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Black Tea Kombucha Consumption: Effect on Cardiometabolic Parameters and Diet Quality of Individuals with and without Obesity

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Abstract: Background: Kombucha, a fermented tea, has been suggested as an adjuvant in the treatment of obesity. Although animal and in vitro studies indicate its promising benefits, exploring kombucha's impact on human health is necessary. Methods: This quasi-experimental pre–post-intervention assessed the effect of black tea kombucha consumption on cardiometabolic parameters for 8 weeks, considering the quality of the diet of individuals with and without obesity. Diet quality was assessed through the Dietary Inflammatory Index® and Dietary Total Antioxidant Capacity. Paired *t*-test/Wilcoxon was applied to compare differences between pre- and post-intervention ($\alpha = 0.05$). Results: After the intervention, individuals with obesity showed a decrease in insulin, HOMA-IR, and GGT; those without obesity showed an increase in total cholesterol and alkaline phosphatase, but this was only observed in those with a worsened diet quality. Conclusion: kombucha intake demonstrated positive impacts on the metabolic health of individuals with obesity beyond the importance of combining it with healthy eating patterns.

Keywords: obesity; cardiometabolic risk factors; kombucha tea; diet; plant bioactive compound

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1. Introduction

Obesity is characterized by abnormal or excessive fat accumulation associated with metabolic disturbances [1–3]. It may exert a major effect on metabolic health and social and psychological factors, affecting all age and socioeconomic groups worldwide [4]. Obesity has reached epidemic proportions around the globe; in 2020, the proportion of the global population with overweight or obesity (BMI $\geq$ 25 kg/m^2) was 38%, and it is projected that this rate alarmingly will increase up to 51% by 2035. If this trend continues, it will impose an even greater burden on health and economic systems [5]. Despite obesity's complex and multifactorial etiology, it is considered preventable in most cases [6]. Its control requires social and environmental changes and must consider adaptations in diet and lifestyle [7,8].

Fermented foods rich in bioactive compounds have been highlighted as a dietary approach that can minimize susceptibility to excess weight and decrease the existing

metabolic imbalance in obesity [9,10]. Among them, there is kombucha, a refreshing fermented drink obtained via the infusion of Camellia sinensis and sugars, fermented using a symbiotic culture of bacteria and yeasts (SCOBY) [11,12]. This drink originated in China (Manchuria) in 220 BC [13] but has only recently become popular worldwide [14]. It is now recognized as one of the world's main low-alcohol fermented beverages (LAFBs) [15].

Kombucha tea has a composition full of many substances, including vitamins (C, B1, B2, B6, and 12), minerals (manganese, iron, copper, zinc, calcium, and magnesium), amino acids, and carbohydrates. Moreover, the fermentation process enriches the composition by degrading complex components, which increases the concentration of phenolic compounds, such as theaflavin and thearubigin, together with the production of organic acids (acetic acid, gluconic and glucuronic acids) [11–13]. Kombucha is produced using a variety of microorganisms, including yeasts and bacteria. Generally, the more prevalent yeast species are *Dekkera* sp., *Saccharomyces* (*S. cerevisiae*), *Brettanomyces* sp., *Candida* sp., and *Zygosaccharomyces* sp., and among the most abundant prokaryotes, there are the acetic acid bacteria, such as *Acetobacter* (*A. aceti*, *A. pasteurianus*, *A. nitrogenifigens*, *A. xylium*) and *Gluconobacter* (*G. sacchari*, *G. oxydans*, *G.* sp. *A4*, *G. kombuchae*) [12].

Phenolic compounds and organic acids are the main functional compounds present in kombucha that provide antioxidant and anti-inflammatory properties to the beverage [16,17], as well as the modulation of the intestinal microbiota [18,19], protection against hepatic steatosis [20], and the improvement in lipid and glucose metabolism [21–24]. In this manner, it is believed that this drink can assist in the treatment of non-communicable chronic diseases (NCDs), such as obesity [25].

Although previous animal and in vitro studies indicate promising results of kombucha consumption for health, investigations in humans are still scarce and needed to confirm its benefits [14,26]. As many factors are involved in obesity, including the individuals' eating habits, clinical studies that consider the dietary context's influence on the effect of regular kombucha consumption are extremely important, especially concerning the Dietary Inflammatory Index (DII), dietary antioxidant capacity (DTAC), and dietary total polyphenols (DTP). Our hypothesis is that after kombucha ingestion, both groups will improve cardiometabolic parameters, especially in individuals who presented better diet quality indices. Thus, this quasi-experimental pre–post-intervention study aimed to evaluate the effect of the regular ingestion of black tea kombucha on cardiometabolic markers, considering the quality of the diet of individuals with and without obesity.

2. Materials and Methods

2.1. Subjects

Recruitment of participants was carried out via social networks between August 2020 and May 2021. An online pre-screening questionnaire was sent to those who expressed interest. Individuals who met the established inclusion criteria were invited to undergo screening at the Laboratory of Energy Metabolism and Body Composition (LAMECC/Federal University of Viçosa) and confirm their participation in the study.

Men and women aged 18–45 years were included. The participants were allocated according to their Body Mass Index (BMI), body fat (BF) measured via InBody®, model 230, BiospaceCo, and waist circumference (WC). When BMI was between 18.5 and 24.9 kg/m^2, BF was up to 25% for men and up to 30% for women, and WC < 88 cm for women and <102 cm for men, participants were considered to be in the group without obesity. On the other hand, individuals in the obesity group had BMI $\geq$ 30 kg/m^2, BF > 27% for men and >34% for women, and WC > 88 cm for women and >102 cm for men.

Individuals with other chronic diseases or anemia were not included. Other criteria for the exclusion of individuals were the regular use of any kind of drugs; the habit of nutritional supplement intake; the use of antibiotics less than three months before the beginning of the study; infectious or allergic episodes in the last month, including SARS-CoV-2; restrictive or compulsive eating behavior; unstable weight in the last three months (up to 3 kg fluctuation); restrictive diet for weight loss; regular kombucha consumption; an aver-

sion or allergy to kombucha; alcohol intake higher than 21 units (≈168 g) per week; smokers; and pregnant and lactating women. Individuals who expressed interest in participating signed an informed consent form. The participants could withdraw from the study at any time they wanted without any negative repercussions.

2.2. Study Design

This is a quasi-experimental, pre–post-intervention clinical study that lasted eight consecutive weeks. This study is in accordance with the Transparent Reporting of Evaluations with Nonrandomized Designs (TREND) guideline to control the risk of biases and assure its transparency [27] (Table S1—Supplementary Materials). Subjects were evaluated before the start of the study and at the end of treatment and analyses were performed by comparing these two moments (Figure 1). At the end of the trial, everyone received biochemical and anthropometric results. Nutritional care was also offered for 30 days with guidance on healthy eating habits.

The software G*Power 3.1.9.2 was used to calculate the a priori sample size [28], considering an effect size of 0,45 between two dependent means in one of the cardiometabolic parameters (fasting glucose was considered) [29] with a significance level of 5% and a power of 80%. The minimum sample size generated was 20 individuals per group; when adding a 15% loss, the final minimum value for each group was 23 participants.

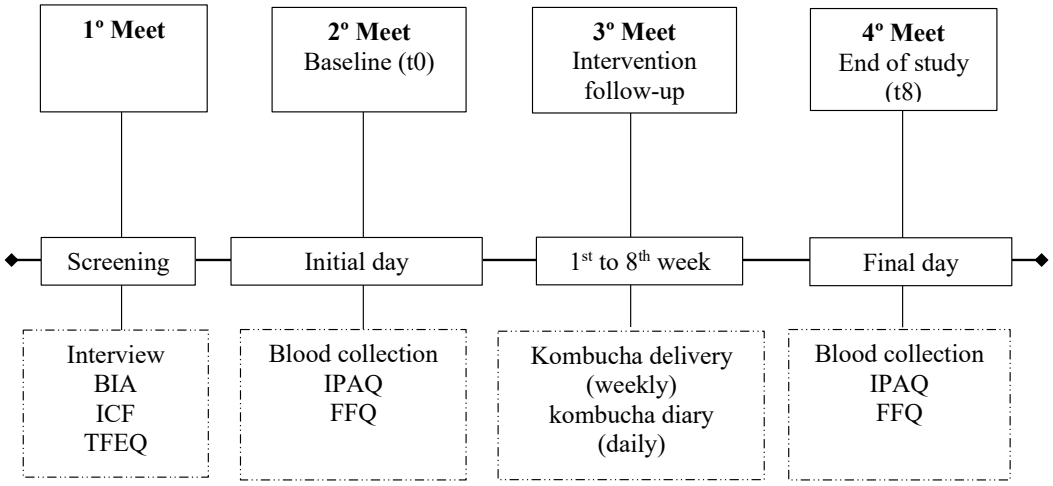

Figure 1. Illustration of the experimental design of the study. This intervention lasted eight weeks. Participants had to drink 200 mL/day of kombucha and maintain their usual diet and physical activity pattern throughout the study. Blood samples and a Food Frequency Questionnaire were collected at the beginning and end of the study. BIA: bioimpedance body composition analysis; IPAQ International Physical Activity Questionnaire; FFQ: Food Frequency Questionnaire; ICF: informed consent form; TFEQ: Three-Factor Eating Questionnaire.

