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Metabolic and Genetic Alterations Induced by High-Fat and High-Glucose Diets in Wistar Rats

Alteraciones metabólicas y genéticas inducidas por dieta alta en grasa y glucosa en ratas Wistar

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Abstract

Introduction: Diet-induced obesity (DIO) in laboratory rats serves as a pathophysiological model for obesity research, yet remains poorly understood. Different types of diets, varying in their concentrations of carbohydrates, fats, and proteins, can induce distinct anatomical, metabolic, and genetic changes in Wistar rats. **Objective:** This study aims to identify the bodily, metabolic, and genetic changes resulting from different ad libitum diets in each sex separately. **Methodology:** Sixty-four Wistar rats were divided into four groups: Standard Diet (SD), Glucose Standard Diet (GSD), High Fat Diet (HFD), and Glucose High Fat Diet (GHFD). Pre- and post-measurements of weight, snout-tail length, and serum levels (glucose, cholesterol, triglycerides, high-density lipoprotein (HDL)) were taken. Post-sacrifice, liver, pancreas, visceral, brown, subcutaneous, and skeletal muscle adipose tissue samples were extracted. Gene expression of insulin receptor (INSR), fatty acid synthase (FASN), peroxisome proliferator-activated receptor gamma (PPARγ), and lipoprotein lipase (LPL) in subcutaneous adipose tissue was assessed by real-time PCR. **Results:** The GSD group exhibited significant increases in body mass, body mass index, and weights of visceral and subcutaneous adipose tissue. Higher cholesterol levels were observed in the HFD group, while GHFD induced the most significant increase in blood glucose and lipid profiles. **Conclusion:** The GSD, HFD, and GHFD diets simulate high-fat and high-sugar eating patterns, resulting in substantial increases in body mass and adipose tissue in the GSD group. The GHFD diet elevates blood glucose and lipid levels the most, while promoting lipogenic gene expression (FASN, LPL) and reducing the expression of insulin receptors (INSR) and PPARγ, thereby inducing insulin resistance and obesity.

Keywords: Obesity; Insulin Resistance; Hypercholesterolemia; Genes; Diet

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Resumen

Introducción: La obesidad inducida por dieta (DIO) en ratas de laboratorio sirve como un modelo fisiopatológico para la investigación en obesidad, aunque su estudio aún es limitado. Diferentes tipos de dietas, con variaciones en las concentraciones de carbohidratos, grasas y proteínas, pueden inducir cambios anatómicos, metabólicos y genéticos distintos en ratas Wistar. **Objetivo:** Este estudio busca identificar los cambios corporales, metabólicos y genéticos resultantes de diferentes dietas *ad libitum* en cada sexo por separado. **Metodología:** Sesenta y cuatro ratas Wistar fueron divididas en cuatro grupos: Dieta Estándar (SD), Dieta Estándar con Glucosa (GSD), Dieta Alta en Grasas (HFD) y Dieta Alta en Grasas con Glucosa (GHFD). Se realizaron mediciones pre y post de peso, longitud hocico-cola y niveles séricos (glucosa, colesterol, triglicéridos, lipoproteína de alta densidad (HDL)). Tras el sacrificio, se extrajeron muestras de hígado, páncreas, tejido adiposo visceral, marrón, subcutáneo y músculo esquelético. Se evaluó la expresión génica del receptor de insulina (INSR), sintasa de ácidos grasos (FASN), receptor gamma activado por proliferadores de peroxisomas (PPARγ) y lipoproteína lipasa (LPL) en tejido adiposo subcutáneo mediante PCR en tiempo real. **Resultados:** El grupo GSD mostró incrementos significativos en la masa corporal, índice de masa corporal y pesos del tejido adiposo visceral y subcutáneo. En el grupo HFD se observaron niveles más altos de colesterol, mientras que la dieta GHFD indujo el mayor aumento en glucosa sanguínea y perfiles lipídicos. **Conclusión:** Las dietas GSD, HFD y GHFD simulan patrones de alimentación altos en grasa y azúcar, resultando en aumentos considerables de masa corporal y tejido adiposo en el grupo GSD. La dieta GHFD eleva en mayor medida los niveles de glucosa y lípidos en sangre, al tiempo que promueve la expresión de genes lipogénicos (FASN, LPL) y reduce la expresión de receptores de insulina (INSR) y PPARγ, induciendo resistencia a la insulina y obesidad.

Palabras clave: Obesidad; Resistencia a la Insulina; Hipercolesterolemia: Genes: Dieta

Introduction

According to the World Health Organisation (WHO), obesity has emerged as a critical global health concern in the 21st century, with its associated morbidity and mortality on the rise. Projections indicate that by 2030, over 1 billion people, including 650 million adults, 340 million adolescents, and 39 million children worldwide, may grapple with obesity [1]. Obesity is a multifactorial condition that requires extensive research, and its increase suggests that novel therapeutic strategies to control the disease are needed. Obesity typically results from excessive caloric intake [2], leading to fat accumulation, which is associated with an increased risk of diabetes and elevated body mass index (BMI) [2,3]. Therefore, studies using animal models are necessary better to understand the pathophysiology of obesity and its metabolic consequences.

