent 5001 standard diet, LabDiet (38% carbohydrates, 24% protein, and 5% fat), and had free access to the sweetened solutions.

2.3.1. Total Energy Intake

The total energy intake per mouse was calculated at the end of the experiment using Equation (1) [15]. For this calculation, the energy contribution of the diet (LabDiet 5001, 4.07 kcal/g) and that of commercial sweeteners were considered: SC (0.4 kcal/g), GLU63 (0.328 kcal/g), AS (0.16 kcal/g), SUC (0 kcal/g), and SGT (0 kcal/g).

$$\text{Energy intake} = \left(\text{LabDiet 5001 g} \times 4.07\ \frac{\text{kcal}}{\text{g}}\right) + \left(\text{Sweeteners}\ \frac{\text{kcal}}{\text{g}}\right) \quad (1)$$

2.3.2. Metabolic Parameters

Biochemical parameters were evaluated monthly, between 8 and 9 a.m. on the day of sample collection. Blood samples were collected by tail nick. Glucose was measured using the One-Touch® Ultra device (LifeScan Inc., Milpitas, CA, USA). The total cholesterol and triglycerides were determined with the Accutrend® Plus (Roche, Basel, Switzerland) The results were evaluated monthly and expressed in mg/dL.

2.3.3. Quantification of Orexigenic and Anorexigenic Peptides

After 24 weeks, at noon, the mice were anesthetized with Pisabental® (5 mg/kg) (PISA, Nuevo León, Mexico). Blood samples were collected by retro-orbital sinus bleeding; the serum was stored at −20 °C until further use. Then, the animals were euthanized by cervical dislocation. The stomach, large intestine, and liver were removed and stored at −20 °C for further processing. To quantify insulin (anorexigenic peptide), the serum was prepared according to the instructions from the ELISA kit employed (NBP2-62853, Novus Biologicals®, Centennial, CO, USA). On the other hand, for gastric ghrelin (orexigenic peptide), GLP-1, and gastric leptin (anorexigenic peptides), the stomach and large intestine were prepared following the methodology reported by Sánchez et al. [16] and Santiago-García et al. [17], respectively, with minor modifications. The large intestine and stomach are important reservoirs of such peptides. For example, GLP-1 is an incretin located in the "L" cells of the small and large intestine. On the other hand, leptin and gastric ghrelin are secreted in the stomach, with activity in the fundus, cardia mucosa, and pylorus [18,19].

The quantification was carried out using ELISA kits and following the manufacturer instructions: (1) gastric ghrelin (MBS722422, MyBioSource®, San Diego, CA, USA), (2) GLP-1 (MBS260820, MyBioSource®, San Diego, CA, USA), (3) gastric leptin (Ab100718, Abcam®, Cambridge, UK), and (4) insulin (NBP2-62853, Novus Biologicals®, Centennial, CO, USA). The results for gastric ghrelin, GLP-1, and gastric leptin were expressed in pg/mL and insulin in ng/mL, according to the kit instructions.

2.3.4. Quantification of Biomarkers of Oxidative Stress

Biomarkers of oxidative damage were measured in the liver because it is the main organ that metabolizes sugars. In addition, possible liver functional damage due to the accumulation of lipids following chronic consumption of commercial sweeteners can be observed in this organ [20,21]. Thus, carbonyl content was determined using the Protein Carbonyl Content Assay Kit (MAK094, Sigma-Aldrich®, Darmstadt, Germany). The results were expressed in nmol. The malondialdehyde content (MDA) was determined using the TBARS Assay Kit (10009055, Cayman Chemical®, Ann Arbor, MI, USA). The results were expressed in μM, according to the kit instructions.

2.4. Statistical Analysis

Data were analyzed with the Statgraphics 8.0 software. A multi-factor ANOVA with a Tukey post hoc test was used to evaluate the effect of the sweeteners and their thermal treatment on the response variables. Additionally, a *t*-Student test was used to compare the mean values obtained with the thermal treatment for each response variable. Differences with a $p < 0.05$ value were considered significant.