2.3. Black Tea Kombucha Production and Consumption

The drink was produced in accordance with the parameters established by the Ministry of Agriculture, Livestock and Supply (MAPA) without adding optional ingredients, aromas, flavorings, or carbon dioxide [11]. Black tea kombucha production is illustrated in Figure 2. In the end, 100 mL/L of previously produced kombucha was added in order to reduce the pH and, thus, inhibit the growth of pathogenic microorganisms [13]. The symbiotic culture of bacteria and yeasts (SCOBY) was purchased from a certified company (Enziquímica Produtos Químicos Ltda., São Paulo, Brazil), free of any contamination. The volume of kombucha offered to the participants complied with the US Centers for Disease Control and Prevention's recommendations [30]. Regarding toxicity, the kombucha offered to the participants was previously tested in Wistar rat hepatocytes [17]. The characterization analysis of the beverage is described in the Appendix A. The final composition of the drink is described in Table 1.

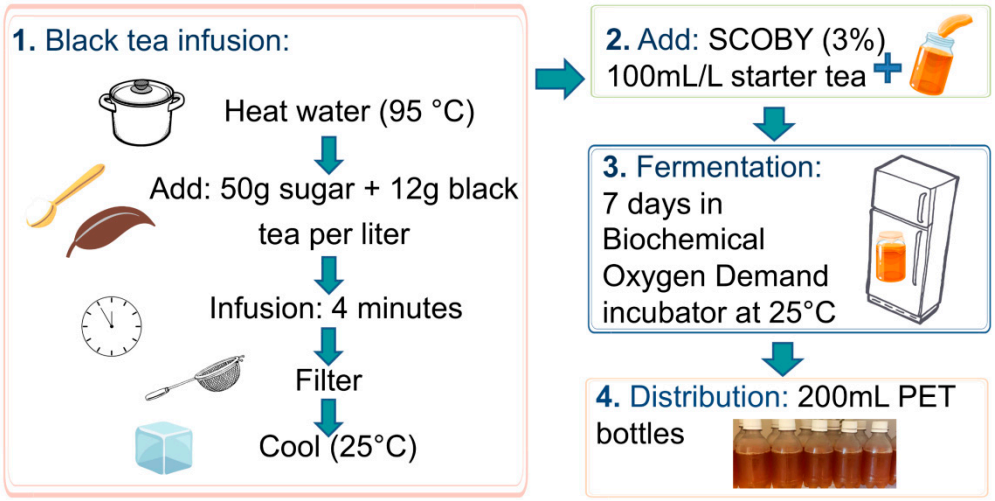

Figure 2. Step-by-step process of black tea kombucha production. It was produced using 12 g of black tea and 50 g of crystal sugar for each liter of drinking water. The black tea leaves were steeped for 5 min after the drinking water reached 95 °C. Then, the tea was strained and cooled until it reached 25 °C. Once cooled, 3% of SCOBY was added to the total volume of black tea produced. Additionally, 100 mL/L of previously produced kombucha was also added in order to reduce the pH and, thus, inhibit the growth of pathogenic microorganisms. Fermentation was carried out in a BOD (Biochemical Oxygen Demand) incubator for temperature control (25 °C) for seven days. After fermentation, the kombucha was strained, packed in 200 mL PET bottles, and stored in a refrigerator until it was distributed to the participants.

Table 1. Black tea kombucha characterization.

Analysis	Mean (SD)
pH	3.48 (0.05)
Volatile acidity (meq/L)	30.0 (3.75)
Total acidity (%, acetic acid-g/100 mL)	0.31 (0.0095)
Acetic acid (g/L)	0.99 (0.015)
Total phenolics (mg/mL)	0.69 (0.02)
Theaflavin (g/100 mL)	0.12 (0.003)
Thearubigin (g/100 mL)	1.88 (0.07)
Yeast (log UFC/mL)	6.10 (0.21)
Acetic bacteria (log UFC/mL)	5.80 (0.28)
Lactic bacteria (log UFC/mL)	6.20 (0.14)
Ethanol (g/L)	4.53 (0.066)
Sucrose (g/L)	13.22 (0.221)
Glucose (g/L)	4.24 (0.079)
Fructose (g/L)	8.54 (0.188)

SD: Standard Deviation.

Each participant received seven weekly bottles containing 200 mL of black tea kombucha. They were instructed to consume one bottle per day over the eight weeks at any time of the day. A diary of kombucha consumption was carried out using a form for daily recording. Participants who stopped consuming kombucha for three consecutive days were excluded from the study.

2.4. Assessment of Cardiometabolic Markers

In this study, the cardiometabolic markers were considered the primary outcomes. The biochemical markers were analyzed with a Mindray/BS-200® Chemistry Analyzer (Shenzhen, China) following the methodology of the commercial kits (Bioclin Bioquímica). The markers evaluated were the plasma concentration of glucose, insulin, total cholesterol (TC), LDL-C, HDL-C, triglyceride (TG), creatinine, urea, liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP). Insulin resistance was assessed by calculating the

Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) [31] and the TyG index (Triglyceride/Glucose index) [32]. The Fatty Liver Index (FLI) relative to the liver fat content was calculated using the algorithm recommended by Bedogni et al. [33].

2.5. Assessment of Physical Activity Pattern and Food Intake

Participants were also instructed to maintain their usual diet and physical activity patterns throughout the study. The participants' physical activity patterns were assessed using the International Physical Activity Questionnaire (IPAQ)—the short version—proposed in 1998 by the World Health Organization and validated for the Brazilian population [34]. Physical activity levels were classified according to the IPAQ categories: very active, active, irregularly active A, irregularly active B, and sedentary.

The participants' eating behaviors were evaluated in the screening to investigate if the participant presented any restrictive or compulsive eating behavior according to the 21-item Three-Factor Eating Behavior Questionnaire (TFEQ-R21), translated and validated for the Brazilian population by Natacci and Ferreira Júnior [35].

A food frequency questionnaire (FFQ) was administered by trained nutritionists to assess an individual's usual food consumption before and after the intervention; the FFQ applied in the end of intervention considered theintake during the last two months to analyze the quality of the diet. The consumption analysis of dietary surveys was performed using the ERICA-REC24h software(version 24_05_2022). When any food or food preparation was not included in this database, the Brazilian Food Composition Table (TBCA) from the University of São Paulo (USP) was used [36].

2.6. Diet Quality Indices

Dietary Inflammatory Index (DII®) scores were calculated according to the previously described, proposed, and validated method [37,38]. Briefly, the Z score of each DII component was calculated. The calculation of the Z score was obtained by subtracting the mean global consumption from the individual consumption and then dividing this result by the standard deviation of the mean global consumption. The global consumption averages and standard deviations presented by Shivappa et al., 2014 were used. The Z score was converted into a proportion (values from 0 to 1) to minimize the effect of asymmetry to the right. Subsequently, to achieve a symmetric distribution with values centered at 0 and limited to −1 (maximum anti-inflammatory) and +1 (maximum pro-inflammatory), each proportion was doubled and then 1 was subtracted. Finally, the centered proportion of each component was multiplied by its respective inflammatory effect, resulting in the final score for each parameter. After calculating all components individually, the final scores were added, resulting in the DII of each participant.

