Dietary Processing and Macronutrient Content Dissociate Adiposity from Metabolic Dysfunction in Wistar Rats

Pedro Rocha Tenório[1]
https://orcid.org/0000-0003-1812-4792

Gabriel Smolak Sobieski e Silva[1]
https://orcid.org/0000-0002-9944-3519

Débora Hipólito Quadreli[2]
https://orcid.org/0009-0000-8323-3712

Juliany Carolina Duma de Castro[1]
https://orcid.org/0009-0000-6527-4860

Glaura Scantamburlo Alves Fernandes[3]
https://orcid.org/0000-0002-6043-1131

Fábio Goulart de Andrade[4]*
https://orcid.org/0000-0002-0206-4986

[1]State University of Londrina, Department of Physiological Sciences, Londrina, Paraná, Brazil; [2]State University of Londrina, Department of Pathology Sciences, Londrina, Paraná, Brazil; [3]State University of Londrina, Department of General Biology, Londrina, Paraná, Brazil; [4]State University of Londrina, Department of Histology, Londrina, Paraná, Brazil.

HIGHLIGHTS

- Diet purification contributes to adipose tissue and weight gain.
- Diet purification alone does not induce metabolic damage.
- Diet composition is the main contributor to the development of metabolic syndrome.

Abstract: Obesity is a multifactorial condition influenced not only by diet composition but also by food processing. While high-fat and high-sugar diets are widely used in rodent models of metabolic syndrome, the independent effects of diet purification remain poorly understood. This study evaluated the impact of a grain-based control (C/GB), a balanced semi-purified (B/SP), and a high-fat/high-sugar semi-purified (HFS/SP) diet on obesity development and metabolic alterations in adult male Wistar rats over 10 weeks. Morphometric, biochemical, histological, metabolic, and oxidative parameters were assessed. Rats fed the B/SP diet exhibited greater body weight and adiposity despite similar caloric intake, yet these changes were largely compensatory, with enhanced fat mobilization, redistribution toward subcutaneous depots, and improved antioxidant defenses. In contrast, HFS/SP-fed rats consumed fewer calories but developed visceral adiposity without additional body weight, in association with reduced fat mobilization and oxidation. Only the HFS/SP group displayed features of metabolic syndrome, including impaired glucose control, dyslipidemia, hepatic steatosis, and systemic as well as tissue oxidative stress. The liver emerged as a central organ mediating oxidative burden, reinforcing its key role in obesity-related metabolic impairment. In conclusion, our findings demonstrate that both nutrient composition and diet purification shape distinct obesity phenotypes, but only high-fat/high-sugar intake determines the risk of metabolic dysfunction.

Keywords: Semi-purified diet; High-fat diet; Obesity; Metabolic Syndrome; Oxidative Stress.

INTRODUCTION

The global prevalence of obesity has been steadily increasing since 1975 contributing to 8.9% of global mortality. Recognized as a pandemic by the World Health Organization in 1995, obesity is projected to affect 51% of the global population by 2030, thus posing one of the 21st century's most pressing public health challenges [1].

The most widely accepted pathophysiological mechanism of obesity involves excessive adipose tissue accumulation coupled with impaired oxidative capacity. This imbalance results in elevated production of reactive oxygen species, primarily within mitochondria and cellular membranes. Initially localized, oxidative stress can become systemic as reactive species disseminate via the bloodstream. In insulin-sensitive organs such as the liver, systemic OS disrupts metabolic homeostasis, impairs insulin signaling, and promotes hypercholesterolemia and insulin resistance. These metabolic disturbances establish a positive feedback loop that can culminate in steatotic liver disease and metabolic syndrome, including comorbidities such as type 2 diabetes and cardiovascular disease [2].

Obesity is a multifactorial condition, with dietary habits playing a crucial role in their pathogenesis. Excessive consumption of sugars and fats has been demonstrated to promote adipose tissue expansion and reactive species generation through glycation and lipotoxicity [3]. Ultra-processed food consumption has also been implicated in disease development, although the epidemiological evidence remains inconclusive [4]. Animal models frequently employed in the study of obesity and metabolic syndrome rely on purified diets, composed of standardized ingredients such as isolated proteins, refined carbohydrates, and purified fats, mimicking ultra-processed human diets and offering experimental reproducibility [5]. However, such diets are deficient in biologically active compounds, such as phytochemicals, that can modulate digestion and metabolism, thereby limiting their physiological representativeness and translational relevance [6].

Comparative studies of PDs and natural ingredient diets yield conflicting results. While some studies demonstrate that PDs induce metabolic harm [7], others report no adverse effects [8] or even improvements relative to chow diets [9]. Semi-purified diets, combining natural and refined ingredients, may offer greater translational relevance, though research remains scarce. Notably, no study has explored OS dynamics in SD-fed models [10, 11].

Although high-fat/high-sugar diets are widely used to induce metabolic dysfunction in rodents [3], it remains unclear whether food processing itself, independent of macronutrient excess, contributes to these pathologies. To address this knowledge gap, this study investigates whether food processing alone by comparing natural-ingredient and semi-purified balanced diets or in combination with high-fat/high-sugar content semi-purified hypercaloric diet promotes obesity, comorbidities, and oxidative stress in adult rats.

MATERIAL AND METHODS

Animal Housing and Management.

Given the lack of consensus on rodent obesity classification and the high inter-study variability in Wistar rat weight gain, a conservative estimate of a 25% excess weight gain over a 10-week experimental period was adopted as the benchmark [12]. This corresponds to a mean additional weight gain of 20 g in adult animals, based on the characteristics of the animals housed in our vivarium. Assuming a standard deviation of 10 g, with $\alpha = 0.05$ and statistical power set at 0.80, the minimum required sample size was calculated as $n = 4.3$ animals per group [13].

Fifteen 90-day-old male Wistar rats were randomly assigned to three dietary groups (n = 5 per group) using random sequence generation: a control commercial grain-based diet (C/GB) (Nuvilab CR1®, Quimtia, Brazil), a balanced semi-purified diet (B/SP), and a high-fat/high-sugar semi-purified diet (HFS/SP). The animals were housed in the same vivarium, in groups of 2-3 animals per cage, under controlled conditions (22 ± 2ºC, 12 h light/dark cycle) with *ad libitum* access to water and food for a period of 73 days.

On the 74th day, following a 6-8 hour fast, between 02:00 and 04:00 pm, the animals were anesthetized via intraperitoneal administration of ketamine (100 mg/kg) and xylazine (10 mg/kg). Blood samples were collected from the inferior vena cava into tubes containing a coagulation activator (FristLab, Brazil). The liver, inguinal and perirenal adipose tissues were dissected, washed in ice-cold PBS, fractioned, and fixed in 4% paraformaldehyde or snap frozen and stored in -80ºC. The collected blood was centrifuged at 12,000 × g for 10 minutes, after which the serum was separated and stored at -20°C.