3. Results

3.1. Effect of the Thermal Treatment on the Composition of Commercial Sweeteners

Table 1 shows the impact of the thermal treatment (121 ± 2 °C, 15 min) on the composition of commercial sweeteners diluted in water (10 °Brix). Regarding the caloric sweeteners, in SC, thermal treatment had no effect ($p > 0.05$) on its concentration of simple sugars. On the contrary, GLU63 decreased ($p < 0.05$) its sucrose and glucose content by 5%. Similarly, the thermal treatment decreased ($p < 0.05$) the fructose content of AS by 19%. Moreover, STG and SUC (non-caloric sweeteners) did not show changes ($p > 0.05$) in their composition after thermal treatment.

Table 1. Effect of the thermal treatment (121 ± 2 °C, 15 min) on the composition of commercial sweeteners diluted to 10 °Brix.

	Sucrose (ppm)	Glucose (ppm)	Fructose (ppm)	Rebaudioside A (ppm)	Sucralose (ppm)
SC (WT)	60.42 ± 0.02 [a]				
SC (TT)	59.90 ± 0.44 [a]				
GLU63 (WT)	3.97 ± 0.05 [b]	2.58 ± 0.01 [b]			
GLU63 (TT)	3.76 ± 0.00 [a]	2.44 ±0.02 [a]			
AS (WT)			5.93 ± 0.30 [b]		
AS (TT)			4.78 ± 0.09 [a]		
STG (WT)				0.52 ± 0.05 [a]	
STG (TT)				0.51 ± 0.03 [a]	
SUC (WT)					0.191 ± 0.03 [a]
SUC (TT)					0.188 ± 0.00 [a]

WT: without thermal treatment; TT: thermal treatment. Different lowercase letters (a and b) indicate significant differences between treatments of the same sweetener ($p < 0.05$) using the post hoc Tukey test. Values represent the Mean ± Standard Deviation (SD).

3.2. Food and Drink Intake, Weight Gain, and Energy Intake

As for the effect of the type of sweetener on food intake (Figure 1(I)), except AS ($p > 0.05$), all sweeteners caused changes in this variable. In the CTL group, caloric sweeteners (SC and GLU63) decreased food intake close to 45%. Non-caloric sweeteners (SUC and SGT) increased this variable by 36%. As for the effect of the thermal treatment on food intake (Figure 1(I)), this factor increased food intake by 10–13% in the AS, SUC, and SGT groups.