Dietary Total Antioxidant Capacity (DTAC) was estimated based on data published by Carlsen et al. [39], which contain TAC values determined via the Ferric Reducing Antioxidant Power (FRAP). An individual DTAC was calculated by multiplying the amount of food consumed by the corresponding FRAP value and subsequently adding all the values of food sources [40]. Food preparations containing more than one ingredient were disaggregated according to standard recipes, and the TAC value was calculated from the content of each ingredient. When the TAC values of raw food were unavailable, the values referring to botanically similar foods were used.

Dietary total polyphenols (DTP) was calculated through the content of polyphenols in foods presented in the Phenol-Explorer database version 3.6 (www.phenol-explorer.eu) [41,42]. Foods of animal origin or that did not contain polyphenols were excluded from the analysis. For consumed foods that could match with various entries in the Phenol-Explorer, the one that is most consumed in Brazil was chosen. The total polyphenol intake for each food was calculated by multiplying the total polyphenol content by the daily consumption of the food (in grams) divided by 100. The values determined using the Follin–Ciocalteu analytical method were considered for the total polyphenol content. When calculating polyphenols in processed foods that only appeared on the list in their raw form, their

Retention Factor (RF) was used to compensate for losses or gains of nutrients during food processing [41,43]. The total polyphenol intake for each subject was finally calculated as the sum of all individual polyphenol intakes from all food sources encountered.

2.7. Statistics

A Shapiro–Wilk test and graphical analysis were conducted to verify the normality of the quantitative variables. Means and standard deviations (SD) were used for variables with normal distribution, as well as the median, minimum, and maximum values for those without normal distribution.

A paired *t*-test (or Wilcoxon) was applied to compare differences between pre- and post-intervention for the same individual. Stratified analyses were conducted for each group considering the variation in the DII and DTAC. The index variations were obtained via subtraction between the values at the end and beginning of the study and then categorized into an increase (with a positive result) and a decrease (with a negative result).

Analyses were performed using SPSS software (IBM SPSS, v. 25, Armonk, NY, USA). In addition, the Jamovi 2.3.28 program was used to analyze the effect size. Cohen's d (d) or rank-biserial correlation (rrb) was used as an indicator of effect size when significant differences were observed. A statistical significance level (α) of 0.05 was adopted for all analyses.

3. Results

3.1. Subjects

Of the 62 individuals screened, 46 were selected to participate in the study—23 in each group. In total, 36 individuals completed the study, 20 without obesity (13 women and 7 men) and 16 with obesity (10 women and 6 men). The exclusion and abandonment reasons of participants are described in Figure 3. No differences between the gender distribution were observed (p = 0.88). The data from excluded participants were not considered in the statistical analysis. Significant age differences were observed (without obesity: 26.5 ± 3.5 years; with obesity: 35.4 ± 6.4 years, $p < 0.001$).

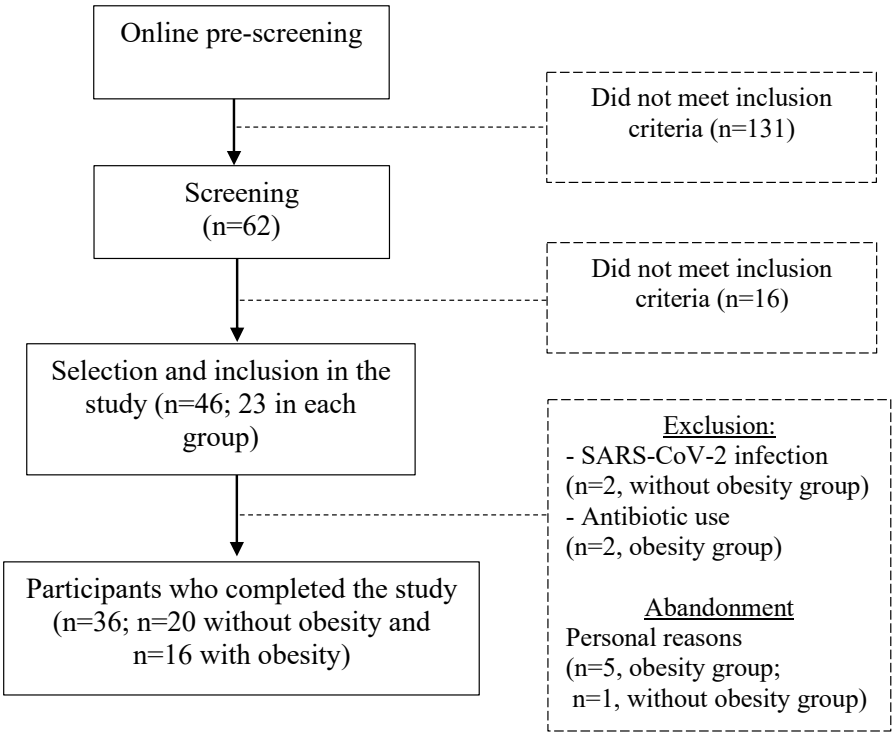

Figure 3. Flowchart of study participants selection. In the online pre-screening, 193 people responded to the form, and of these, 62 passed the screening eligibility criteria and were invited to a face-to-face

screening. Of these, 46 met the inclusion criteria and were selected to participate in the study, 23 in each group. In the group without obesity, two individuals were excluded due to suspected or confirmed infection of SARS-CoV-2, and one withdrew for personal reasons. In the obesity group, seven individuals did not complete all stages, two were excluded due to antibiotics use, and the others withdrew for personal reasons.

3.2. Cardiometabolic Parameters and Physical Activity

At baseline, the cardiometabolic parameters cholesterol, LDL-C, insulin, triglycerides, HOMA-IR, and TyG indices were statistically higher among participants with obesity compared to the other group, probably because obesity is associated with metabolic alterations. The other variables did not show significant differences between the groups (Table 2).

After the intervention, participants in the group without obesity increased total cholesterol ($p = 0.02$; $d = 0.552$, 95%CI: 0.074–1.018), ALP ($p = 0.04$; $d = 0.481$, 95%CI: 0.012–0.940), and DII ($p = 0.02$; $d = 0.600$, 95%CI: 0.116–1.071). The group with obesity showed a significant decrease in insulin ($p = 0.01$; $r_{rb} = -0.752$), HOMA-IR ($p = 0.02$; $r_{rb} = -0.769$), GGT ($p = 0.02$; $r_{rb} = -0.667$), and DII after their consumption of black tea kombucha ($p = 0.02$; $d = 0.631$, 95%CI: 0.084–1.161) (Table 2) (Figure 4).

The DTAC did not show a significant difference in any group when comparing the results at baseline and after eight weeks (Table 2). Regarding DTP, without considering the total phenolic content of the drink, there was a significant decrease ($p = 0.03$; $d = -0.532$, 95%CI: −0.995–−0.056) compared to the baseline in the group without obesity (Table 2). However, when considering the total phenolic content present in the consumed kombucha (0.686 mg/mL), the total dietary polyphenols did not show a significant difference after the intervention ($p = 0.97$) (Table 2).

Table 2. Comparison of cardiometabolic data and diet quality between participants at the beginning and end of the study, according to allocation group.