Diets Composition

The semi-purified diets were prepared weekly in the laboratory. Both semi-purified diets contained 100 g of soy protein isolate, 25 g of wheat gluten, 25 g of dried whole egg, 100 g of maltodextrin, 100 g of degermed cornmeal, 50 g of wheat bran, 25 g of psyllium husk, 100 g of defatted coconut flour, 5 g of Brazilian nut flour, 25 g of brewer's yeast, and 40 g of a mineral and vitamin mix. The B/SP diet was supplemented with 474 g of cornstarch, whereas the HFS/SP diet included 100 g of sucrose and 155 g of lard. All ingredients were thoroughly mixed, oven-dried at 65°C for 72 hours and stored in sealed

containers. Nutrient composition was formulated in accordance with NRC-95G guidelines to prevent nutritional deficiencies [14]. Table 1 presents the detailed nutrient composition of diets.

Table 1. Nutritional composition of diets

Nutritional Facts	C/GB	B/SP	HFS/SP
Energy (kcal/kg)	3468	3697	4625
Carbohydrates (g/kg) (% kcal)	523 (66)	597 (71)	364 (36)
Added Sugars (g/kg) (% kcal)	0 (0)	0 (0)	117 (10)
Lipids (g/kg) (% kcal)	40 (10)	57 (14)	254 (49)
Protein (g/kg) (% kcal)	223 (24)	151 (15)	188 (15)
Fiber (g/kg)	67	72	86
Cholesterol (mg/kg)	0	397	673
Vitamin E (mg/kg)	27	47	60

[1] **Legend:** C/GB = Comercial grain base diet; B/SP = Balanced semi-purified diet; HFS/SP = High-fat/high-sugar semi-purified diet.

Murinometric and Dietary Parameters

Body weight, food intake, and water consumption were monitored twice-weekly using the residual intake method and adjusted for the number of animals per cage. Daily chow and water intake were calculated as the average consumption per measurement, with a total of 60 measurements each. Data pertaining to body weight and food intake were utilized to calculate food efficiency, defined as grans of body weight gain per kcal consumed. The adiposity index was calculated as the relative weight of adipose tissues divided by body weight, while the visceral/subcutaneous ratio was expressed as the ratio of perirenal adipose tissue to inguinal adipose tissue.

Serum Metabolic Profile and Liver Markers

To assess glucose homeostasis, fasting glucose was measured together with the TyG index as a marker of insulin sensitivity and fructosamine as a marker of medium-term glucose control. Free fatty acids and glycerol were determined as indicators of fat mobilization, ketone bodies as markers of fat oxidation, and triglycerides and cholesterol as markers of lipid homeostasis. Corticosterone was quantified due to its role in glucose and lipid homeostasis. Alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase were assessed as indicators of liver function.

Fasting glucose, serum fructosamine, triglycerides, glycerol, total cholesterol, HDLc, albumin, alanine aminotransferase, aspartate aminotransferase, and gamma glutamyl transferase were measured using commercial kits (Vida Biotecnologia, Brazil). Free fatty acids were measured using diphenyl carbazide method [15]. Ketone body concentration was determined after serum deproteinization by the conversion of β-Hydroxy butyric acid into acetoacetic acid using John's reagent and subsequent measurement with sodium nitro prussiate in an alkaline glycine solution [16]. The TyG index was calculated as the log of fasting glucose multiply by triglycerides divided by 2 [17]. Corticosterone concentrations were measured using NBT colorimetric method [18].

Tissues Histology, Metabolism, and Composition

Liver and adipose tissue fixed samples were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. The degree of hepatic steatosis, hepatic ballooning, and inflammation were assessed and scored accordingly. Mean adipocyte diameter was calculated from a minimum of 500 adipocytes per sample and tissue cellularity was subsequently estimated [19]. Histological images were captured using a BA310MET-T light microscope (Motic, China) equipped with a Moticam A5 digital camera and analyzed with ImageJ software (v1.53, National Institutes of Health, USA) using the Adiposoft plugin (v1.16).

Total lipoprotein lipase (LPL) and heparin-released LPL activity, considered the functional fraction of the enzyme involved in lipid uptake, together with lipolysis capacity, were measured as previously described [20] with minimal modifications. For heparin-released LPL, an additional incubation step with 5 U/mL heparin was included. Lipolysis was assessed using 2,3-dimercapto-1-propanol tributyrate as the substrate and 5,5-dithiobis-2-nitrobenzoic acid as the color reagent. 1 U equals 1μmol of free fatty acid released per minute.

To assess tissue composition, triglycerides, cholesterol, glycogen, and glucose were extracted from frozen liver samples using the Folch method, followed by digestion of residual tissue debris in 20% (w/v) KOH with heating. Triglycerides, cholesterol, and glucose were quantified using commercial kits, whereas glycogen content was determined by the phenol–sulfuric acid method.

Oxidative Markers

Serum and tissue samples homogenized in PBS (pH 7.4) were used to determine antioxidant capacity by ferric-reducing antioxidant power [21] and total free thiols [22], as well as oxidative stress markers, including total reactive oxygen species (modified FOX-1 assay) [23], lipid peroxidation [24], and protein carbonylation [25]

Statistical Analysis

Residual normality and homoscedasticity were assessed using the Shapiro-Wilk and Brown-Forsythe tests, respectively. When the assumptions were met, one-way ANOVA followed by Tukey's HSD post hoc test was used. In cases of heteroscedasticity, Welch's ANOVA with Games-Howell correction was applied. For data with non-normal residuals, log transformation was performed, followed by reassessment of residual normality and variance homogeneity; if assumptions were met, ANOVA or Welch's methods were applied accordingly. When normality was not achieved even after transformation, the Kruskal-Wallis test with Dunn's multiple comparisons post hoc was employed. Steatosis scores and MASH prevalence were analyzed using Fisher's exact test. All analyses were performed in RStudio (v4.2, Posit, USA) using the 'onewaytests' and 'ggplot2' packages. Data were presented as mean ± standard deviation. Statistical significance was set at $p<0.05$.

RESULTS

No animals were excluded in any analysis.

Murinometric and Dietary Parameters

Initial body weights were comparable across groups, confirming similar baseline conditions. Final body weight was significantly higher in the B/SP group compared to both C/GB and HFS/SP, with no difference between C/GB and HFS/SP (Fig. 1a). Marked differences in adipose tissue distribution were observed across dietary interventions. The adiposity index was significantly higher in animals fed semi-purified diets, with the greatest increase observed in B/SP (Fig. 1b). Moreover, the visceral to subcutaneous adipose ratio was significantly lower in B/SP compared to the other groups, indicating a shift toward subcutaneous fat deposition (Fig. 1c).

Figure 1. Effects of dietary interventions on morphometric parameters: Body weight gain throughout time (a), adiposity index (b), visceral-to-subcutaneous adipose tissue ratio (c). Diet groups: C/GB = commercial grain-based diet, B/SP = balanced semi-purified diet, HFS/SP = high-fat high-sugar semi-purified diet. Statistical analysis: One-way ANOVA or two-way ANOVA with repeated measures with Tukey's HSD post hoc. Data are presented as box plots as median and interquartile ranges; white dots indicate group means. [a] indicates $p<0.05$ of C/GB vs B/SP; [b] indicates $p<0.05$ of B/SP vs HFS/SP.

Water intake was significantly lower in animals fed SP diets (Fig. 2a). The HFS/SP group demonstrated the lowest total and mean daily chow intake when compared to both the C/GB and B/SP groups (Fig. 2b). Although caloric intake was similar between C/GB and B/SP, food efficiency was significantly higher in B/SP animals; in contrast, no significant difference in food efficiency was observed between the C/GB and HFS/SP groups (Fig. 2c and d).