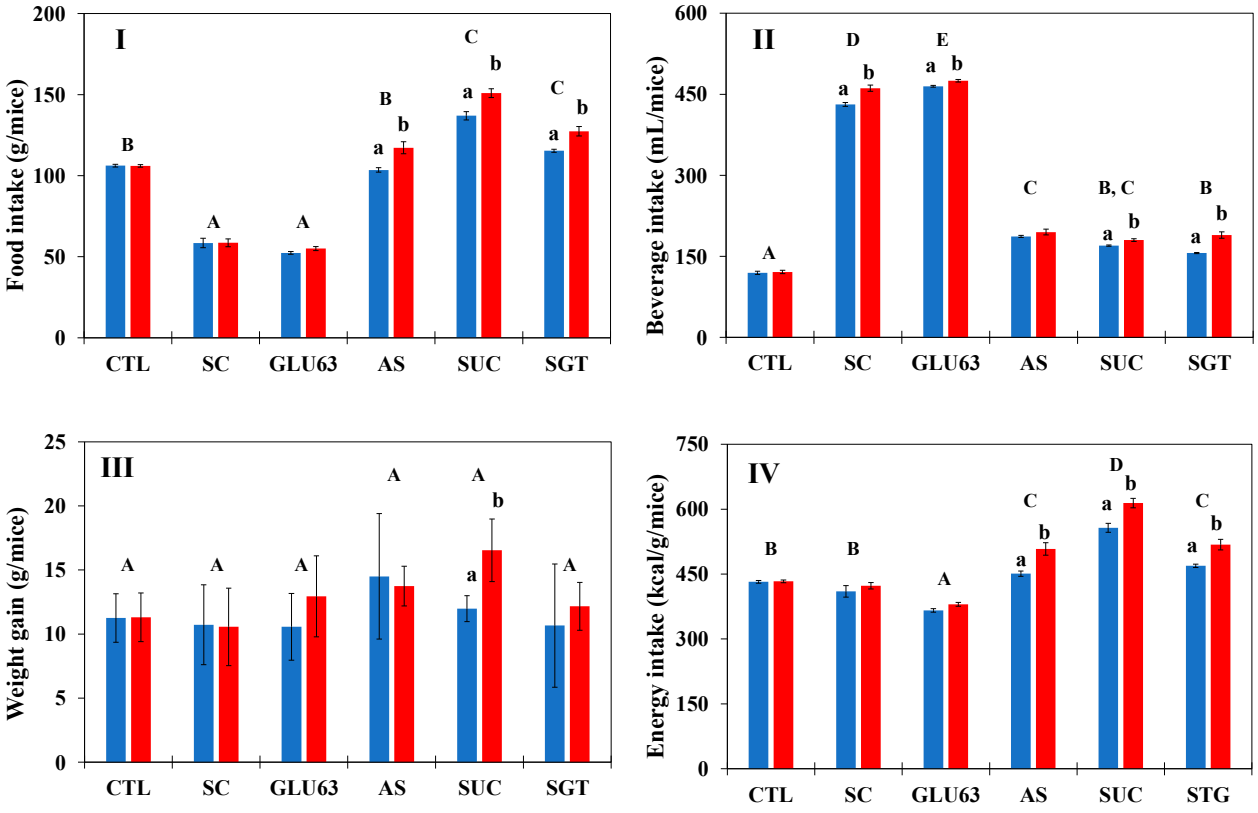

Figure 1. Effect of the thermal treatment (121 ± 2 °C, 15 min) of commercial sweeteners on the food intake (**I**), drink intake (**II**), weight gain (**III**), and energy intake (**IV**) in C57BL/6 mice during 24 weeks of experimentation. Blue bars indicate samples without thermal treatment. Red bars indicate samples subjected to heat. Different lowercase letters (a and b) show significant differences caused by the thermal treatment ($p < 0.05$). Different capital letters (A, B, C, D, and E) represent significant differences between sweeteners ($p < 0.05$).

As for drink intake (Figure 1(II)), compared to the CTL, sweeteners increased this variable ($p < 0.05$). However, these increases were higher (~291%) in the SC and GLU63 groups. Regarding thermal treatment, except for the AS group, all the groups treated with sweeteners subjected to thermal treatment increased their beverage intake; this increase ranged from 2% (SUC) to 21% (SGT) (Figure 1(II)).

As for weight gain (Figure 1(III)), despite the effect observed on food and drink intake, sweeteners had no effect ($p > 0.05$) on weight gain during the 24 weeks of treatment. As for the sweeteners subjected to thermal treatment, only SUC increased ($p < 0.05$) weight gain by 38% (Figure 1(III)).

Regarding energy intake (Figure 1(IV)), except for SC ($p > 0.05$), all sweeteners caused changes in this variable. Compared to the CTL group, GLU63 reduced ($p < 0.05$) caloric intake by 14%. On the other hand, SUC increased caloric intake by 36%, following SGT (14%) and AS (11%). Sweeteners subjected to thermal treatment increased ($p < 0.05$) energy intake in the groups treated with AS (13%), SUC (10%), and STG (10%) (Figure 1(IV)).

3.3. Blood Biochemical Parameters

Regarding the effect of the different types of sweeteners on glucose levels (Figure 2(I)), SUC and SGT reduced this variable by 9% compared to the CTL group. Moreover, thermal treatment reduced ($p < 0.05$) glucose levels by 8% in the AS group and increased glucose by 17% and 11% in the SC and SGT groups, respectively (Figure 2(I)).