Over the course of many years, experimental animals have been recognised as invaluable tools for understanding pathophysiology and developing therapeutic strategies for various diseases [4]. In particular, rat models of diet-induced obesity (DIO) remain foundational in translational research, despite ongoing debates regarding heterogeneity in metabolic responses and reporting standards, which highlights the need for improved study design and the inclusion of sex variables (e.g., recent systematic review, 2023) [5]. Additionally, advanced multimodal models combining ageing and high-fat diets have emerged, offering refined platforms to investigate sarcopenic obesity and its metabolic consequences (e.g., Biogerontology, 2023) [6]. Among these animals, rats have emerged as one of the most extensively utilised subjects for investigating physiology, pharmacology, and metabolism in fundamental medical research due to their manageable dietary requirements and the ability to regulate environmental elements such as temperature, humidity, and lighting. Furthermore, rats typically have a shorter lifespan than humans, which simplifies the monitoring of alterations and adjustments throughout their lifecycle or even across multiple generations [4,7].

We hypothesise that certain types of diets, varying in the concentration of carbohydrates, fats, and proteins, will cause anatomical, metabolic, and genetic changes in the rats being studied. Therefore, the present study aims to compare the effects of four types of diet, differing in the concentrations of fat and glucose supplied to male and female Wistar rats, and subsequently evaluate the physiological response to each of these diets by analysing the anatomical, biochemical, and genetic changes.

Methodology

Animals

All animal experimentation was conducted in accordance with the Colombian Guide for the Care and Use of Laboratory Animals (Ministry of Health and Social Protection, Resolution No. 008430, April 10, 1993). The study was approved by the Research Ethics Committee of the Universidad Industrial de Santander (record number 4110, 20 Sept. 2019).

Wistar (*Rattus norvegicus*; WI IOPS AF/Han type) rats were maintained under controlled environmental conditions (room temperature, 23°C, on a 12-h light/dark cycle) and provided with water *ad libitum* and their respective experimental diet.

Experimental design

Sixty-four Wistar rats (thirty-two males and thirty-two females, 3 weeks old, body weight 30–40 g) were divided into four experimental groups (n=16 per diet; 8 males and 8 females each), using a simple randomisation method. Exclusion criteria included congenital malformations, clinical illness, persistent weight loss during the acclimatisation period, refusal to consume the assigned diet, or death before the start of the experimental period.

The sample size was determined following the guidelines of Arifin and Zahiruddin (2017) [7] for a repeated measures design, which indicated that a minimum of eight animals per sex per group would provide 80% power to detect a medium effect size ($\alpha = 0.05$). This calculation was consistent with previous studies on diet-induced obesity in rodents [8]. Consequently, using 64 animals in total was justified considering these precedents.

The four experimental diets were formulated based on the macronutrient ratios previously described by Kleiber (1947) [10], with modifications (Supplementary Table S1). The standard diet (SD) contained 50% carbohydrates (mainly starch and fiber), 30% fat, and 20% protein, providing 4.8 kcal/g. The standard glucose diet (GSD) consisted of 60% carbohydrates, with glucose as the primary source of simple carbohydrates, 25% fat, and 15% protein, providing 5.0 kcal/g. The high-fat diet (HFD) contained 45% carbohydrates, 40% fat, and 15% protein, providing 5.3 kcal/g. The high-fat, high-glucose diet (GHFD) contained 55% carbohydrates with a high glucose content, 35% fat, and 10% protein, providing 7.2 kcal/g. The term "glucose diet" refers to the use of glucose as the primary source of carbohydrates, in contrast to the starch in the standard diet, which increases the simple sugar load and caloric density.

Glucose was selected as the primary simple carbohydrate instead of sucrose to ensure a direct effect of a rapidly absorbed monosaccharide on glycemia and insulin signalling. This approach avoids the confounding metabolic effects of fructose, which is present in sucrose and has distinct hepatic metabolism.

Parametric and biochemical measurements

Body weight was measured daily throughout the study using a digital balance Model PX224/E Pioneer ™ (OHAUS). At baseline and after six weeks, body mass index (BMI) was calculated.

Blood samples were collected at baseline and post-diet following a 5-hour fasting period. Glycemia, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein (LDL) were determined using commercial kits (Wiener Lab, Argentina). The manufacturer validates these kits and has been routinely used in our laboratory, along with internal quality controls, to ensure accuracy and reproducibility. Measurements were performed using a BioTek Synergy H1 Microplate Reader (BioTek Instruments, Winooski, VT, USA).

At the end of the experiment, animals were euthanised by decapitation. Organs (liver, pancreas and skeletal muscle) and adipose tissue (brown, BAT; visceral, VAT; and subcutaneous, SAT) were dissected and weighed.