Figure 2. Dietary parameters among different diets. Diet groups: C/GB = commercial grain-based diet, B/SP = balanced semi-purified diet, HFS/SP = high-fat high-sugar semi-purified diet. Statistical analysis: One-way ANOVA with Tukey's HSD post hoc, Welch's ANOVA with Games–Howell correction or Kruskal-Wallis with Dunn's multiple comparisons post hoc. Data are presented as box plots as median and interquartile ranges; white dots indicate group means.

Serum Metabolic Profile and Liver Markers

Markers of glucose control, including fasting blood glucose, fructosamine, and the TyG index, were elevated in the HFS/SP group, along with lipid metabolism markers such as triglycerides, total cholesterol,

and corticosterone levels, while a reduction in glycerol, free fatty acids, and ketone bodies was observed. In contrast, the B/SP diet reduced the TyG index but had no effect on other glycemic control parameters. However, it modulated lipid metabolism, as evidenced by decreased triglyceride levels and increased circulating glycerol, free fatty acids, ketone bodies, and total cholesterol. Hepatic injury markers are reduced in semi-purified diets independent of diet composition, except the more sensitive marker alanine aminotransferase, which was elevated in the HFS/SP group compared to the B/SP group (Table 2).

Table 2. Serum metabolic panel

Metabolic Parameters	C/GB	B/SP	HFS/SP
Fasting Glucose (mmol/L)	6.79 ± 0.16	6.83 ± 0.45	7.65±0.52 [a, b]
Fructosamine (µmol/g of alb.)	1.08 ± 0.12	1.14 ± 0.23	2.01 ± 0.17 [a, b]
TyG index	3.89 ± 0.07	3.55 ± 0.04 [a]	4.10 ± 0.05 [a, b]
Triglycerides (mmol/L)	1.45 ± 0.25	0.66 ± 0.08 [a]	2.08 ± 0.25 [a, b]
Free Fatty Acids (mmol/L)	0.61 ± 0.07	0.69 ± 0.15	0.47 ± 0.07 [b]
Glycerol (mmol/L)	0.50 ± 0.02	0.77 ± 0.16 [a]	0.38 ± 0.03 [a, b]
Ketone Bodies (mmol/L)	0.41 ± 0.04	0.58 ± 0.10 [a]	0.30 ± 0.04 [b]
Total Cholesterol (mmol/L)	2.40 ± 0.39	3.31 ± 0.35 [a]	4.83 ± 0.67 [a, b]
HDLc (mmol/L)	1.16 ± 0.24	1.46 ± 0.36	1.21 ± 0.23
Non-HDLc (mmol/L)	1.24 ± 0.21	1.85 ± 0.56	3.62 ± 0.48 [a, b]
Corticosterone (µmol/L)	0.96 ± 0.11	0.99 ± 0.15	1.73 ± 0.33 [a, b]
Albumin (g/L)	38.64 ± 3.15	38.02 ± 3.18	38.39 ± 5.96
ALT (U/L)	32.29 ± 4.62	16.28 ± 2.97[a]	33.00 ± 9.05 [b]
AST (U/L)	64.68 ± 9.67	39.21 ± 6.12 [a]	37.20 ± 12.62 [a]
γGT (U/L)	12.88 ± 1.80	5.35 ± 2.06 [a]	5.73 ± 0.95 [a]

[2] **Legend:** C/GB = Commercial grain-based diet; B/SP = Balanced semi-purified diet; HFS/SP = High-fat/high-sugar semi-purified diet, ALT = Alanine transaminase; AST = Aspartate transaminase; γGT = Gamma-glutamyltransferase. Statistical analysis: One-way ANOVA with Tukey's HSD post hoc. a $p < 0.05$ compared to C/GB, b $p < 0.05$ compared to B/SP.

Tissues Histology, Metabolism and Composition

Semi-purified diets promoted adipose tissue accumulation at both sites; however, the relative weight of perirenal and inguinal adipose tissue was greater in the B/SP group compared to HFS/SP (Fig. 3a, g). Histological analysis revealed similar adipocyte hypertrophy in animals fed semi-purified diets regardless of diet composition. In contrast, only the high-fat, high-sugar diet altered tissue cellularity, inducing adipocyte hyperplasia in perirenal adipose tissue and causing a non-significant reduction in adipocyte number in inguinal adipose tissue (Fig. 3b, c, h, i). In perirenal adipose tissue, both total LPL and heparin-released LPL fractions were elevated in HFS/SP animals, accompanied by reduced lipolysis, whereas B/SP showed increased lipolysis (Fig. 3d–f). In B/SP, total LPL was elevated without a corresponding increase in heparin-released LPL or lipolysis; conversely, HFS/SP exhibited an increase only in heparin-released LPL, along with reduced lipolysis (Fig. 3j–l).

Liver weight did not differ among groups; however, biochemical analysis revealed a significant accumulation of triglycerides, cholesterol, and glycogen, along with a non-significant increase in glucose, in HFS/SP animals (Fig. 3m–q). Histological analysis corroborated these findings, showing extensive areas of mixed macrovesicular and microvesicular steatosis and hepatocyte ballooning in HFS/SP animals, which resulted in an increased histological score (Fig. 3r).

Figure 3. Histological, biochemical, and metabolic evaluation of adipose tissues and liver. Representative hematoxylin and eosin-stained perirenal adipose tissues (a), inguinal adipose tissue (h), and liver sections (o); relative weight of perirenal adipose tissue (b), inguinal adipose tissue (i), and liver (p); mean adipocyte diameter in perirenal (c), and inguinal adipose tissue (j); estimated adipocyte number in perirenal (d), and inguinal adipose tissue (k); total lipoprotein lipase activity in perirenal (e), and inguinal adipose tissue (l); heparin-released fraction of lipoprotein lipase in perirenal (f), and inguinal adipose tissue (m); *in vitro* lipolysis activity in perirenal (g), and inguinal adipose tissue (n); hepatic triglyceride content (q); hepatic cholesterol content (r); hepatic glycogen content (s); hepatic glucose content (t); total histological lesion score of the liver(u). Diet groups: C/GB = commercial grain-based diet, B/SP = balanced semi-purified diet, HFS/SP = high-fat high-sugar semi-purified diet. Statistical analysis: One-way ANOVA with Tukey's HSD post hoc, Welch's ANOVA with Games–Howell correction or Kruskal-Wallis with Dunn's multiple comparisons post hoc. Data are presented as box plots as median and interquartile ranges; white dots indicate group means. Scale bar = 25 µm; (↖) arrows indicate lipid droplet, (▲) arrowhead indicates ballooning.

Oxidative Markers

In the serum, semi-purified diets enhanced antioxidant capacity to a similar extent, regardless of diet composition (Fig. 4a). Oxidative markers were comparable between C/GB and B/SP but were elevated in the HFS/SP group (Fig. 4b-e). In the liver, antioxidant capacity was reduced, and oxidative markers were increased in animals fed the high-fat, high-sugar diet (Fig. 4f-j). In perirenal tissue, antioxidant capacity was not affected by diet; however, reactive species production was reduced by semi-purified diets, while oxidative stress markers were elevated in the HFS/SP group (Fig. 4k-o). In inguinal adipose tissue, both antioxidant capacity and reactive species production were reduced, whereas oxidative stress markers were elevated in HFS/SP. Notably, oxidative status, assessed by total free thiols, was improved in the B/SP diet (Fig. 4p-t).