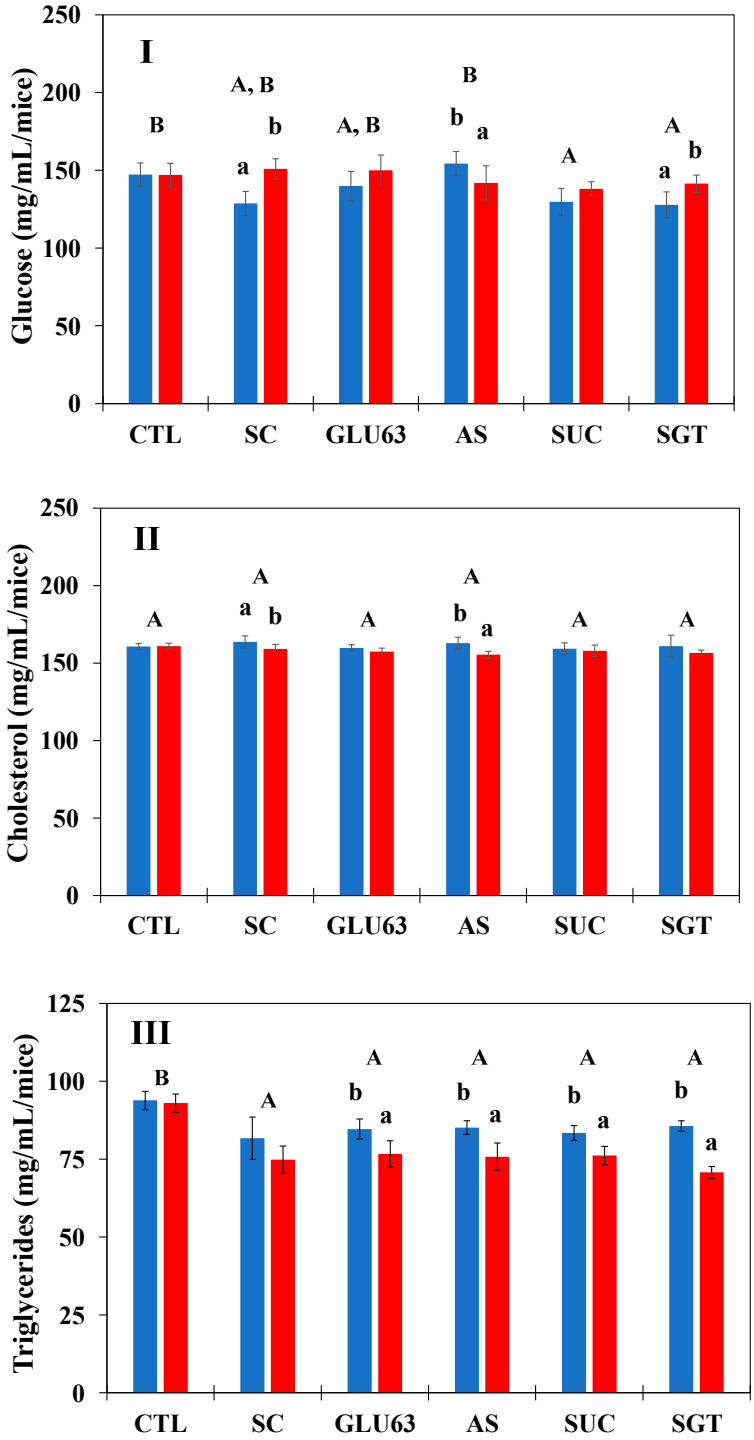

Figure 2. Effect of the thermal treatment (121 ± 2 °C, 15 min) of commercial sweeteners on blood glucose (**I**), cholesterol (**II**), and triglycerides (**III**) of C57BL/6 mice during 24 weeks of experimentation. Blue bars indicate samples without thermal treatment. Red bars indicate samples subjected to heat. Different lowercase letters (a and b) show significant differences caused by the thermal treatment ($p < 0.05$). Different capital letters (A and B) represent significant differences between sweeteners ($p < 0.05$).