Real-time PCR and gene expression profile

Genes encoding the following proteins implicated in human obesity were selected. One of these is the insulin receptor (INSR), which is involved in the development of systemic insulin resistance linked to obesity, a significant contributing factor to type 2 diabetes mellitus. When insulin signalling is diminished, it leads to insulin resistance, thereby affecting insulin's metabolic functions [12]. Peroxisome proliferator-activated receptor gamma (PPARγ) transcription factor is a gene mostly expressed in adipose tissue. In patients with obesity and diabetes, increased expression of its mRNA is observed, mainly due to its role in regulating the expression of several genes related to lipid metabolism, obesity-associated inflammation, and metabolic syndrome [13]. The fatty acid synthesis enzyme (FASN) gene plays a crucial role in the body's fat formation process, known as lipogenesis. Its inhibition leads to a rapid decrease in fat stores, suggesting that this enzyme plays a crucial role in maintaining energy balance and regulating food intake [14]. Finally, lipoprotein lipase (LPL), another relevant enzyme. If this gene is overexpressed, it can alter the distribution of triglycerides in the blood between muscle and adipose tissue, potentially impacting insulin resistance and obesity.

RNA was isolated from adipose tissue using TRIzol Reagent® (Ambion Life Thecnology, Waltham, MA, USA) according to the manufacturer's instructions. RNA concentration and purity were determined with a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), followed by DNase I treatment (Promega, Madison, Wisconsin, USA). cDNA was synthesised using M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA) and random hexamers (Roche, Penzberg, Alemania) in a final reaction volume of 20 μl according to the manufacturer's instructions. Real-time PCR was performed on a Bio-Rad CFX96 System (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Gene expression was normalised using 16S rRNA as the reference gene. Both primers and probes were designed using Biosearch Technologies Software (AMLTDA). The sequence for primers is shown in Supplementary Table 1. The data analysis was performed using BioTek's Gen5 data analysis software.

Statistic analysis

All results were expressed as mean ± standard error (SEM). Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test to correct for multiple comparisons. The use of ANOVA was appropriate given the design, which involved more than two independent groups. Given that our primary objective was to evaluate diet within each sex separately, analyses were stratified by sex rather than using a factorial model. Assumptions of normality and homogeneity of variance were verified using the Shapiro-Wilk test and Levene's test, respectively. A value of $p<0.05$ was considered statistically significant. Statistical analyses were performed. Using SPSS/Windows version 15.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism® Version 8.0 (GraphPad Software, San Diego, CA, USA).

Results

The GHFD diet caused the most significant increase in body weight, BMI and Lee's Index.

All animals survived without showing abnormal symptoms or behavioural changes throughout the observation period. At baseline, rats in all four groups had similar initial body weights and body mass indices (BMIs). A significant increase in both body weight and BMI was observed in all rats on the GHFD diet ($p < 0.001$, according to ANOVA/Tukey) (**Table 1**). Significant differences were found between the body weights of the GSD, HFD, and GHFD groups compared to the SD group, with the most important difference in the GSD group ($p = 0.001$). There were also differences between the GHFD group and the other two groups ($p < 0.01$). Rats fed the GHFD diet experienced a significant increase in BMI value ($p < 0.001$), unlike those fed the other three diets. Regarding Lee's index, a validated indicator of adiposity in rodents calculated as [cube root of body weight (g) × 10 / nose–anus length (mm)], was significantly increased in males in the post of the GHFD diet compared to the post values of the SD, GSD and HFD diets ($p<0.001$; $p=0.002$ and $p=0.001$, respectively). Likewise, in females, the diet that presented the most significant changes with respect to the post-values of the other diets (SD, GSD, and HFD) was the GHFD, with values of $p < 0.001$, $p = 0.001$, and $p < 0.001$, respectively.

Daily food intake and food efficiency were monitored throughout the study. No significant differences were observed between groups (data not shown).

Table 1. Weight, body mass index (BMI) and Lee Index in Wistar rats according to diet and sex

Diet	Weight (g)		BMI (g/cm^2)		Lee Index	
	Basal	Post	Basal	Post	Basal	Post
			Males			
SD	342 ± 8.7	407.8 ± 11.9 ^{a}	0.209 ± 0.0	0.232 ± 0.0	0.285 ± 0.0	0.291 ± 0.0
GHFD	332.6 ± 6.0	491 ± 8.9 ^{a,b,c}	0.218 ± 0.0	0.360 ± 0.0 ^{b,d,e}	0.273 ± 0.0 ^{c,f}	0.329 ± 0.0 ^{b,d}
HFD	354.2 ± 1.4	428 ± 2.7 ^{a}	0.198 ± 0.0	0.220 ± 0.0	0.286 ± 0.0 ^{c,e}	0.305 ± 0.0 ^{b,f}
GSD	392.2 ± 8.7	457.9 ± 8.2 ^{a,b}	0.208 ± 0.0	0.242 ± 0.0	0.305 ± 0.0 ^{a}	0.309 ± 0.0 ^{b}
			Females			
SD	224.3 ± 2.18	244.7 ± 1.2 ^{a}	0.164 ± 0.0	0.165 ± 0.0	0.278 ± 0.0	0.288 ± 0.0
GHFD	237.5 ± 1.3	327.6 ± 10.0 ^{a,b}	0.161 ± 0.0^{f}	0.237 ± 0.0 ^{b,d,e}	0.266 ± 0.0 ^{a,c,e}	0.312 ± 0.0 ^{b,e}
HFD	235.6 ± 1.0	259.0 ± 2.6 ^{a}	0.158 ± 0.0^{e}	0.192 ± 0.0 ^{b}	0.284 ± 0.0	0.286 ± 0.0
GSD	234.8 ± 1.6	262.0 ± 7.1 ^{a,b}	0.168 ± 0.0	0.182 ± 0.0 ^{b}	0.289 ± 0.0	0.289 ± 0.0 ^{f}