Figure 4. Oxidative markers in the serum, liver, and adipose tissues in animals fed grain-based and semi purified diets. Antioxidant capacity measured by ferric-reducing antioxidant power in ascorbic acid equivalent (AntiOx.) (a, f, k, and p), total free thiols (TFT) (b, g, l, and q), total oxidative status in equivalent of H_2O_2 (TOS) (c, h, m, and r), lipid peroxidation in equivalent of H_2O_2 (LOOH) (d, l, n, and s), and protein carbonylation (PCO) (e, j, o, and t). Diet groups: C/GB = commercial grain-based diet, B/SP = balanced semi-purified diet, HFS/SP = high-fat high-sugar semi-purified diet. Statistical analysis: One-way ANOVA with Tukey's HSD post hoc, Welch's ANOVA with Games–Howell correction or Kruskal-Wallis with Dunn's multiple comparisons post hoc. Data are presented as box plots as median and interquartile ranges; white dots indicate group means.

DISCUSSION

High-fat diets are widely used in rodent models of diet-induced obesity, yet our findings highlight that the effects of diet composition are strongly modulated by the degree of processing. Animals fed the HFS/SP diet consumed fewer calories but presented similar weight gain to those fed the non-purified diet, developing greater adiposity without excess body mass. Although a lack of weight gain in high-fat diets has been occasionally reported with purified diets, the phenotype of visceral fat accumulation has not, suggesting that the degree of diet purification modulates the obesogenic potential of macronutrient-dense formulations [26]. In contrast, the B/SP diet promoted greater body weight and adiposity than all other groups, despite comparable caloric intake to a grain-based diet. While carbohydrate-rich diets typically fail to induce obesity without additional metabolic challenges [27], our data demonstrates that ad libitum intake of a semi-purified, carbohydrate-rich diet can indeed trigger obesity, possibly through increased metabolizable energy and reduced energy expenditure [28].

Increased processing/purification of the diet alters global energy metabolism in a manner dependent on diet composition. Only an imbalanced composition, such as high-fat and high-sugar intake, induces metabolic disturbances consistent with type 2 diabetes, dyslipidemia, and metabolic syndrome, independent of adipose tissue accumulation, adipocyte hypertrophy, or excessive weight gain. High-fat/high-sugar diets promote insulin resistance, as evidenced by elevated markers of glucose impairment. However, reduced fat mobilization and oxidation suggest partial preservation of insulin signaling, particularly in visceral fat, as supported by the observed hyperplasia. This may help explain the absence of further weight gain, as tissue-specific insulin resistance reduces glucose uptake in skeletal muscle and limits fat mobilization in adipose tissue, leading to decreased energy availability for muscle. Combined with hypercortisolemia, which enhances protein degradation, this results in skeleton muscle loss and ultimately prevents additional weight gain [29].

In the absence of imbalanced diet composition, the metabolic alterations induced by diet purification appear to be compensatory. Increased fat mobilization from visceral adipose tissue, redistribution of fat toward subcutaneous depots, and enhanced fat oxidation may mitigate the strain on internal organs and prevent the metabolic damage typically associated with greater body weight and adipose tissue accumulation [30]. One dietary component that may contribute to this phenotype is coconut, a rich source of medium-chain fatty acids, which have been shown to attenuate diet-induced obesity by increasing lipolysis and β-oxidation, in agreement with our findings [31].

In addition to the high-fat content of the diet, other nutritional factors may have contributed to the metabolic profiles. Sugar-induced insulin resistance is primarily linked to fructose intake and its ability to induce hepatic metabolic dysregulation and steatosis [32], a dietary element presented only in the HFS/SP diet. Dietary cholesterol is another factor implicated in insulin resistance, primarily via hepatic steatosis [33] and visceral adipose tissue inflammation [34]. Although the cholesterol levels in this study (0.04% and 0.07%) were well below the doses typically linked to metabolic dysfunction in rodents (0.5% to 1%), its combination with dietary fructose may have exerted a synergistic effect.

Although adipose dysfunction is a central feature of obesity-related metabolic changes [2], our findings suggest that adiposity alone does not fully explain the observed outcomes. The fat redistribution in B/SP animals may partly underlie their metabolically protected profile; however, the similar proportion of visceral adipose tissue between the two semi-purified groups indicates that other mechanisms contribute to metabolic disturbances. Oxidative stress is a central mechanism in obesity-related comorbidities [2]. Since the redox system is influenced by nutritional status, obesity, and their comorbidities, we assessed systemic and tissue-specific oxidative stress to clarify its role in the metabolic alterations observed.

Systemic antioxidant capacity was increased in both semi-purified diets, likely due to lower levels of potential contaminants and better alignment of these diets with international nutritional recommendations for rodents [14]. Previous studies have shown that commercial grain-based diets are inadequate to support optimal antioxidant defenses, leading to elevated oxidative damage markers compared to standardized purified diets, and suggested that this effect may be related to insufficient vitamin E content [35]. Indeed, the semi-purified diets provide more than twice the amount of vitamin E compared to the commercial diet, a nutrient that not only acts as a direct antioxidant but also contributes to the upregulation of antioxidant enzymes such as superoxide dismutase and catalase [36].

Increasing the degree of diet purification does not lead to higher systemic or tissue oxidative stress, even in the presence of increased obesity. Markers of antioxidant capacity and oxidative stress were either unchanged or improved in animals fed a balanced diet, regardless of the level of purification/processing. Only a nutritionally imbalanced diet induced systemic and tissue oxidative stress, likely resulting from lipotoxicity and mitochondrial dysfunction, which elevate oxygen radical production through excessive fat accumulation in tissues. Fructose also contributes to oxidative stress by promoting mitochondrial overproduction of reactive oxygen species, activating NADPH oxidase, and enhancing purine degradation. Moreover, excessive sugar intake fosters protein glycation, resulting in radical species generation via advanced glycation end-products [2], consistent with the elevated fructosamine levels observed in our HFS/SP group.

Beyond dietary influences, our data demonstrate that neither adiposity nor adipose tissue plasticity fully explains the metabolic damage associated with nutrition, underscoring the central role of metabolically active organs, particularly the liver, in regulating whole-body homeostasis and driving metabolic dysfunction. The liver, capable of generating reactive oxygen species in response to both endogenous and exogenous stimuli, was the only tissue showing elevated reactive species production, which was associated with systemic dissemination of oxidative burden [37]. These findings highlight the liver as a key organ in this model, with hepatic alterations emerging as the principal determinants of metabolic impairment. Consistently, human studies have reported that adiposity contributes to metabolic disease only when accompanied by increased liver fat [38]. Furthermore, the patterns observed here emphasize the combined impact of diet and hepatic function on oxidative stress, aligning with lipotoxicity-driven mechanisms associated with ectopic fat accumulation and insulin resistance [26, 39].

The distinct metabolic and oxidative profiles observed in this study closely resemble clinical obesity subtypes, such as metabolically healthy obesity and normal-weight obesity syndrome, also referred to as metabolically unhealthy non-obese. Metabolically healthy obesity is considered a transient state that often progresses to metabolic complications; thus, the ~10-week obesity induction period applied here may not have been sufficient for adiposity alone to trigger alterations in glucose and lipid metabolism. Although 10 weeks is generally adequate for the development of metabolic dysfunction in male Wistar rats, such changes are typically associated with diets rich in fat or sugar. This suggests that diet composition, rather than excess adiposity per se, is the primary driver of rapid metabolic alterations, providing insight into the origins of the metabolically healthy obesity phenotype [12].