Variables	Without Obesity (n = 20)			With Obesity (n = 16)		
	Baseline	**After 8 Weeks**	***p*-Value**	**Baseline**	**After 8 Weeks**	***p*-Value**
Cardiometabolic markers						
FBG, mg/dL	88 (79–99)	87 (79–101)	0.2	91 (83–123)	92 (82–115)	0.89
Insulin, IU/L	5.3 (2.2–7.6) [a]	5.9 (2.7–8.7)	0.50	15 (6.3–23.6) [a]	12 (6–18)	**0.01**
TC, mg/dL	166.35 (32.92) [a]	177.05 (34.83)	**0.02**	189.94 (25.4) [a]	194.4 (22.1)	0.20
LDL-C, mg/dL	86.45 (26.56) [a]	90.80 (24.43)	0.25	104.19 (18.2) [a]	106.3 (17.2)	0.64
HDL-C, mg/dL	60.35 (13.81)	61.65 (12.65)	0.59	52.81 (16.2)	52.25 (16.5)	0.76
TG, mg/dL	67 (39–136) [a]	74 (36–195)	0.30	106 (62–298) [a]	97 (56–380)	0.30
ALT, U/L	17 (7–30)	16 (6–51)	0.23	21 (9–130)	20 (9–70)	0.23
AST, U/L	15 (9–22)	14 (10–25)	0.06	17 (9–57)	14 (7–32)	0.06
ALP, U/L	71.10 (18.0)	76.7 (15.8)	**0.04**	82.44 (16.0)	83.0 (21.1)	0.89
GGT, U/L	16 (8–58)	16 (8–61)	0.65	23 (9–142)	22 (8–108)	**0.02**
Creatinine, mg/dL	0.94 (0.09)	0.92 (0.12)	0.49	0.93 (0.14)	0.94 (0.16)	0.72
Urea, mg/dL	22 (16–44)	23 (15–36)	0.84	23 (15–39)	25 (12–48)	0.84
HOMA-IR	1.15 (0.48–1.67) [a]	1.30 (0.59–1.84)	0.52	3.75 (1.89–4.89) [a]	2.78 (2.34–3.60)	**0.02**
TyG	4.32 (4.07–4.76) [a]	4.37 (4.01–4.89)	0.41	4.64 (4.30–5.17) [a]	4.55 (4.27–5.41)	0.41
FLI	0.1 (0.02–0.7) [a]	0.9 (0.02–1.0)	0.10	11.3 (0.9–77.9) [a]	12.9 (0.4–84.8)	0.33
Diet quality						
DII	1.82 (1.28)	2.25 (1.02)	**0.02**	1.23 (0.96)	1.75 (1.31)	**0.02**
DTAC	5.84 (2.18–9.36)	4.77 (2.31–9.99)	0.07	5.64 (4.12–11.96)	5.57 (1.52–17.09)	0.80
DTP	1165.9 (449.4)	1031.1 (420.8)	**0.03**	1234.6 (414.9)	1219.9 (516.3)	0.91
DTP+KTP	1165.9 (449.4)	1168.3 (420.8)	0.97	1234.6 (414.9)	1357.1 (516.3)	0.34

Data expressed as means (SD) or medians (minimum–maximum). p-value: comparison between the same individual at the beginning and end of the study (paired *t*-test or Wilcoxon; $p < 0.05$). Significant differences are indicated in bold. [a]: baseline differences between groups (independent *t*-test or Mann–Whitney U test). ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; DII: Dietary Inflammatory Index; DTAC: Dietary Total Antioxidant Capacity; DTP: dietary total polyphenols; TC: total cholesterol; FBG: Fasting Blood Glucose; FLI: Liver Fat Index; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance; KTP: kombucha total polyphenols;; LDL: low-density lipoprotein; TG: triglycerides; TyG: Triglyceride/Glucose Index.

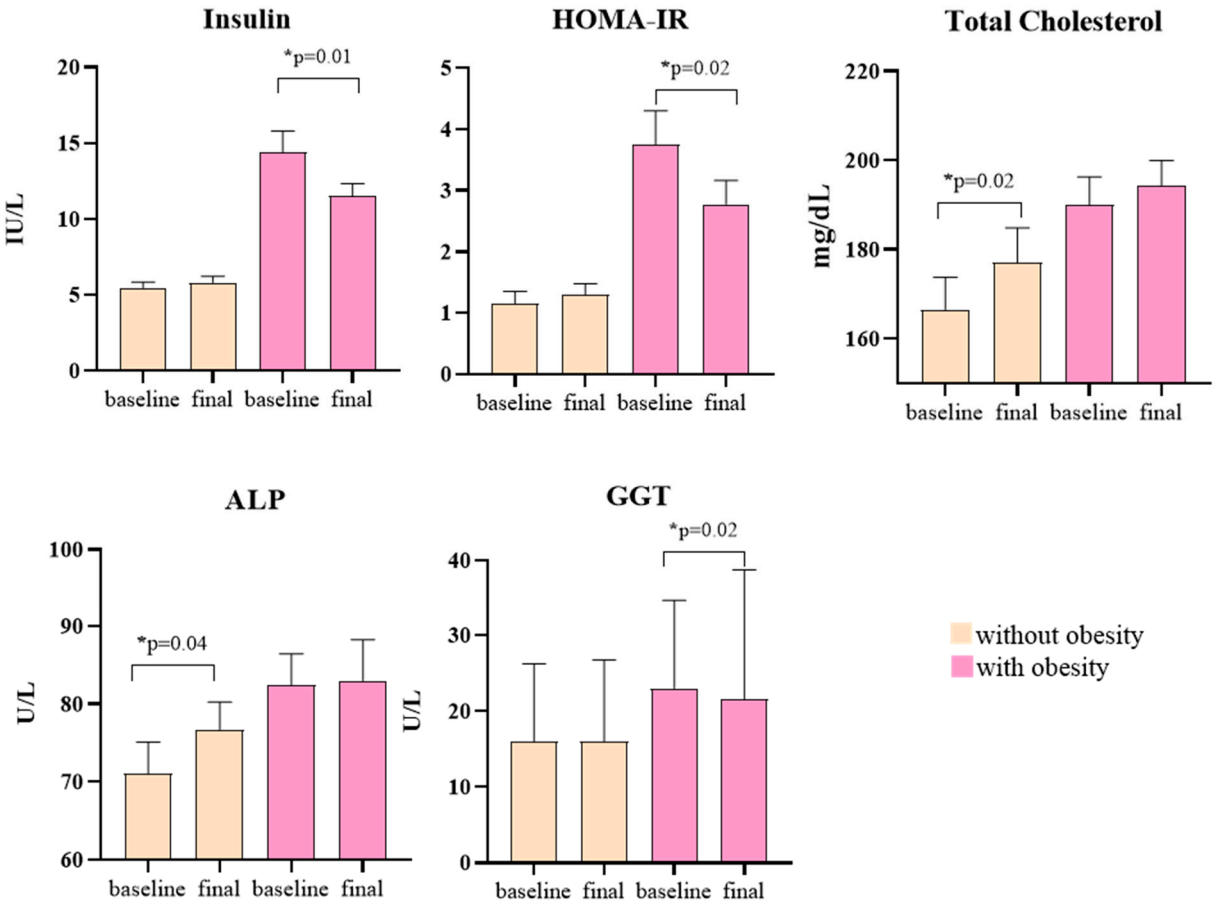

Figure 4. Illustration comparing cardiometabolic markers at the beginning and end of the study, according to allocation group (without and with obesity). Data expressed as means with SEM for total cholesterol, ALP, and GGT (normal distribution, paired *t*-test) and medians with interquartile range for insulin and HOMA-IR (without normal distribution, Wilcoxon test). Significance expressed by * $p < 0.05$. Orange color: participants without obesity and pink color: participants with obesity. ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase.

The IPAQ results indicated that 61.1% of the participants were active before the intervention, 16.7% were very active, 5.6% were irregularly active A, and 16.7% were irregularly active B. At the end of the eight weeks, only three individuals changed their physical activity pattern—two in the group without obesity and 1 in the obese group—with 13.9% of individuals being classified as very active, 13.9% as irregularly active B, and 5.6% as sedentary. The other categories maintained the pattern of physical activity.

3.3. Stratified Analysis by Group, Considering the Increase or Decrease in the Parameters of Diet Quality

Among individuals without obesity, those who showed an increase in DII score also experienced an increase in TC ($p = 0.004$; $d = 1.061$, 95%CI: 0.331–1.762) (Figure 5), TG ($p = 0.015$; rrb = 0.795), TyG index ($p = 0.04$; rrb = 0.667), and FLI ($p = 0.03$; rrb = 0.718), in addition to a lower DTAC ($p = 0.02$; rrb = −0.795) (Table 3).

In contrast, participants with obesity who decreased DII had lower HOMA-IR values ($p = 0.04$; rrb = −1.00) and a tendency to decrease insulin ($p = 0.08$) (Figure 5). Moreover, when they showed an increase in DII, no significant differences were observed in all variables (Table 3). Although a tendency to reduce insulin ($p = 0.07$) was shown, this emphasizes that independent of the DII variation, the insulin of participants with obesity tended to lower after the intervention (Figure 5).