*Body weight, body mass index (BMI) and Lee Index in male and female Wistar rats according to diet. Values are means ± SEM (n=8 per sex per diet). Basal = week 0; Post = end of week 6. Statistical analysis: one-way ANOVA with Tukey's post-test. Diets: SD = Standard Diet; GHFD = Glucose High Fat Diet; GSD = Glucose Standard Diet; HFD = High Fat Diet; Superscripts indicate significant differences: a *vs.* pre SD, b *vs.* post SD, c *vs.* pre GSD, d *vs.* post GSD, e *vs.* post HFD, f *vs.* post GHFD.

The GHFD diet caused the most significant increase in both organ and adipose tissue weights.

Figure 1 shows the comparison of liver, pancreas, and skeletal muscle weights in rats fed GSD, HFD, and GHFD diets relative to the SD group. Liver weight: Both male and female GHFD rats exhibited a significant increase in weight ($p < 0.05$ and < 0.001, respectively); meanwhile, male rats fed GSD and HFD showed an increase ($p < 0.01$) in liver weight, but this was not observed in females. The notable variations in liver weights across the experimental groups indicate the induction of hepatomegaly: in males, SD = 11.61 ± 0.11 *vs.* HFD = 14.51 ± 0.09 ($p < 0.001$), *vs.* GSD = 14.90 ± 0.21, and *vs.* GHFD = 15.76 ± 0.19 ($p = 0.3834$); and in females, SD = 8.67 ± 0.10 *vs.* HFD = 8.16 ± 0.31 ($p = 0.839$, and *vs.* GHFD = 9.85 ± 0.25 ($p = 0.029$); *vs.* GSD = 9.88 ± 0.52 ($p = 0.0765$). Spleen and skeletal muscle: A significant increase in spleen weight was observed in males fed GHFD ($p < 0.001$), GSD, and HFD ($p < 0.01$), but not in females. No significant differences were found in muscle weight under any condition.

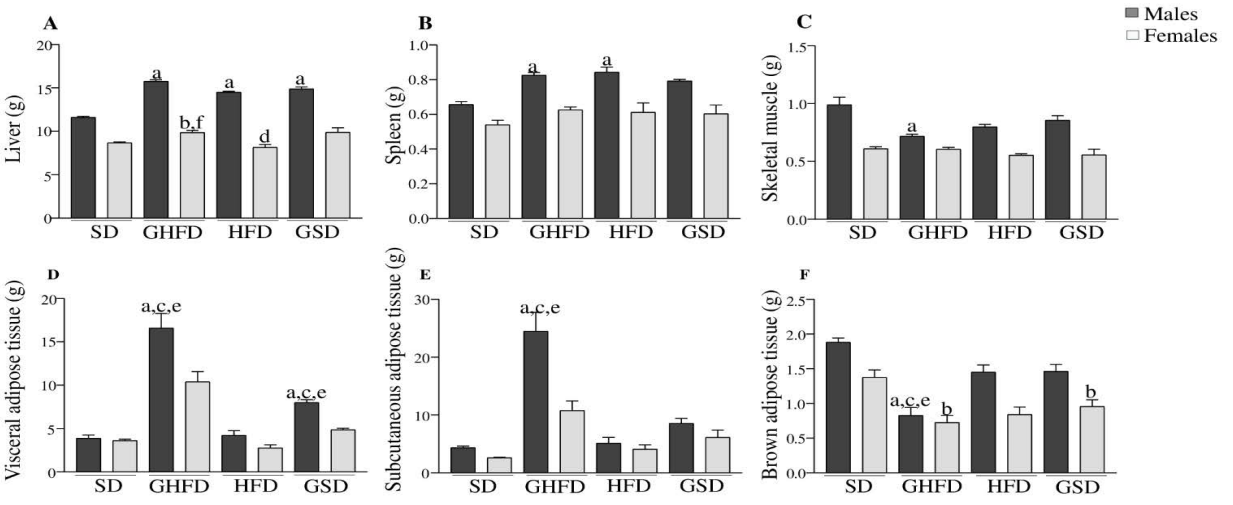

Figure 1. - Organ (top: 1A-C) and adipose tissue (bottom: 1D-F) relative weights in Wistar according to diet and sex (Diets: SD = Standard Diet; GHFD = Glucose High Fat Diet; GSD = Glucose Standard Diet; HFD = High Fat Diet). Data are means ± SEM (n=8/sex/diet) at week 6 (post). One-way ANOVA with Tukey's test. a *vs.* males SD, b *vs.* females SD, c *vs.* males GSD, d *vs.* females GSD, e *vs.* males HFD, f *vs.* females HFD.