The dissociation between obesity and metabolic complications, seen in phenotypes like metabolically healthy obesity and normal-weight obesity syndrome, has been reported in other animal models, but is usually strain-specific. Wistar rats are typically described as either developing metabolic syndrome with obesity or remaining metabolically healthy. Remarkably, this study is the first, to our knowledge, to identify both obese animals without metabolic dysfunction and normal-weight animals with significant metabolic impairments within the same Wistar cohort [12]. This expands the utility of Wistar rat model as a tool to study diverse metabolic states within a single strain, increasing both translational relevance and experimental efficiency.

Although the commercial diet differs from the experimental diets, it broadly aligns with NRC-95R recommendations. In fact, the caloric contribution of protein in the commercial diet was higher; nonetheless, both diets provided more than sufficient protein to ensure optimal development and health. Therefore, unexpected effects are unlikely to result from excesses or deficiencies, aside from those previously noted [14]. All diets shared a similar matrix, with most ingredients derived from soy, corn, and wheat, minimizing variability in nutrient availability and metabolic impact [40]. Moreover, the two semi-

purified diets were nutritionally matched, supporting the conclusion that the metabolic differences observed stem from processing and macronutrient composition.

Certain questions remain to be addressed. One important aspect is the influence of sexual dimorphism on nutritional responses, as this study included only male rats [12, 26]. Moreover, as this study focused on evaluating oxidative stress as a mechanism underlying metabolic damage in response to diet purification and composition, other mechanisms, such as specific genetic regulatory pathways and the crosstalk between gut microbiota and host metabolism, warrant further investigation [6, 31]. Previous studies have shown that both factors contribute to the development of obesity and metabolic dysfunction in relation to diet purification and composition and may help clarify some of the unresolved questions raised in this work.

CONCLUSION

In summary, our results highlight that both dietary composition and the degree of food processing shape distinct obesity phenotypes and influence the risk of metabolic dysfunction, beyond weight or adiposity alone. While fat gain is primarily associated with diet purification and processing, metabolic damage is largely driven by a high intake of fat and sugar, with the liver emerging as a central organ mediating these effects and acting as a major contributor to systemic oxidative stress and the development of metabolic syndrome in male Wistar rats. These findings reinforce the importance of evaluating not only macronutrient profiles but also the degree of diet purification when assessing morphometric and metabolic outcomes in diet-induced obesity models. Furthermore, this study provides insights into the mechanisms linking nutrition, obesity phenotypes, and metabolic disease risk.

Author Contributions:

Pedro Rocha Tenorio conceived and designed the study, performed all experiments, analyzed the data, and wrote the manuscript. Gabriel Smolak Sobieski e Silva assisted with animal care, experiments, and manuscript revision. Débora Hipólito Quadreli and Glaura Scantamburlo Alves Fernandes contributed to the oxidative stress assays. Fábio Goulart de Andrade participated in all phases of the project, acting as the project supervisor. All authors read and approved the final version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The animal study protocol was conducted in strict adherence to the Ethical Principles in Animal Research endorsed by the Brazilian College of Animal Experimentation and was approved by the Ethics Committee on Animal Use of the State University of Londrina (Official Letter Nº 074/2022 Protocol Nº 032.2022)

Data Availability Statement: Research data are only available upon request from the corresponding author.

Acknowledgments: The authors would like to express their sincere gratitude to the Central Animal Facility of the Center for Biological Sciences, State University of Londrina, for providing and maintaining the laboratory animals.

Conflicts of Interest: The authors declare no conflict of interest.