Table 3. Comparison of diet quality and cardiometabolic markers of the groups, according to the variation in the Dietary Inflammatory Index (DII) and obesity status between the end and beginning of the study.

Variables	Without Obesity (n = 20)						With Obesity (n = 16)					
	↑ DII			↓ DII			↑ DII			↓ DII		
	Baseline	**Post 8 Weeks**	***p*-Value**	**Baseline**	**Post 8 Weeks**	***p*-Value**	**Baseline**	**Post 8 Weeks**	***p*-Value**	**Baseline**	**Post 8 Weeks**	***p*-Value**
FBG, mg/dL	85 (79–99)	86 (79–94)	0.96	88 (88–99)	87 (82–101)	0.07	91 (83–123)	92 (86–115)	0.96	85 (83–123)	92 (82–112)	0.90
Insulin, IU/L	5.2 (3.9–9.2)	6.3 (3.4–9.3)	0.21	6.3 (2.2–7.6)	4.9 (2.7–7.4)	0.67	10.6 (6–21)	10.5 (6–14)	0.07	17 (12–24)	13 (11–18)	0.08
TC mg/dL	163 (33)	179 (42)	**0.004**	172 (34)	174 (21)	0.84	193 (24)	195 (23)	0.69	185 (29)	194 (23)	0.14
LDL, mg/dL	87 (31)	91 (29)	0.39	86 (21)	91 (18)	0.47	109 (14)	111 (20)	0.82	96 (22)	99 (8)	0.68
HDL, mg/dL	59 (15)	60 (13)	0.56	63 (12)	64 (12)	0.80	54 (16)	52 (13)	0.32	52 (18)	54 (22)	0.63
TG, mg/dL	67 (39–136)	84 (45–195)	**0.02**	66 (42–126)	67 (36–116)	0.58	101 (62–298)	95 (56–270)	0.09	123 (74–269)	127 (79–380)	0.75
ALT, U/L	17 (9–30)	18 (11–51)	0.21	16 (7–27)	13 (6–21)	0.17	20 (9–44)	18 (11–32)	0.39	26 (10–130)	28 (9–70)	0.42
AST, U/L	15 (9–20)	16 (10–25)	0.13	14 (12–22)	14 (10–23)	0.73	17 (9–23)	13 (8–19)	0.07	18 (10–57)	20 (7–32)	0.28
ALP, U/L	76 (20)	83 (14)	0.11	64 (12)	67 (14)	0.25	87 (16)	90 (21)	0.61	75 (14)	72 (18)	0.72
GGT, U/L	19 (8–58)	21 (10–61)	0.65	12 (8–45)	13 (8–43)	1.00	22 (9–49)	21 (8–60)	0.11	28 (15–142)	29 (11–108)	0.10
Cr, mg/dL	0.9 (0.1)	0.9 (0.1)	0.37	0.1 (0.1)	0.9 (0.1)	0.22	0.9 (0.1)	0.9 (0.1)	0.49	1.0 (0.2)	0.9 (0.2)	0.76
Urea, mg/dL	21 (16–33)	24 (15–36)	0.59	23 (18–44)	22 (17–28)	0.23	24 (19–33)	26 (12–34)	0.74	21.5 (15–39)	23 (18–48)	0.27
HOMA-IR	1.12 (0.84–1.24)	1.38 (0.68–1.85)	0.20	1.40 (0.48–1.67)	1.15 (0.59–1.53)	0.61	3.56 (1.89–4.89)	2.61 (2.34–3.21)	0.13	3.76 (2.68–4.89)	2.92 (2.68–3.56)	**0.04**
TyG Index	4.3 (4.1–4.8)	4.4 (4.1–4.9)	**0.04**	4.3 (4.1–4.7)	4.3 (4.0–4.6)	0.33	4.6 (4.3–5.2)	4.5 (4.3–5.0)	0.11	4.6 (4.4–5.1)	4.7 (4.5–5.4)	0.60
FLI	0.10 (0.04–0.71)	0.11 (0.06–1.00)	**0.03**	0.06 (0.02–0.19)	0.08 (0.02–0.12)	0.67	10 (0.93–52.87)	7.5 (0.38–43.07)	0.29	27.83 (9.05–77.93)	23.0 (8.87–84.81)	0.75
DTAC	6.26 (3.10–9.36)	4.56 (2.33–9.99)	**0.02**	4.51 (2.18–7.16)	5.06 (2.31–8.46)	0.58	5.15 (4.12–11.96)	4.44 (1.52–10.73)	0.39	6.51 (4.91–9.68)	8.03 (4.89–17.09)	0.12
DTP	1150.3 (340.8)	980.6 (312.3)	0.06	1189.2 (603.9)	1106.9 (562.5)	0.29	1364.9 (429.3)	1170.7 (537.5)	0.19	1017.5 (307)	1302 (516.3)	0.22

Data expressed as means (SD) or medians (minimum–maximum). *p*-value: comparison between the same individual at the beginning and end of the study (paired *t*-test or Wilcoxon; $p < 0.05$) according to the variation in the Diet Inflammatory Index after the intervention. Statistically significant differences are indicated in bold. ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Cr: creatinine; DII: Dietary Inflammatory Index; DTAC: Dietary Total Antioxidant Capacity; DTP: dietary total polyphenols; TC: total cholesterol; FBG: fasting blood glucose; FLI: Liver Fat Index; GGT: gamma-glutamyl transferase; HDL: high-density lipoprotein; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance; DII: Dietary Inflammatory Index;; LDL: low-density lipoprotein; TG: triglycerides; TyG: Triglyceride/Glucose Index. ↑: increase compared to the initial and final values of the study; ↓: decrease compared to the initial and final values of the study.