As for cholesterol levels, compared to the control, sweeteners had no effect ($p > 0.05$) on this variable during the 24 weeks of treatment (Figure 2(II)). On the other hand, thermal treatment reduced ($p < 0.05$) cholesterol levels in the SC (3%) and AS (5%) groups (Figure 2(II)). Finally, for triglyceride levels (Figure 2(III)), all sweeteners decreased ($p < 0.05$) this variable

by approximately 16% compared to the CTL group. Sweeteners subjected to thermal treatment also reduced ($p < 0.05$) triglyceride levels in the groups treated with GLU63 and SUC (9%), AS (11%), and SGT (17%) (Figure 2(III)).

3.4. Concentration of Orexigenic and Anorexigenic Peptides

Regarding the effect of the different types of sweeteners on ghrelin levels (Figure 3(I)), all sweeteners increased ($p < 0.05$) this variable compared to the CTL group. These increases ranged between 30 and 50% for SC and GLU63 and 75–90% for AS, SUC, and STG. As for GLP-1 (Figure 3(II)) and gastric leptin (Figure 3(III)), the type of sweetener had no effect ($p > 0.05$) on these variables, except for the increase ($p < 0.05$) of GLP-1 in the SUC group (78%) and gastric leptin on the SUC and SGT groups (>70%). Finally, SC and SUC increased ($p < 0.05$) insulin (Figure 3(IV)) by more than 100%. The other sweeteners had no effect ($p > 0.05$) on insulin levels compared to the CTL.