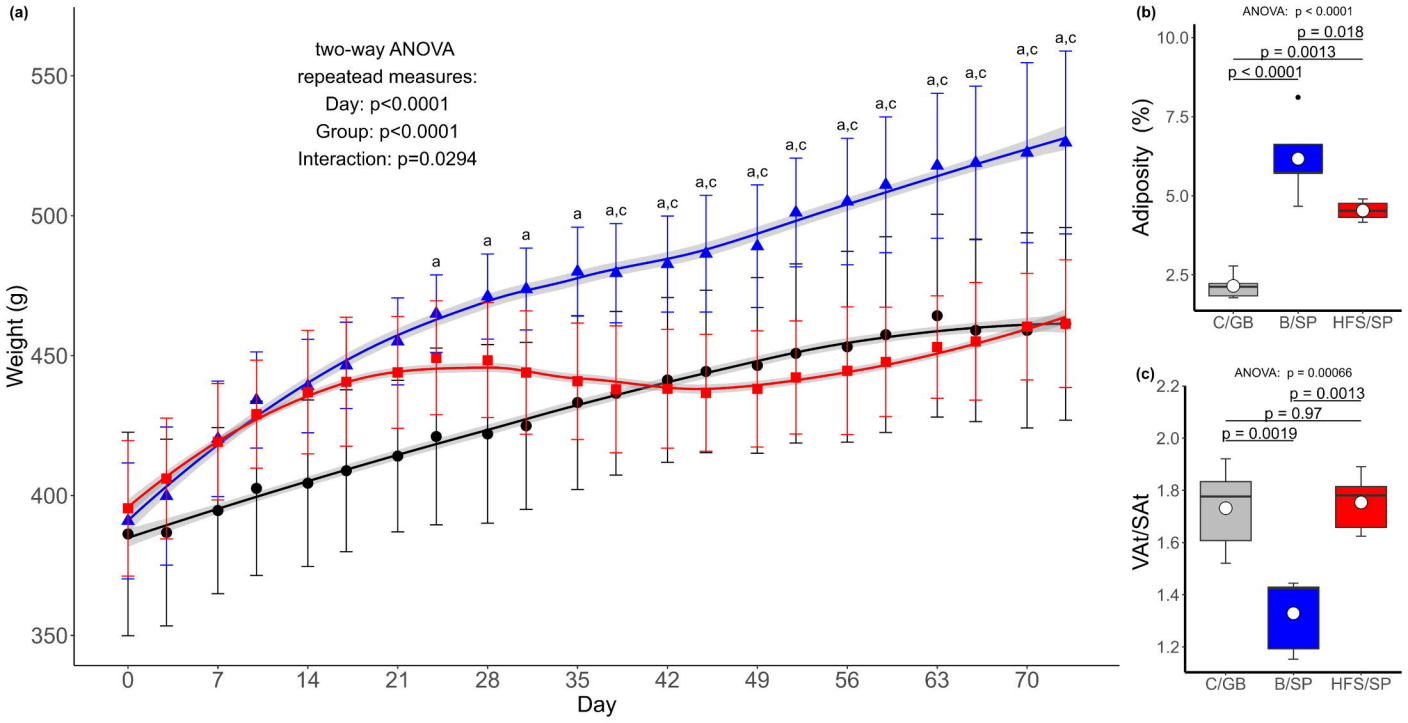
REFERENCES

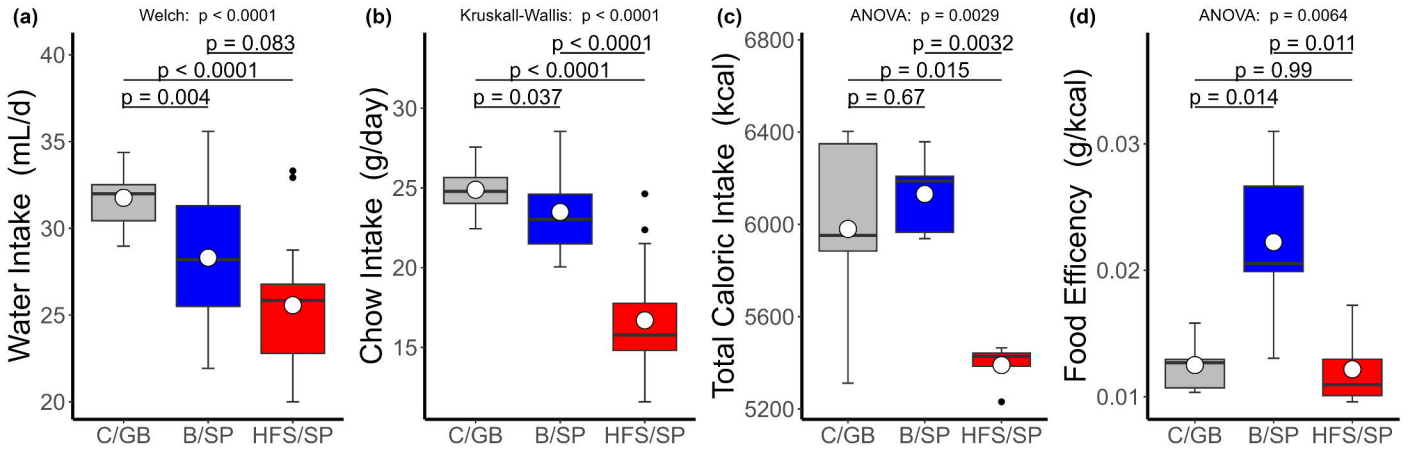
1. Finkelstein EA, Khavjou OA, Thompson H, Trogdon JG, Pan L, Sherry B, Dietz W (2012) Obesity and severe obesity forecasts through 2030. Am J Prev Med 42:563–70
2. Jin X, Qiu T, Li L, Yu R, Chen X, Li C, Proud CG, Jiang T (2023) Pathophysiology of obesity and its associated diseases. Acta Pharm Sin B 13:2403–2424
3. Suleiman JB, Mohamed M, Bakar ABA (2020) A systematic review on different models of inducing obesity in animals: Advantages and limitations. J Adv Vet Anim Res 7:103–114
4. Valicente VM, Peng C-H, Pacheco KN, Lin L, Kielb EI, Dawoodani E, Abdollahi A, Mattes RD (2023) Ultraprocessed Foods and Obesity Risk: A Critical Review of Reported Mechanisms. Adv Nutr 14:718–738
5. Bastías-Pérez M, Serra D, Herrero L (2020) Dietary Options for Rodents in the Study of Obesity. Nutrients 12:3234
6. Zhang L, Li X, Liu X, et al (2022) Purified diet versus whole food diet and the inconsistent results in studies using animal models. Food Funct 13:4286–4301
7. Cheng HS, Phang SCW, Ton SH, Abdul Kadir K, Tan JBL (2019) Purified ingredient-based high-fat diet is superior to chow-based equivalent in the induction of metabolic syndrome. J Food Biochem 43:e12717
8. Almeida-Suhett CP, Scott JM, Graham A, Chen Y, Deuster PA (2019) Control diet in a high-fat diet study in mice: Regular chow and purified low-fat diet have similar effects on phenotypic, metabolic, and behavioral outcomes. Nutr Neurosci 22:19–28
9. Shahidi F, Pan Y (2022) Influence of food matrix and food processing on the chemical interaction and bioaccessibility of dietary phytochemicals: A review. Crit Rev Food Sci Nutr 62:6421–6445