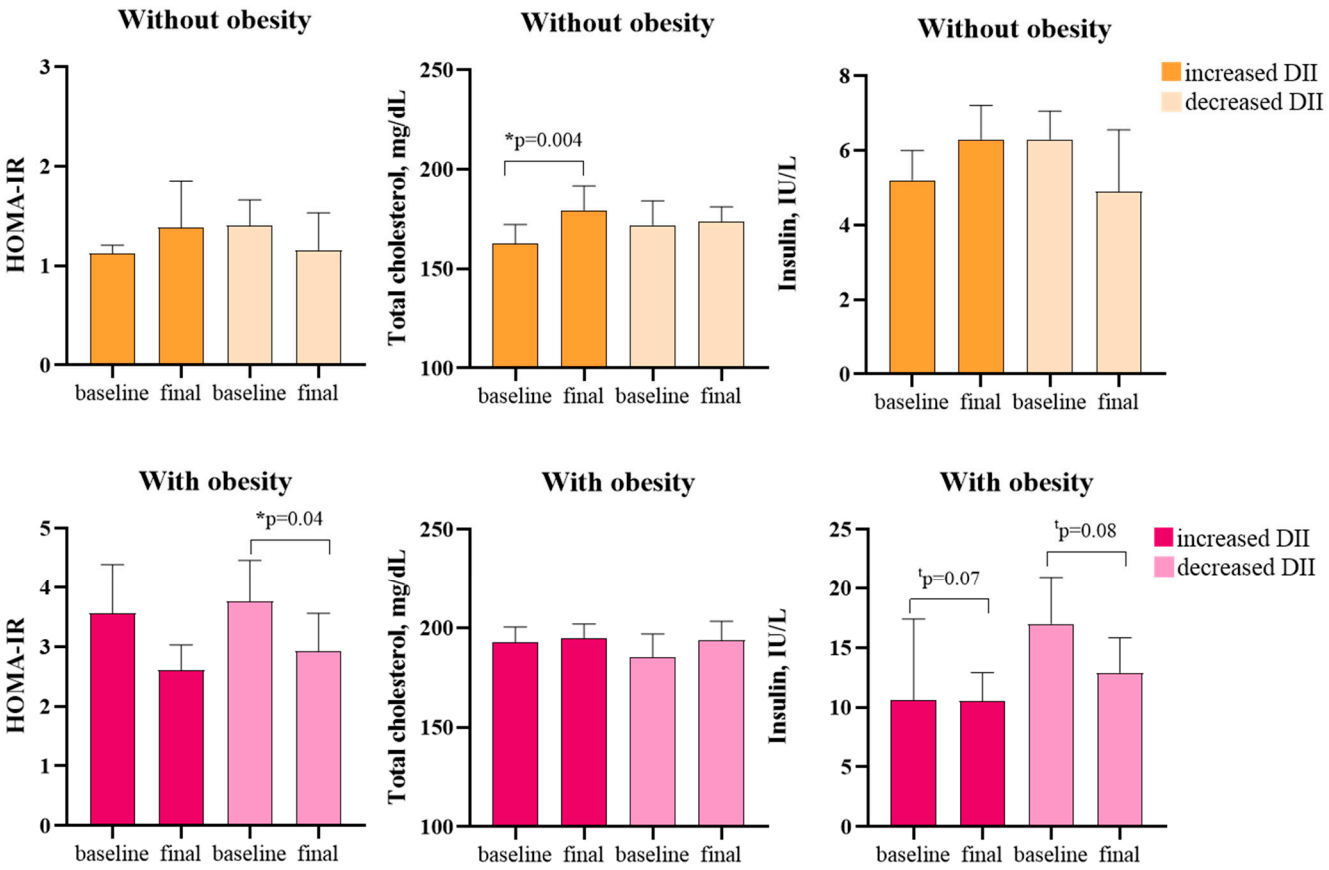

Figure 5. Illustration comparing main cardiometabolic markers, according to the variation in the Dietary Inflammatory Index (DII) and obesity status between the end and beginning of the study. Data expressed as means with SEM for total cholesterol (normal distribution, paired *t*-test) and medians with interquartile range for insulin and HOMA-IR (without normal distribution, Wilcoxon test). Significance expressed by * $p < 0.05$, *t* = tendency < 0.1. Orange/beige color: participants who increased/decreased DII. Pink/light-pink color: participants who increased/decreased DII.

Regarding the variation in DTAC, those in the group without obesity who decreased DTAC had lower DTP ($p = 0.005$; $d = -0.856$, 95%CI: −1.44–−0.250) and higher values for TC ($p < 0.001$; $d = 0.800$, 95%CI: 0.204–1.374) (Figure 6), TG ($p = 0.04$; rrb = 0.600), and DII ($p = 0.001$; rrb = 0.817) (Table 4).

In the group with obesity, participants who increased DTAC had lower GGT ($p = 0.04$; rrb = −0.238) and a tendency for lower values of HOMA-IR ($p = 0.07$) (Figure 6). On the other hand, those who worsened the antioxidant capacity of their diet had higher values of ALP ($p = 0.04$; $d = 0.761$, 95%CI: 0.035–1.455) and DII ($p = 0.002$; $d = 1.382$, 95%CI: 0.481–2.246) and lower values of DTP ($p = 0.007$; $d = -1.100$, 95%CI: −1.878–−0.285) (Table 4). Moreover, independent of the alterations in DTAC, participants with obesity tended to reduce insulin, the same pattern that occurred in the DII stratification (Figure 6).

Table 4. Comparison of diet quality and cardiometabolic markers of the groups, according to the variation in the Dietary Total Antioxidant Capacity (DTAC) and obesity status between the end and beginning of the study.

Variables	Without Obesity (n = 20)						With Obesity (n = 16)					
	↑ DTAC			↓ DTAC			↑ DTAC			↓DTAC		
	Baseline	**After 8 Weeks**	***p*-Value**	**Baseline**	**After 8 Weeks**	***p*-Value**	**Baseline**	**After 8 Weeks**	***p*-Value**	**Baseline**	**After 8 Weeks**	***p*-Value**
Gl, mg/dL	88 (83–99)	88 (82–101)	0.72	88 (79–99)	85 (79–94)	0.44	85 (83–123)	90 (82–112)	0.60	93 (83–123)	92 (86–115)	0.95
Insulin, IU/L	6.0 (2.7–7.6)	4.2 (2.7–7.1)	0.27	5.3 (2.2–9.2)	6.2 (3.4–9.3)	0.16	17 (6.3–23.6)	13 (6–18)	0.14	13 (8.4–21.3)	11 (6.4–14.3)	0.07
TC mg/dL	188 (20)	188 (19)	0.97	159 (34)	173 (38)	**<0.001**	176 (21)	181 (12)	0.50	198 (25)	203 (23)	0.31
LDL-C, mg/dL	102 (11)	102 (15)	0.98	81 (28)	87 (26	0.17	95 (17)	97 (8)	0.83	110 (17)	112 (19)	0.68
HDL, mg/dL	66 (12)	67 (10)	0.86	58 (14)	60 (13)	0.54	53 (19)	52 (14)	0.75	53 (15)	53 (19)	0.93
TG, mg/dL	75 (50–98)	62 (42–94)	0.35	62 (39–136)	79 (36–195)	**0.04**	118 (72–167)	92 (56–224)	0.46	106 (62–298)	100 (58–380)	0.44
ALT, U/L	18 (7–19)	13 (6–19)	0.34	16 (8–30)	17 (8–51)	0.38	29 (10–130)	26 (12–142)	0.23	20 (9–44)	18 (9–32)	0.68
AST, U/L	15 (13–20)	14 (12–22)	1.00	15 (9–22)	15 (10–25)	0.22	17 (13–57)	16 (7–32)	0.08	17 (9–23)	14 (8–24)	0.40
ALP, U/L	70 (7)	73 (13)	0.53	71 (21)	78 (17)	0.06	79 (18)	67 (13)	0.20	85 (15)	92 (20)	**0.04**
GGT, U/L	12 (10–24)	14 (8–26)	0.48	17 (8–58)	18 (10–61)	0.89	23 (12–142)	22 (9–108)	**0.04**	23 (9–49)	22 (8–60)	0.17
Cr, mg/dL	0.9 (0.1)	0.8 (0.1)	0.21	0.9 (0.1)	0.9 (0.1)	0.90	0.9 (0.1)	0.8 (0.1)	0.41	0.9 (0.2)	1.0 (0.2)	0.10
Urea, mg/dL	23 (21–44)	21 (18–27)	0.14	20 (16–34)	25 (15–36)	0.50	22 (15–27)	23 (15–29)	0.75	25 (18–39)	26 (12–48)	0.95
HOMA-IR	1.30 (0.59–1.65)	1.05 (0.59–1.54)	0.27	1.12 (0.48–1.66)	1.34 (0.68–1.85)	0.17	3.89 (3.75–4.89)	3.24 (2.76–3.56)	0.07	3.37 (1.89–4.89)	2.64 (2.33–3.21)	0.12
TyG Index	4.37 (4.19–4.54)	4.29 (4.07–4.51)	0.13	4.26 (4.07–4.76)	4.44 (4.01–4.89)	0.13	4.59 (4.36–4.97)	4.49 (4.27–4.91)	0.46	4.65 (4.30–5.17)	4.61 (4.35–5.41)	0.72
FLI	0.07 (0.03–0.13)	0.05 (0.02–0.13)	0.35	0.07 (0.02–0.71)	0.10 (0.02–1.00)	0.05	22.1 (0.9–52.9)	19.6 (0.4–43.1)	0.35	10.72 (4.86–77.93)	10.39 (3.76–84.81)	0.72
DII	2.34 (1.32)	2.31 (0.79)	0.92	1.43 (1.36)	2.21 (1.09)	**0.001**	0.77 (0.88)	0.38 (1.34)	0.20	1.27 (1.19)	2.34 (1.10)	**0.002**
DTP	1036.4 (502.3)	1123.8 (549.9)	0.18	1209 (440.5)	1000 (387)	**0.005**	1218.2 (332.9)	1628.2 (359.2)	0.12	1244.5 (474.4)	975 (441.3)	**0.007**

Data expressed as means (SD) or medians (minimum–maximum). *p*-value: comparison between the same individual at the beginning and end of the study (paired *t*-test or Wilcoxon; $p < 0.05$) according to the variation in the dietary antioxidant capacity after the intervention. Significant differences are indicated in bold. ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Cr: creatinine; DII: Dietary Inflammatory Index; DTAC: Dietary Total Antioxidant Capacity; DTP: dietary total polyphenols; TC: total cholesterol; ALP: alkaline phosphatase; FBG: fasting blood glucose; FLI: Liver Fat Index; GGT: gamma-glutamyl transferase; HDL-C: high-density lipoprotein; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance; DII: Dietary Inflammatory Index; BMI: Body Mass Index; LDL-C: low-density lipoprotein; TG: triglycerides; TyG: Triglyceride/Glucose Index. ↑: increase compared to the initial and final values of the study; ↓: decrease compared to the initial and final values of the study.