The GHFD diet resulted in the most significant changes in both serum glucose levels and lipid profiles.

The data were analysed by comparing concentration changes from baseline to post-diet samples within and between treatment groups **(Table 2)**. In both male and female rats, all diets (GSD, HFD, GHFD) significantly increased glucose concentrations post-diet compared to baseline (GSD: male p=0.013, female p <0.001; HFD: male p=0.001, female p=0.001; GHFD: male p=0.0017, female p<0.001). Notably, GHFD induced more significant differences in glucose levels compared to SD (male p=0.0195, female p<0.001), GSD (male p=0.999, female p<0.001), and HFD (male p=0.0016, female p<0.001) post-diet. HFD and GHFD exhibited hyperglycemia at the end compared to SD, with sex influencing glucose changes, especially in females.

Table 2. Biochemical parameters in Wistar rats according to diet and sex

Biochemical Parameters. (mg/dL)	SD		GHFD		HFD		GSD	
	Basal	Post	Basal	Post	Basal	Post	Basal	Post
Glucose								
Male	207.6 ± 5.7	206.4 ± 19	188.6 ± 13	278 ± 12 [a,b,c]	198.1 ± 7.7	266.4 ± 16	220.2 ± 4.9	270.7 ± 4.1
Female	180.2 ± 5.6	188.7 ± 1.7	194.5 ± 11	327.2 ± 5.1 [a,b,c]	202 ± 2.2	255 ± 4.5	192.3 ± 4.0	256 ± 5.7
Triglyceride								
Male	52.2 ± 13.6	64.2 ± 1.3	46.6 ± 3.1	91.4 ± 9.5	64.6 ± 6.7	170.3 ± 7.2 [a,b]	83 ± 5.8	57.7 ± 11
Female	66 ± 4.8	69.6 ± 3.4	62.2 ± 8.8	148.8 ± 10	65.8 ± 6.0	95 ± 5.5 [a,b]	62 ± 6.8	80.5 ± 4.5
Cholesterol								
Male	56.5 ± 6.3	57.2 ± 2.2	62.3 ± 1.3	119 ± 9.4	51.2 ± 2.5	57.2 ± 3.1	64 ± 2.8	57 ± 1.5
Female	52.2 ± 2.5	51.7 ± 1.3	53.4 ± 2.5	85.1 ± 4.7	52.6 ± 3.0	67.5 ± 2.9	51.4 ± 3.6	56.5 ± 2.6
LDL cholesterol								
Male	231.8 ± 2.1	227.5 ± 8.8	263 ± 2.7	370.3 ± 7.8	258 ± 4.0	336.5 ± 5.9	239.2 ± 5.8	322.8 ± 6.8 [a,b]
Female	250.3 ± 20.2	254.3 ± 12	287 ± 10	376 ± 2.9	260.3 ± 3.7	364.3 ± 5.1	265.7 ± 8.7	342.3 ± 16
HDL cholesterol								
Male	300.5 ± 4.7	277 ± 6.5	267 ± 51	333.3 ± 9.6	277.2 ± 9.0	305.3 ± 7.8	305 ± 6.7	289.7 ± 1.3
Female	341.6 ± 23	362.4 ± 32	389.7 ± 29	444. 3 ± 8.9	390 ± 31	404.6 ± 38	348.8 ± 28	402.5 ± 30 [a,b]

*Biochemical parameters (glucose, triglycerides, total cholesterol, LDL, low-density lipoprotein, HDL, high-density lipoprotein) in male and female Wistar rats according to diet. Diets: SD = Standard Diet; GHFD = Glucose High Fat Diet; GSD = Glucose Standard Diet; HFD = High Fat Diet. Values are means ± SEM (n = 8 per sex per diet). Basal = week 0; Post = end of week 6. Statistical analysis: one-way ANOVA with Tukey's post-test. Superscripts indicate significant differences: a *vs.* pre-SD, b *vs.* post SD, c *vs.* pre GSD.

Regarding lipid profiles, GHFD significantly increased cholesterol levels in both male and female rats compared to other diets (male: $p < 0.001$, female: SD $p < 0.001$, GSD $p = 0.0003$, HFD $p = 0.003$). GHFD also significantly increased triglyceride (TG) levels compared to HFD (male, $p < 0.001$; female, $p < 0.001$). In females, it showed significant differences compared to SD, GSD, and HFD ($p < 0.001$, $p < 0.001$, $p < 0.001$, respectively). HFD induced significant TG increases compared to SD and GSD in males ($p<0.001$) and exhibited elevated TG levels compared to baseline in both sexes.