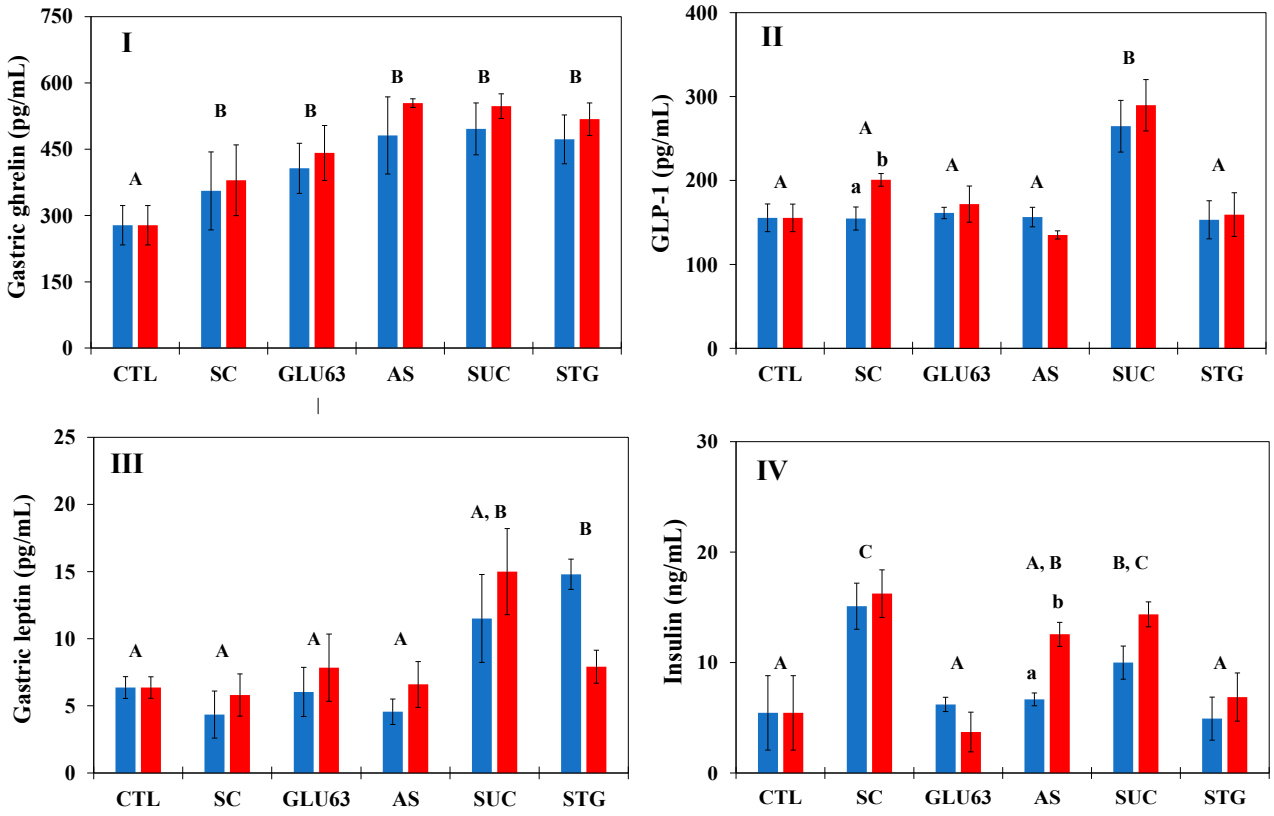

Figure 3. Effect of the thermal treatment (121 ± 2 °C, 15 min) of commercial sweeteners on gastric ghrelin (**I**), GLP-1 (**II**), gastric leptin (**III**), and insulin (**IV**) of C57BL/6 mice during 24 weeks of experimentation. Blue bars indicate samples without thermal treatment. Red bars indicate samples subjected to heat. Different lowercase letters (a and b) show significant differences caused by the thermal treatment ($p < 0.05$). Different capital letters (A, B, and C) represent significant differences between sweeteners ($p < 0.05$).

The thermal treatment had no effect ($p > 0.05$) on the levels of ghrelin (Figure 3(I)) and gastric leptin (Figure 3(III)) during the 24 weeks of treatment. On the contrary, thermal treatment increased GLP-1 and insulin by 30% in the SC group (Figure 3(II)) and 88% in the AS group (Figure 3(IV)).

3.5. Quantification of Oxidative Markers

As for the effect of the type of sweetener on the carbonyl content (Figure 4(I)) and malondialdehyde levels (TBARS) (Figure 4(II)), the GLU63 group showed an increase of

100% in carbonyl content. The AS group showed a decrease of 36% in the TBARS levels. The other sweeteners did not affect the oxidative markers analyzed in this study.