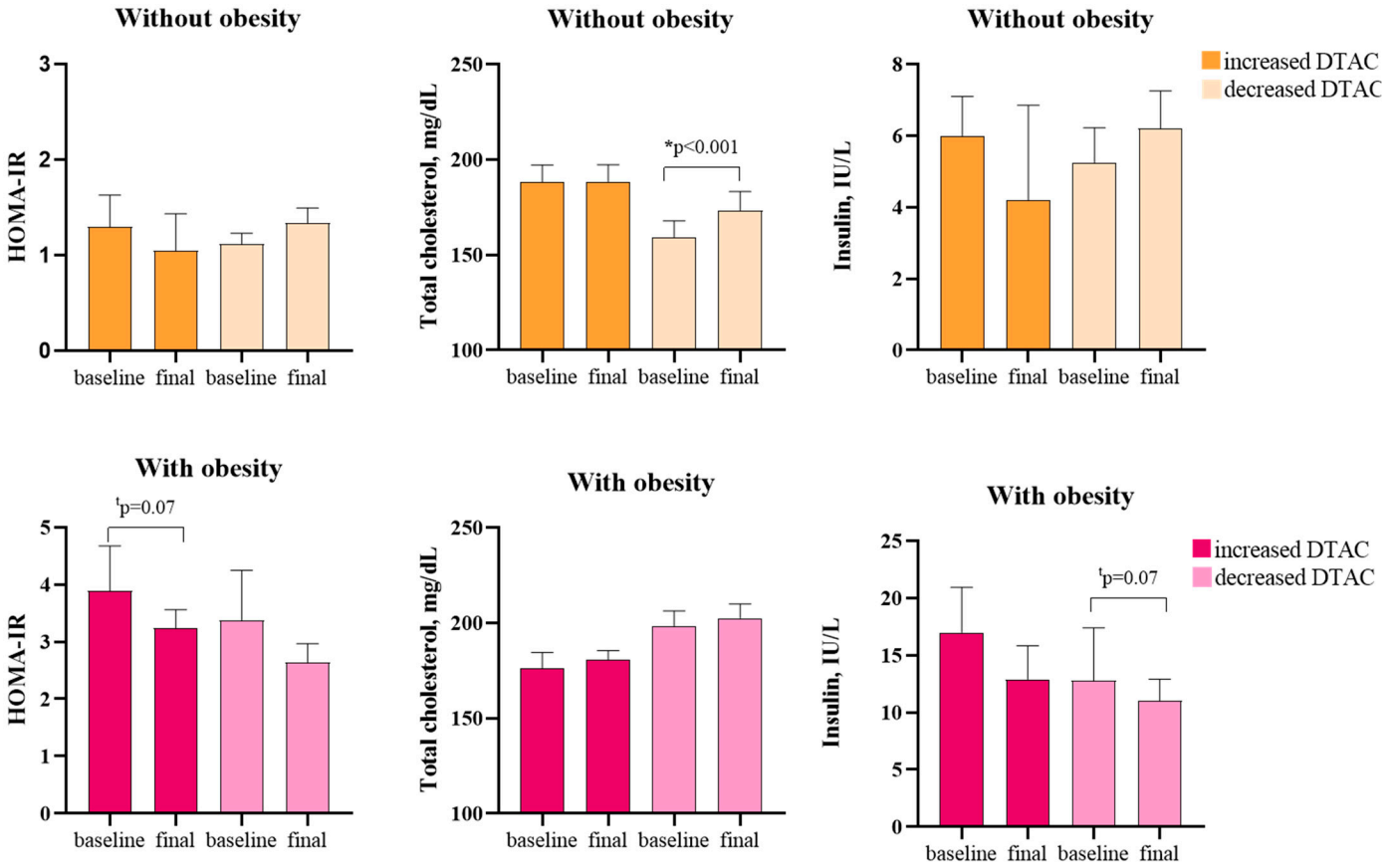

Figure 6. Illustration comparing main cardiometabolic markers, according to the variation in the Dietary Total Antioxidant Capacity (DTAC) and obesity status between the end and beginning of the study. Data expressed as means with SEM for total cholesterol (normal distribution, paired *t*-test) and medians with interquartile range for insulin and HOMA-IR (without normal distribution, Wilcoxon test). Significance expressed by * $p < 0.05$, t = tendency < 0.1. Orange/beige color: participants who increased/decreased DTAC. Pink/light-pink color: participants who increased/decreased DTAC.

4. Discussion

Kombucha is sold as a functional product with probiotic and antioxidant properties worldwide; however, there are few clinical trials that support it so far [23,24]. Thus, we wanted to evaluate whether the regular consumption of kombucha within the context of a habitual diet and lifestyle would be able to affect the health of the participants. In this manner, data from this study indicate that the consumption of black tea kombucha positively impacted the metabolic health of individuals with obesity as we hypothesized. Regardless of the variations in the diet quality, participants with obesity showed a significant decrease in insulin, HOMA-IR, and the liver enzyme GGT after drinking the beverage. However, the same did not happen with eutrophic participants since worsening in some of the cardiometabolic markers, such as total cholesterol occurred. But when we analyzed the effect of kombucha consumption considering the quality of the diet of these individuals, the results showed us that those participants without obesity and with the increased dietary inflammatory potential and decreased Dietary Total Antioxidant Capacity were the ones who presented worsening in their cardiometabolic markers after the intervention. One possible explanation for the changes in the cardiometabolic results in eutrophic individuals may be due to the variation in eating patterns and not the kombucha intake. This suggests that the beverage failed to limit the increase in cardiometabolic markers in the participants who worsened their diet quality.

Corroborating the results of this research, an experimental study with 36 albino rats observed that the consumption of black tea kombucha promoted a decrease in insulin

concentrations in the animals with induced diabetes and with greater weight compared to healthy rats [44]. To our knowledge, only two clinical trials evaluated the impact of kombucha consumption on human health. In a randomized controlled pilot investigation, kombucha's intake (240 mL/day) presented an anti-hyperglycemic effect with a significant decrease in fasting blood glucose after 4 weeks compared to in individuals with type 2 diabetes [23]. Furthermore, Atkinson et al. [24] found that kombucha reduced glycemic and insulin indices in healthy subjects after they consumed a standardized high-glycemic-index meal. This demonstrates that kombucha may indeed be an emerging agent in glucose metabolism.

The rich composition of kombucha has been identified as responsible for its health benefits since the fermentation process enriches the tea's nutritional composition, resulting in a beverage rich in bioactive compounds [45]. The improvement in cardiometabolic parameters after the consumption of the drink in participants with obesity can be explained by several metabolic mechanisms that induce liver detoxification and the attenuation of oxidative stress and inflammation in obesity [25]. Therefore, the action of organic acids produced after fermentation, such as acetic acid, glucuronic, and D-saccharic acid-1,4-lactone (DSL), mainly in the liver, may explain the improvement in the liver enzyme GGT in the participants with obesity in this study. These organic acids present in kombucha are evidenced to reverse the significant increase in liver enzymes, including GGT, and promote hepatoprotective effects via glucuronidation and the inhibition of beta-glucuronidase [44,46–50].

The phenolic compounds found in kombucha are also relevant components that may explain the bioactive effect of the drink. They are involved in the modulation of adipose tissue metabolism and the regulation of insulin resistance through their interaction with cell signaling pathways, the regulation of transcription factor activity, and, consequently, the modulation of epigenetic factors such as micro-RNAs (miRNAs) [51,52]. Finally, phenolic compounds, together with microorganisms in the beverage, may act as pro- and prebiotics and exert an anti-obesity effect by modulating the intestinal microbiota [18,19,53].