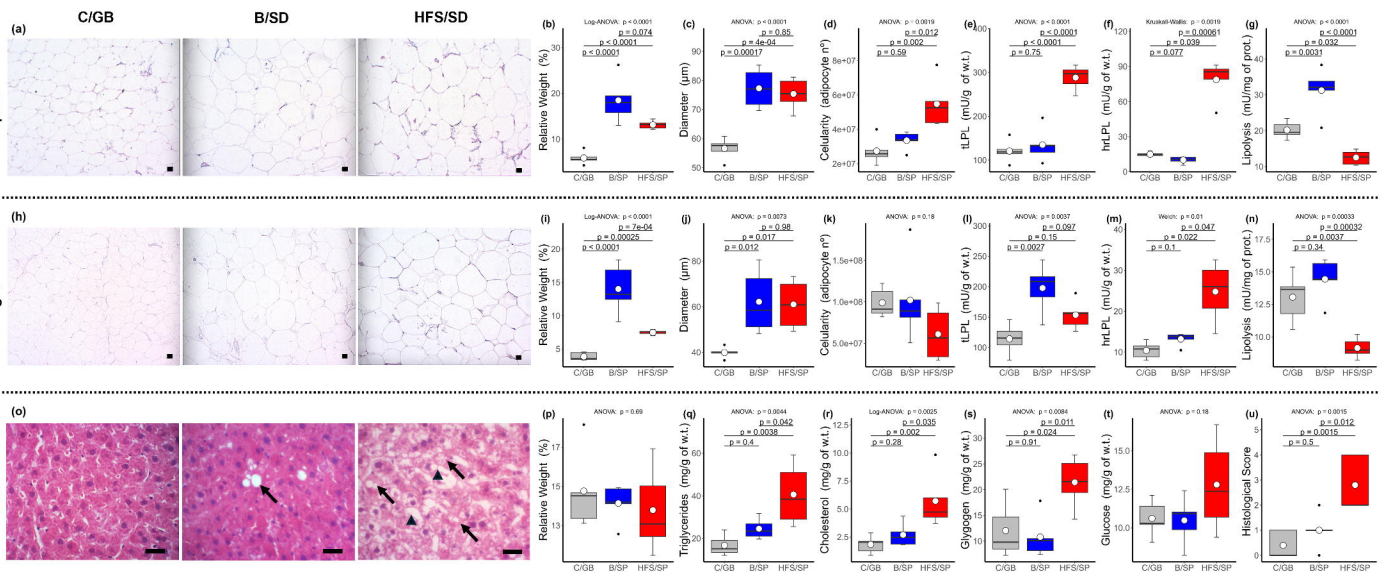
10. Del Bas JM, Caimari A, Ceresi E, Arola-Arnal A, Palou A, Arola L, Crescenti A (2015) Differential effects of habitual chow-based and semi-purified diets on lipid metabolism in lactating rats and their offspring. Br J Nutr 113:758–69
11. Parkman JK, Mao X, Dillon K, Gudivada A, Moustaid-Moussa N, Saxton AM, Kim JH (2016) Genotype-dependent Metabolic Responses to Semi-Purified High-Sucrose High-Fat Diets in the TALLYHO/Jng vs. C57BL/6 Mouse during the Development of Obesity and Type 2 Diabetes. Exp Clin Endocrinol Diabetes 124:622–629
12. Preguiça I, Alves A, Nunes S, Fernandes R, Gomes P, Viana SD, Reis F (2020) Diet-induced rodent models of obesity-related metabolic disorders-A guide to a translational perspective. Obes Rev 21:e13081
13. Charan J, Biswas T (2013) How to calculate sample size for different study designs in medical research? Indian J Psychol Med 35:121–6
14. National Research Council (US) Subcommittee on Laboratory Animal Nutrition (1995) Nutrient Requirements of Laboratory Animals: Fourth Revised Edition, 1995.
15. Konobu K, Takao H (1978) An improved colorimetric micromethod with diphenylcarbazide for serum free fatty acids determination. Yakugaku Zasshi 98:226–30
16. Itaya K, Ui M (1965) Colorimetric determination of free fatty acids in biological fluids. J Lipid Res 6:16–20
17. Wan H, Cao H, Ning P (2024) Superiority of the triglyceride glucose index over the homeostasis model in predicting metabolic syndrome based on NHANES data analysis. Sci Rep 14:15499
18. Tu E, Pearlmutter P, Tiangco M, Derose G, Begdache L, Koh A (2020) Comparison of Colorimetric Analyses to Determine Cortisol in Human Sweat. ACS Omega 5:8211–8218
19. Eriksson-Hogling D, Andersson DP, Bäckdahl J, Hoffstedt J, Rössner S, Thorell A, Arner E, Arner P, Rydén M (2015) Adipose tissue morphology predicts improved insulin sensitivity following moderate or pronounced weight loss. Int J Obes (Lond) 39:893–8
20. Pérez-Torres I, Gutiérrez-Alvarez Y, Guarner-Lans V, Díaz-Díaz E, Manzano Pech L, Caballero-Chacón SDC (2019) Intra-Abdominal Fat Adipocyte Hypertrophy through a Progressive Alteration of Lipolysis and Lipogenesis in Metabolic Syndrome Rats. Nutrients.
21. Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal Biochem 239:70–6
22. Koning AM, Meijers WC, Pasch A, Leuvenink HGD, Frenay A-RS, Dekker MM, Feelisch M, de Boer RA, van Goor H (2016) Serum free thiols in chronic heart failure. Pharmacol Res 111:452–458
23. Erel O (2005) A new automated colorimetric method for measuring total oxidant status. Clin Biochem 38:1103–11
24. Jiang ZY, Hunt J V, Wolff SP (1992) Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. Anal Biochem 202:384–9
25. Colombo G, Clerici M, Garavaglia ME, Giustarini D, Rossi R, Milzani A, Dalle-Donne I (2016) A step-by-step protocol for assaying protein carbonylation in biological samples. J Chromatogr B Analyt Technol Biomed Life Sci 1019:178–90
26. de Moura E Dias M, Dos Reis SA, da Conceição LL, Sediyama CMN de O, Pereira SS, de Oliveira LL, Gouveia Peluzio M do C, Martinez JA, Milagro FI (2021) Diet-induced obesity in animal models: points to consider and influence on metabolic markers. Diabetol Metab Syndr 13:32
27. Lackey DE, Lazaro RG, Li P, Johnson A, Hernandez-Carretero A, Weber N, Vorobyova I, Tsukomoto H, Osborn O (2016) The role of dietary fat in obesity-induced insulin resistance. American Journal of Physiology-Endocrinology and Metabolism 311:E989–E997
28. Barr SB, Wright JC (2010) Postprandial energy expenditure in whole-food and processed-food meals: implications for daily energy expenditure. Food Nutr Res 54:5144
29. Calderón-DuPont D, Torre-Villalvazo I, Díaz-Villaseñor A (2023) Is insulin resistance tissue-dependent and substrate-specific? The role of white adipose tissue and skeletal muscle. Biochimie 204:48–68
30. Ibrahim MM (2010) Subcutaneous and visceral adipose tissue: structural and functional differences. Obes Rev 11:11–8
31. Xia J, Yu P, Zeng Z, Ma M, Zhang G, Wan D, Gong D, Deng S, Wang J (2021) Lauric Triglyceride Ameliorates High-Fat-Diet-Induced Obesity in Rats by Reducing Lipogenesis and Increasing Lipolysis and β-Oxidation. J Agric Food Chem 69:9157–9166
32. Baena M, Sangüesa G, Dávalos A, Latasa M-J, Sala-Vila A, Sánchez RM, Roglans N, Laguna JC, Alegret M (2016) Fructose, but not glucose, impairs insulin signaling in the three major insulin-sensitive tissues. Sci Rep 6:26149
33. Püschel GP, Henkel J (2018) Dietary cholesterol does not break your heart but kills your liver. Porto Biomed J 3:e12
34. Chung S, Parks JS (2016) Dietary cholesterol effects on adipose tissue inflammation. Curr Opin Lipidol 27:19–25
35. Caetano AC, da Veiga LF, Capaldi FR, de Alencar SM, Azevedo RA, Bezerra RMN (2013) The antioxidant response of the liver of male Swiss mice raised on a AIN 93 or commercial diet. BMC Physiol 13:3
36. Newaz MA, Nawal NN (1998) Effect of alpha-tocopherol on lipid peroxidation and total antioxidant status in spontaneously hypertensive rats. Am J Hypertens 11:1480–5
37. Lee J, Kim J, Lee R, et al (2022) Therapeutic strategies for liver diseases based on redox control systems. Biomed Pharmacother 156:113764
38. Magkos F, Fabbrini E, Mohammed BS, Patterson BW, Klein S (2010) Increased whole-body adiposity without a concomitant increase in liver fat is not associated with augmented metabolic dysfunction. Obesity (Silver Spring) 18:1510–5

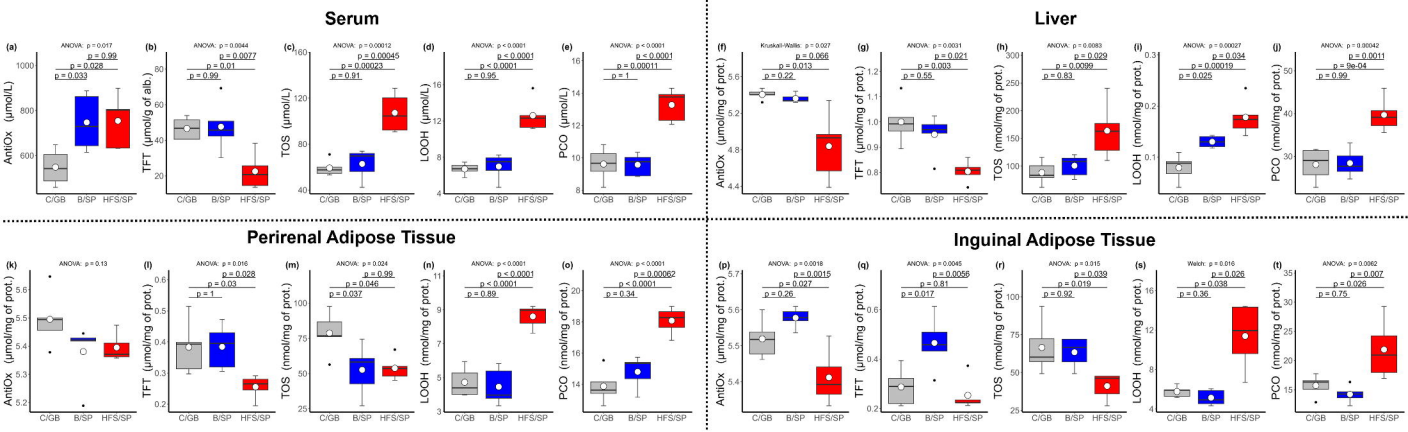
39. Ahmed B, Sultana R, Greene MW (2021) Adipose tissue and insulin resistance in obese. Biomed Pharmacother 137:111315
40. van den Berg LA, Mes JJ, Mensink M, Wanders AJ (2022) Protein quality of soy and the effect of processing: A quantitative review. Front Nutr 9:1004754