In terms of LDL, all treatment groups (GSD, HFD, GHFD) showed significant increases post-diet compared to baseline, with GHFD exhibiting more pronounced differences compared to the other diets in males (GSD, $p < 0.001$; HFD, $p = 0.004$). HDL levels remained similar across all experimental groups, with no significant differences observed.

Overall, rats treated with GHFD developed hyperlipidemia, characterised by elevated cholesterol, triglycerides, and LDL, suggesting that GHFD has a pronounced impact on disrupting glucose and lipid homeostasis in both male and female rats.

The GHFD diet caused the most significant changes in gene expression.

Insulin receptor expressions decreased significantly in all diets compared to SD in both male ($p=0.011$ GSD, $p=0.002$ HFD, $p<0.001$ GHFD) and female ($p=0.001$ GSD, $p<0.001$ HFD, $p<0.001$ GHFD) rats, indicating altered insulin signalling, particularly pronounced in GHFD **(Figure 2A).** FASN mRNA expression significantly increased with GHFD ($p<0.001$), suggesting its role in obesity development and insulin resistance **(Figure 2B)**. In PPARγ mRNA expression, the levels were significantly lower in all diets compared to SD ($p < 0.001$), especially in GHFD, indicating an association with obesity-related complications **(Figure 2C)**. LPL gene expression showed significant differences, particularly in males, with GHFD exhibiting the highest expression ($p < 0.001$). In females, GHFD also showed significant differences compared to other diets ($p<0.001$), suggesting its role in modulating LPL gene expression **(Figure 2D)**.

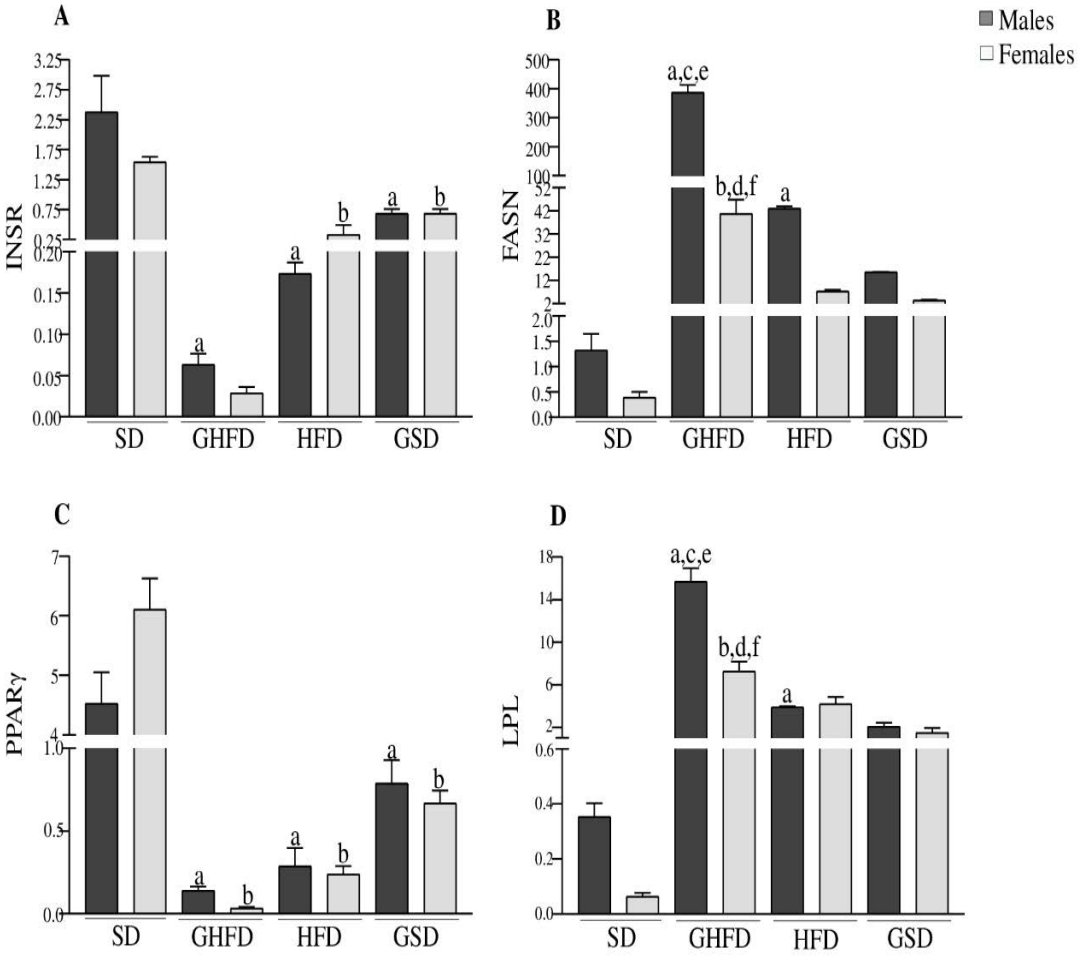

Figure 2. Gene expressions associated with obesity in Wistar rats according to diet (Diets: SD = Standard Diet; GHFD = Glucose High Fat Diet; GSD = Glucose Standard Diet; HFD = High Fat Diet). Abbreviations: INSR (2A), insulin receptor; FASN (2B), fatty acid synthase; PPARγ (2C), peroxisome proliferator-activated receptor gamma; LPL (2D), lipoprotein lipase. Data are means ± SEM (n=8/sex/diet) at week 6 (post). One-way ANOVA with Tukey's test. a *vs.* males SD, b *vs.* females SD, c *vs.* males GSD, d *vs.* females GSD, e *vs.* males HFD, f *vs.* females HFD.