The ideal intake of phenolic compounds in humans necessary to enjoy their benefits is not established. However, it is known that, in addition to the daily amount ingested, its effect also depends on the bioavailability of the metabolites produced by the intestinal microbiota and in the tissues [54]. The amount of total phenolic compounds presents in 200 mL of black tea kombucha in this study contributed to an increase of 137.2 mg in the total phenolics ingested daily. It also collaborated with an increment of 0.236 g and 3.76 g/200 mL in theaflavin and thearubigin, respectively. The group without obesity had a 12% decrease in the dietary total polyphenols (DTP) at the end of the intervention, evidencing a lower consumption of foods rich in these compounds compared to the baseline. When analyzing the DTP, considering the amount present in the drink, an important compensation was observed in individuals without obesity. Thus, it is crucial to maintain a dietary pattern rich in polyphenols through various foods, such as fruits and vegetables, in addition to the consumption of kombucha.

The benefits of kombucha in animal studies, in which feeding is closely supervised, are well established. Nevertheless, in clinical studies with humans, controlling the dietary pattern during the experiment is challenging since each individual has a specific eating habit, which is susceptible to external influences. Therefore, considering indices that reflect global dietary patterns in clinical trials that study isolated dietary components in obesity is necessary not only to eliminate confounding factors in the research but also to contribute with a broader view of the various factors linked to each individual. The participants of this study had changes in their diet after the intervention despite the orientation to maintain the usual dietary pattern, which impacted the results regarding kombucha consumption.

The consumption of kombucha per se in individuals without obesity did not neutralize the harmful effects of a diet with high inflammatory potential. In fact, a more pro-inflammatory diet is associated with a higher prevalence of excess weight, in addition to the increased risk of developing cardiovascular diseases, liver disorders, and cancer [55–58].

In individuals with obesity, kombucha consumption combined with an improvement in the inflammatory potential of the diet directly impacted HOMA-IR positively. Indeed, DII is positively associated with HOMA-IR and presents increased odds of insulin resistance and the development of prediabetes [59]. When evaluating the results of kombucha consumption considering the DTAC, it is observed that all individuals who decreased DTAC presented negative alterations in the cardiometabolic parameters. The DTAC is considered a good tool for assessing the quality of the population's diet, and studies have shown that DTAC is associated with reducing CVD risk factors [60,61]. In fact, a dietary pattern composed of antioxidant foods rich in phenolic compounds helps fight metabolic alterations by increasing plasma antioxidant capacity and mitigating oxidative stress in people with metabolic disturbances [62].

Despite these results, generalizations should be interpreted with caution; this work adhered to a careful nonrandomized quasi-experimental design, evaluating the effect of kombucha on a pre–post-intervention. When this type of study is chosen, it is important to minimize the risk of the existence of bias to increase the confidence of the findings [63]. Furthermore, quasi-experimental studies have better external validity than randomized control trials (RCTs), being more generalizable than RCTs. However, randomization and blinding were not used, which limits the causal effect of the intervention [64]. Moreover, a more extended intervention period is needed to confirm the effects of black tea kombucha on human health.

5. Conclusions

In conclusion, the regular intake of black tea kombucha appears to benefit the metabolic health of individuals with obesity by reducing key cardiometabolic markers, potentially improving their overall health status. This consumption, when combined with the enrichment of diet quality through anti-inflammatory and antioxidant foods, also enhanced these cardiometabolic markers, with a great tendency for insulin.

In contrast, in individuals without obesity, the worsening of the diet quality resulted in an increase in certain cardiometabolic parameters. This indicates that consuming black tea kombucha alone was insufficient to counteract the harmful effects of a diet with higher inflammatory potential and lower antioxidant capacity in this group. Therefore, combining a healthy eating pattern rich in antioxidant and anti-inflammatory foods may be necessary to enjoy the bioactive effect of black tea kombucha in eutrophic individuals.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/fermentation10080384/s1: Table S1: Transparent Reporting of Evaluations with Nonrandomized Designs (TREND).

Author Contributions: G.M.F.: Conceptualization, Methodology, Formal Analysis, Investigation, Writing—Original Draft, and Visualization. M.A.C.C.: Conceptualization, Methodology, Investigation, Writing—Review and Editing. R.R.C.: Conceptualization, Investigation, Writing—Review and Editing. J.R.H.: Software, Formal Analysis, and Writing—Review and Editing. L.Z.: Software, Formal Analysis, and Writing—Review and Editing. V.C.: Methodology, Resources, and Funding Acquisition. A.G.: Methodology, Resources, and Funding Acquisition. F.I.M.: Writing—Review and Editing, and Supervision. F.A.R.B.: Conceptualization, Methodology, Resources, Writing—review and Editing, Supervision, Project Administration, and Funding Acquisition. J.B.: Conceptualization, Methodology, Resources, Writing—Review and Editing, Supervision, Project Administration, and Funding Acquisition. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This project was approved by the Human Research Ethics Committee of the Federal University of Viçosa (UFV) and the National Research Ethics Committee–CONEP

(number: 3.948.033). The procedures described were established under Resolution CNS/466 of 2012 and the Declaration of Helsinki. This project was also registered with the Brazilian Registry of Clinical Trials (REBEC) (number: U1111-1263-9550).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: The data that support the findings of this study are available upon request from the corresponding author. The data are not publicly available because they contain information that could compromise the privacy of the research participants.

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Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. However, James R. Hébert wishes to disclose that he owns a controlling interest in Connecting Health Innovations LLC (CHI), a company that has licensed the right to his invention of the Dietary Inflammatory Index (DII®) from the University of South Carolina in order to develop computer and smartphone applications for patient counseling and dietary intervention in clinical settings. The subject matter of this paper will not have any direct bearing on that work, nor has that activity exerted any influence on this project.

Appendix A

A description of the kombucha characterization analysis is described in Appendix A.

Appendix A.1. Total and Volatile Acidity and pH

The pH was determined by a previously calibrated pH meter (Kasvi, K39-1014B, China) in all samples. The total acidity of kombucha was determined by titration with a standard 0.01 M sodium hydroxide solution, using phenolphthalein as an indicator [65]. The result was expressed as % acetic acid (w/v). The volatile acidity of the samples was performed by the titrimetric method with a 0.1 N sodium hydroxide (NaOH) solution in the presence of 1% phenolphthalein (m/v) in ethyl alcohol as an indicator. The sample was extracted through steam dragging using a TE-0871 volatile acidity tester from the manufacturer Tecnal (Piracivaba, Brazil), according to method 05 of the manual of methods of analysis of beverages and vinegar of the Ministry of Agriculture, Livestock and Supply—MAPA [66]. The result was expressed as milliequivalent acetic acid per kombucha liter (mEq/L).

Appendix A.2. Acetic Acid, Sugar, and Ethanol

The quantification of acetic acid, sugars (glucose, fructose, and sucrose), and ethanol was analyzed by High-Performance Liquid Chromatography (HPLC) (SHIMADZU, model LC-10A VP) coupled to a refractive index detector (RID 6A). 20 µL of a previously filtered sample (0.45 µm filter) was injected into an HPX-87P column (BIORAD, diameter 30 cm × 4.5 mm). Ultrapure water was used as the mobile phase. The flow rate was set to 0.6 mL/min, and the column temperature was 80 °C. Standards of the analyzed compounds were used for identification (retention time) and quantification (external standard). The results were expressed in g/L.

Appendix A.3. Total Phenolics, Theaflavin and Thearubigin

The concentration of total phenolics in kombucha was determined by the colorimetric method of Follin-Ciocalteu, using gallic acid as the standard [67]. Theaflavin and thearubigin concentrations in kombucha samples were determined by spectrophotometer according to the method proposed by Jayabalan and Swaminathan [68]. Absorbance was measured in a spectrophotometer at 760 nm, and the results were expressed in mg of gallic acid equivalent per mL of kombucha (mg GAE/mL).

Appendix A.4. Microbiological Characterization

For counting acetic bacteria, serial dilutions of kombucha samples were plated on GYC agar (50 g/L glucose, 10 g/L yeast extract, 5 g/L calcium carbonate, and 20 g/L agar) and ethanol (70 mL/L). The lactic acid bacteria count was performed on MRS agar (De Man, Rogosa & Sharpe; Merck; Germany), using bromocresol (0.004%) as an indicator. Mesophilic bacteria were counted on PCA agar (Merck, Germany), and yeasts were counted on PDA agar (Merck, Germany). The plates were incubated at 30 °C for three days in aerobiosis, except for lactic bacteria counts, which were incubated in a microaerophilic medium. Results were expressed in colony-forming units per mL (CFU/mL).

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