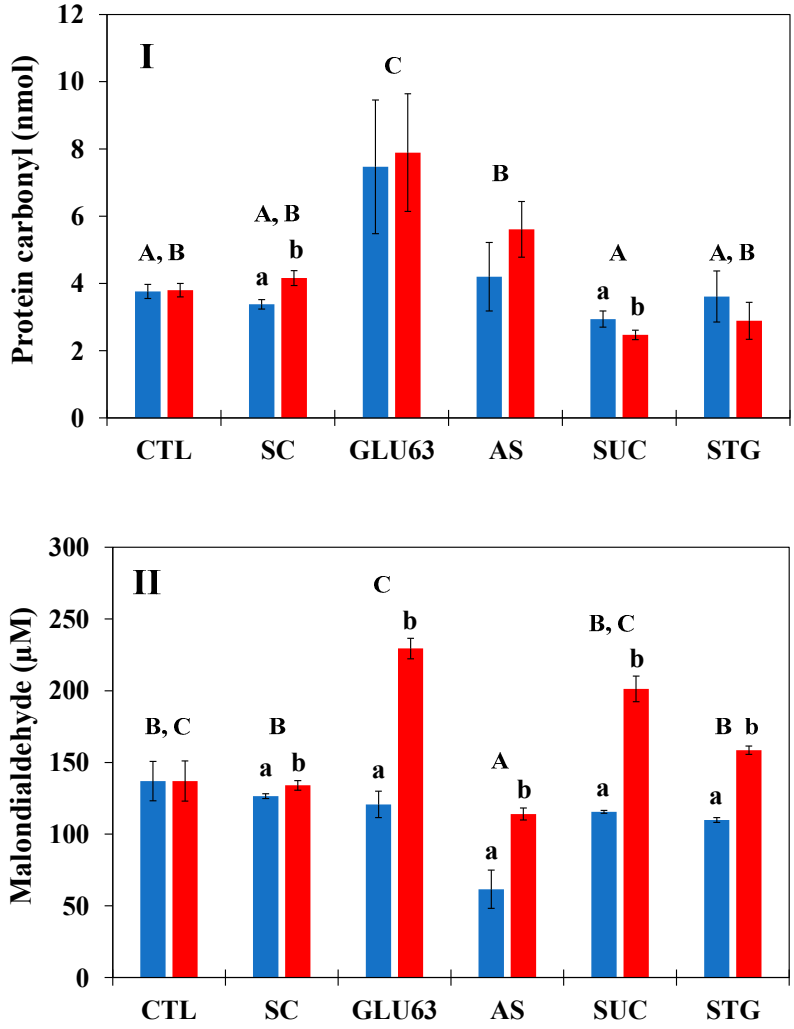

Figure 4. Effect of the thermal treatment (121 ± 2 °C, 15 min) of commercial sweeteners on protein carbonyls (**I**) and malondialdehyde (**II**) content in the liver of C57BL/6 mice during 24 weeks of experimentation. Blue bars indicate samples without thermal treatment. Red bars indicate samples subjected to heat. Different lowercase letters (a and b) show significant differences caused by the thermal treatment ($p < 0.05$). Different capital letters (A, B, and C) represent significant differences between sweeteners ($p < 0.05$).

Moreover, thermal treatment increased ($p < 0.05$) by 23% and decreased by 16% the carbonyl content for the SC and SUC groups, respectively (Figure 4(I)). As for the TBARS levels (Figure 4(II)), thermal treatment increased this variable ($p < 0.05$) in the SC (6%), GLU63 (90%), AS (85%), SUC (74%), and SGT (44%) groups (Figure 4(II)).

4. Discussion

Thermal treatment decreased the simple sugars concentration of caloric sweeteners rich in glucose and fructose (GLU63 and AS). This decrease in simple sugars could be attributed to caramelization reactions which occur at the temperatures we have studied [22]. In addition, thermal treatments above 120 °C have been shown to produce toxic secondary compounds, such as glucose degradation products (glyoxal and methylglyoxal) [12], by-products such as organic acids (formic, lactic, and levulinic acid), and compounds like 5-hydroxymethylfurfural. On the other hand, non-caloric sweeteners (SUC and STG) were stable to the thermal treatments in this study (121 ± 2 °C for 15 min). Several studies have

reported that starting at 250 °C, the STG of *Stevia rebaudiana* Bertoni degrades into partially deglycosylated compounds [10]. Moreover, it has been reported that SUC can degrade during cooking or baking processes (temperatures above 180 °C), generating potentially toxic chlorinated compounds [11].

As for the impact of commercial sweeteners on metabolism, mice in the SUC and SGT (non-caloric) and AS (caloric) groups showed higher food and caloric intake and lower beverage intake compared to the CTL. Additionally, the thermal treatment significantly increased the total energy, food, and beverage intakes in the previously mentioned groups.

Compared to the control, SUC showed increased gastric ghrelin, GLP-1, gastric leptin, and insulin. Moreover, thermal treatment significantly increased the levels of TBARS. Various studies have reported that SUC induces an immediate increase of cytoplasmic Ca^{2+} and cAMP in pancreatic islets, which stimulates the release of neurotransmitters such as insulin [8,23,24]. This gives rise to hyperinsulinemia and hypoglycemia, which generates greater energy intake and increased body adiposity with high leptin levels. Additionally, in mice, SUC increased propionate levels (gluconeogenesis, lipogenesis, and protein synthesis precursor) after eight weeks of treatment [8]. To date, the information about the metabolic effect of the SUC secondary compounds generated from thermal treatment is limited. However, there is evidence that this sweetener, without thermal treatment, forms two less polar and more lipophilic acetylated metabolites, which have been detected in the urine, feces, and adipose tissue of rats [25]. This is relevant because changes in the acetylation of proteins or histones can result in various diseases (obesity, diabetes mellitus, cancer, oxidative stress, and others) [26].