Discussion

Understanding the metabolic implications of different diets is crucial to elucidating the potential effects of various nutrients on health. This study contributes to the comparison of metabolic outcomes in male and female rats subjected to different diets: SD, Standard Diet; GSD, Glucose Standard Diet; HFD, High-Fat Diet; and GHFD, Glucose High-Fat Diet, which mimics the diet-induced obesity model. Notably, preclinical models of diet-induced obesity often exhibit metabolic responses in rodents that are very similar to those observed in humans.

In this study, we evaluated the metabolic consequences using multivariate statistical models in male and female Wistar rats subjected to diets with different proportions of fat and glucose, including the SD, GSD, HFD, and GHFD groups. Our results indicated that the GHFD group underwent significantly greater weight gain compared to the post-SD group; very similar to the consequence evidenced in the study carried out by Sasidharan et al., (2013) [16], where all groups that consumed the high-fat diet (HFD) showed a significant increase in body weight compared to the control group. While weight gain in the SD group did not differ significantly from that in the HFD group, it did vary considerably in the GHFD group. These results also indicate that the GHFD diet significantly affected Lee's index in both male and female rats, showing a marked increase compared to SD, GSD and HFD. These data are consistent with previous research that has established Lee's index as a reliable indicator of obesity in rodent models. For example, Kahn et al. (2104) [18], noted that an elevated Lee's index correlates with a higher percentage of body fat and metabolic dysfunction, critical markers of obesity. Similarly, Lackey et al., (2016) [19] demonstrated that dietary compositions rich in fats and sugars significantly elevate the Lee index, further supporting its usefulness in assessing obesity-related changes.

The significant alterations observed in the GHFD group underscore the detrimental effects of a diet rich in fat and glucose, which not only promotes weight gain but also increases metabolic disorders. Elevated Lee's index reflects the accumulation of adipose tissue and body mass, which was further supported by the increase in VAT and SAT weight in GHFD rats, making this measure valuable for assessing the impact of diet on obesity development. Body weight is a crucial indicator of overall health status, while changes in organ weight may reflect underlying diseases or metabolic disorders.

Our study observed significant increases in organ weight, particularly in the liver of male rats exposed to HFD and GHFD. This phenomenon of liver enlargement is associated with the development of hepatomegaly, a characteristic feature of the diabetic and obesogenic model induced by dietary factors. Significant differences in the spleen were also observed, particularly in diets with high glucose intake, suggesting a crucial role of dietary sugar in its functional impairment and its potential contribution to the development of type 2 diabetes.

The increase in spleen weight observed in glucose-enriched diets may reflect immunometabolic dysregulation. Previous studies have linked hyperglycemia and type 2 diabetes with splenic hypertrophy due to increased inflammatory cell turnover [27, 29]. Similar findings were reported by Jia et al., (2013) [17] regarding the serum levels present in the rats in their respective study. They showed that rats fed HFD for 4 weeks experienced a significant increase in body weight. In addition, they observed significant increases in serum levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL-C). CT/HDL-C and LDL-C/HDL-C ratios, key indicators of abnormalities in lipid metabolism, also increased compared to week 0.

Interestingly, despite consuming a high-fat diet, the GHFD group showed lower VAT and SAT weights compared to the high-glucose diet group. This finding highlights the complexity of adipose tissue biology. At the same time, the reduction in BAT under GHFD suggests impaired thermogenic capacity, since BAT is the primary adipose tissue involved in thermoregulation and energy expenditure, exerting protective effects against obesity. Although GHFD rats showed relatively lower VAT and SAT compared to high-glucose diets, this may indicate impaired adipose tissue expandability rather than protection. Limited storage capacity in these depots can lead to ectopic fat deposition in the liver and muscle, thereby aggravating metabolic dysfunction. Other studies have shown that administering a diet rich in fructose (60% by weight) for approximately a period of at least 10 weeks increased body weight [20, 23] and retroperitoneal fat deposits [22, 23]. Although in Moura et al., (2008) [24], no differences were observed in the body weight of adult rats that received fructose, a greater weight was observed in the retroperitoneal, mesenteric and subcutaneous fat deposits [23].

Our data revealed an inverse correlation between BAT weight and body weight, indicating that as body weight increases, BAT tends to decrease in size, especially in cases associated with a higher fat content in VAT and diets rich in both glucose and fat. This suggests a potential role of adipose tissue, known for modulating both fat and carbohydrate metabolism, highlighting its protective effect against obesity. Furthermore, our results underscore the significance of organ weight as a disease indicator and the potential of non-invasive imaging techniques.