© 2025 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/)


(a)
two-way ANOVA
repeatead measures:
Day: p<0.0001
Group: p<0.0001
Interaction: p=0.0294
Weight (g)
Day
(b)
ANOVA: p < 0.0001
p = 0.018
p = 0.0013
p < 0.0001
Adiposity (%)
C/GB
B/SP
HFS/SP
(c)
ANOVA: p = 0.00066
p = 0.0013
p = 0.97
p = 0.0019
VAt/SAt
C/GB
B/SP
HFS/SP


(a)
Welch: p < 0.0001
p = 0.083
p < 0.0001
p = 0.004
Water Intake (mL/d)
40
35
30
25
20
C/GB
B/SP
HFS/SP
(b)
Kruskall-Wallis: p < 0.0001
p < 0.0001
p < 0.0001
p = 0.037
Chow Intake (g/day)
30
25
20
15
C/GB
B/SP
HFS/SP
(c)
ANOVA: p = 0.0029
p = 0.0032
p = 0.015
p = 0.67
Total Caloric Intake (kcal)
6800
6400
6000
5600
5200
C/GB
B/SP
HFS/SP
(d)
ANOVA: p = 0.0064
p = 0.011
p = 0.99
p = 0.014
Food Efficency (g/kcal)
0.03
0.02
0.01
C/GB
B/SP
HFS/SP


C/GB
B/SD
HFS/SD
(a)
prAt
(b)
Log-ANOVA: p < 0.0001
p = 0.074
p < 0.0001
p < 0.0001
Relative Weight (%)
(c)
ANOVA: p < 0.0001
p = 0.85
p = 4e-04
p = 0.00017
Diameter (µm)
(d)
ANOVA: p = 0.0019
p = 0.012
p = 0.002
p = 0.59
Celularity (adipocyte n°)
(e)
ANOVA: p < 0.0001
p < 0.0001
p < 0.0001
p = 0.75
tLPL (mU/g of w.t.)
(f)
Kruskall-Wallis: p = 0.0019
p = 0.00061
p = 0.039
p = 0.077
hrLPL (mU/g of w.t.)
(g)
ANOVA: p < 0.0001
p < 0.0001
p = 0.032
p = 0.0031
Lipolysis (mU/mg of prot.)
C/GB
B/SP
HFS/SP
(h)
igAt
(i)
Log-ANOVA: p < 0.0001
p = 7e-04
p = 0.00025
p < 0.0001
Relative Weight (%)
(j)
ANOVA: p = 0.0073
p = 0.98
p = 0.017
p = 0.012
Diameter (µm)
(k)
ANOVA: p = 0.18
Celularity (adipocyte n°)
(l)
ANOVA: p = 0.0037
p = 0.097
p = 0.15
p = 0.0027
tLPL (mU/g of w.t.)
(m)
Welch: p = 0.01
p = 0.047
p = 0.022
p = 0.1
hrLPL (mU/g of w.t.)
(n)
ANOVA: p = 0.00033
p = 0.00032
p = 0.0037
p = 0.34
Lipolysis (mU/mg of prot.)
C/GB
B/SP
HFS/SP
(o)
Liver
(p)
ANOVA: p = 0.69
Relative Weight (%)
(q)
ANOVA: p = 0.0044
p = 0.042
p = 0.0038
p = 0.4
Triglycerides (mg/g of w.t.)
(r)
Log-ANOVA: p = 0.0025
p = 0.035
p = 0.002
p = 0.28
Cholesterol (mg/g of w.t.)
(s)
ANOVA: p = 0.0084
p = 0.011
p = 0.024
p = 0.91
Glygogen (mg/g of w.t.)
(t)
ANOVA: p = 0.18
Glucose (mg/g of w.t.)
(u)
ANOVA: p = 0.0015
p = 0.012
p = 0.0015
p = 0.5
Histological Score
C/GB
B/SP
HFS/SP


Serum
Liver
Perirenal Adipose Tissue
Inguinal Adipose Tissue
(a) ANOVA: p = 0.017 p = 0.99 p = 0.028 p = 0.033 AntiOx (µmol/L)
(b) ANOVA: p = 0.0044 p = 0.0077 p = 0.01 p = 0.99 TFT (µmol/g of alb.)
(c) ANOVA: p = 0.00012 p = 0.000045 p = 0.00023 p = 0.91 TOS (µmol/L)
(d) ANOVA: p < 0.0001 p < 0.0001 p < 0.0001 p = 0.95 LOOH (µmol/L)
(e) ANOVA: p < 0.0001 p < 0.0001 p < 0.0001 p = 0.00011 p = 1 PCO (µmol/L)
(f) Kruskall-Wallis: p = 0.027 p = 0.066 p = 0.013 p = 0.22 AntiOx (µmol/mg of prot.)
(g) ANOVA: p = 0.0031 p = 0.021 p = 0.003 p = 0.55 TFT (µmol/mg of prot.)
(h) ANOVA: p = 0.0083 p = 0.029 p = 0.0099 p = 0.83 TOS (nmol/mg of prot.)
(i) ANOVA: p = 0.00027 p = 0.034 p = 0.00019 p = 0.025 LOOH (nmol/mg of prot.)
(j) ANOVA: p = 0.00042 p = 0.0011 p = 9e-04 p = 0.99 PCO (nmol/mg of prot.)
(k) ANOVA: p = 0.13 AntiOx (µmol/mg of prot.)
(l) ANOVA: p = 0.016 p = 0.028 p = 0.03 p = 1 TFT (µmol/mg of prot.)
(m) ANOVA: p = 0.024 p = 0.99 p = 0.046 p = 0.037 TOS (nmol/mg of prot.)
(n) ANOVA: p < 0.0001 p < 0.0001 p < 0.0001 p = 0.89 LOOH (nmol/mg of prot.)
(o) ANOVA: p < 0.0001 p = 0.00062 p < 0.0001 p = 0.34 PCO (nmol/mg of prot.)
(p) ANOVA: p = 0.0018 p = 0.0015 p = 0.027 p = 0.26 AntiOx (µmol/mg of prot.)
(q) ANOVA: p = 0.0045 p = 0.0056 p = 0.81 p = 0.017 TFT (µmol/mg of prot.)
(r) ANOVA: p = 0.315 p = 0.039 p = 0.019 p = 0.92 TOS (nmol/mg of prot.)
(s) Welch: p = 0.016 p = 0.026 p = 0.038 p = 0.36 LOOH (nmol/mg of prot.)
(t) ANOVA: p = 0.0062 p = 0.007 p = 0.026 p = 0.75 PCO (nmol/mg of prot.)
C/GB
B/SP
HFS/SP