STG decreased blood glucose and triglycerides compared to the CTL group. However, it increased the levels of gastric ghrelin and leptin compared to the CTL and SC; it also decreased insulin levels with respect to SC. Furthermore, thermal treatment significantly decreased the levels of triglycerides and increased blood glucose and TBARS levels in the liver. Mice in the STG group followed a similar behavior to those in the SUC group, with some differences in their hormonal profile. Compared to SUC, STG mice showed a lower content of GLP-1 and insulin. This trend could be attributed to the fact that rebaudioside A is resistant to digestive enzymes and is metabolized in the colon by gut microbiota, forming steviol or steviol glucuronide [27] and short-chain fatty acids [8]. These metabolites have an insulinotropic effect by potentiating the transient receptor potential melastatin 5 (TRPM5) on pancreatic β cells, which reduces blood glucose [27]. However, Gupta et al. [28] reported that the metabolite generated from rebaudioside A is stevioside, which can be transformed into glucose and steviol. This could explain the increase in blood glucose levels in mice treated with STG subjected to thermal treatment. Non-caloric sweeteners (SUC and STG) are specifically detected by the T1r3 taste subunit in enteroendocrine cells, leading to the release of neurotransmitters [24]. This could increase the secretion of GLP-1 and insulin, affecting weight, appetite, and glycemia.

AS increased gastric ghrelin levels, but the rest of the hormonal profile was similar to the CTL. However, thermal treatment increased the levels of TBARS in the liver of mice. As for the weight gain reported in the AS group, it has been shown that fructose decreases insulin and leptin levels, attenuating ghrelin suppression, which increases caloric intake [29]. Based on the decrease in insulin, it cannot influence the hepatic metabolism of fructose and its conversion to triose phosphate. Therefore, triose phosphate can transform into fatty acids via lipogenesis [30]. Excess fructose in the intestinal lumen has been shown to result in advanced glycation end products (AGEs), which increase oxidative stress, leading to neurodegenerative diseases, atherosclerosis, and chronic inflammatory diseases [31].

On the other hand, mice treated with SC and GLU63 (caloric sweeteners) showed higher drink intake and lower food intake. Additionally, the thermal treatment caused the beverage intake in these groups to be higher. This could be due to the release of neuroadapters in the reward system, which separates eating behavior from the homeostatic signaling of the hypothalamus (caloric), leading to compulsive eating [31,32]. Thus, the

calories of solid foods are reduced to compensate for the calories of liquids generated in a diet [33]. In the SC group, thermal treatment increased the concentration of blood glucose, GLP-1, and carbonyls. Additionally, in the SC and GLU63 groups, thermal treatment increased the levels of TBARS in the liver of mice. In its early stages, SC increases the preference for sweet taste by activating the taste receptors T1r2 and T1r3 [7]. Wu et al. [34] state that SC increases LDL cholesterol in plasma, insulin, and glucose intolerance in male mice. High-carbohydrate diets have also been shown to induce reactive oxygen species, leading to cell damage and disease progression [35].

5. Conclusions

The thermal process evaluated in this study changes the simple sugar concentration of caloric sweeteners. However, both caloric and non-caloric sweeteners, when subjected to thermal treatment, accentuate the metabolic responses of healthy mice. These changes and the duration of sweetener intake may be related to weight gain and its comorbidities. For future studies, it is important to characterize the chemical substances that result from subjecting sweeteners to temperatures higher than 120 °C, which are used in common thermal processes such as ultra pasteurization, and correlate them with more detailed metabolic profiling. In addition, this information could contribute to elucidate the possible mechanisms related to the impact of chemical compounds generated by the thermal treatment of commercial sweeteners on consumer health. Finally, it is important to extrapolate this investigation to clinical studies.

Author Contributions: E.N.-M.: Methodology, Data curation, Formal analysis, Writing—original draft. A.S.-R.: Statistical analysis, Methodology, Supervision. N.P.: Methodology, Supervision. E.F.-R.: Methodology, Supervision, Writing—review and editing. C.O.: Conceptualization, Funding acquisition, Supervision, Project administration, Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: Data may be made available upon request.

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