Dyslipidemia, characterised by abnormal cholesterol metabolism, can be induced in rats by the administration of a high-fat diet incorporating a diverse range of fats, such as lard, coconut oil, soybean oil, or palm oil. This induction is often associated with the prolonged ingestion of saturated fats, which directly correlates with dyslipidemia and atherosclerosis [4, 25]. In experiments focusing on cholesterol metabolism, Wistar and Sprague-Dawley rats are susceptible to diet-induced obesity and dyslipidemia. Previous studies have shown that feeding diets containing as little as 42% fat can alter peripheral cholesterol levels in both Wistar and Sprague Dawley rats in various contexts [12, 16, 17, 23, 26]. However, influential factors such as age, feeding duration, and dietary fat composition may also affect the experimental results [4, 16], apart from the associated changes in the expression of genes encoding various proteins involved in lipid and glycemic metabolism [27].

The choice of glucose over sucrose as the main simple carbohydrate aimed to isolate the impact of a rapidly absorbed monosaccharide on metabolic outcomes. While sucrose is more representative of human dietary sugar intake, it contributes both glucose and fructose. Fructose metabolism has been linked to increased hepatic lipogenesis, uric acid production, and oxidative stress. Thus, the use of glucose allowed us to attribute the observed effects more specifically to glycemic load. In contrast, sucrose could activate additional fructose-related mechanisms that warrant evaluation in future studies.

Beyond descriptive findings, the reduction in INSR expression, particularly under GHFD, may reflect impaired insulin signalling pathways that contribute to systemic insulin resistance. This alteration, coupled with the upregulation of lipogenic genes such as FASN and LPL, suggests a metabolic shift toward enhanced lipid synthesis and storage in adipose tissue, reinforcing the obesogenic phenotype. Although the current study did not directly measure insulin sensitivity, the magnitude of these transcriptional changes aligns with previous reports linking decreased INSR expression and increased FASN activity to insulin resistance and type 2 diabetes in both rodents and humans [13, 28]. These molecular adaptations may also explain the biochemical alterations observed in glucose and lipid homeostasis [29].

In translational terms, the findings of this study mirror key metabolic disturbances commonly observed in human obesity, including hyperglycemia, dyslipidemia, and impaired insulin receptor signalling. Rodent models, such as this one, are therefore valuable tools for preclinical evaluation of dietary interventions, pharmacological agents, or functional foods aimed at mitigating obesity and metabolic syndrome [3]. Understanding the convergence of glucose and lipid metabolic pathways in animals provides mechanistic insights that can be extrapolated cautiously to clinical scenarios, highlighting potential therapeutic targets such as FASN inhibition or PPARγ modulation.

Nonetheless, certain limitations should be acknowledged. First, the study was restricted to Wistar rats, and species-specific differences may limit generalisation to human physiology. Second, we did not include measurements of inflammatory markers (e.g., TNF-α, IL-6, CRP), which are closely linked to adipose tissue dysfunction and insulin resistance. Inflammation is a critical axis in obesity pathophysiology, and its omission constrains the mechanistic scope of the present work. Future studies should therefore incorporate inflammatory and oxidative stress parameters to provide a more comprehensive and integrative perspective on obesity-related metabolic dysregulation.

In summary, the findings of this study support the notion that diets differing in carbohydrate and fat composition induce anatomical, metabolic, and genetic changes in Wistar rats. The GHFD diet, in particular, elicited the most significant alterations across multiple parameters, including body weight, BMI, organ weights, glucose levels, lipid profiles, and gene expression patterns related to obesity and insulin resistance. These insights provide a valuable foundation for understanding translational mechanisms linking diet-induced obesity in rodents to human metabolic disease.

As a recommendation, gene studies of all the tissues collected could be carried out for future research.

Conclusions

The animals exposed to the GSD, HFD, and GHFD diets simulate the diabetic and obesogenic eating pattern characterised by a high intake of fats and simple carbohydrates (glucose) and a lower protein and fibre content throughout the six weeks of the experimental period. Due to the observed consumption pattern, the GSD group exhibited a significant increase in various obesity parameters (e.g., body mass, body mass index, and weight of visceral and subcutaneous adipose tissue). In the biochemical parameters, higher cholesterol levels were observed in the HFD group, while the GHFD diet produced the most pronounced alterations in glucose and lipid profiles. At the molecular level, GHFD also induced upregulation of lipogenic genes (FASN, LPL) and downregulation of INSR and PPARγ, supporting the development of insulin resistance and obesity.

Beyond the experimental scope, these findings underscore the translational relevance of diet-induced obesity models in elucidating the mechanisms underlying human metabolic diseases. The observed alterations reflect patterns frequently reported in clinical obesity and type 2 diabetes, suggesting that this model can be useful for testing potential nutritional and pharmacological interventions. Considering the increasing global burden of obesity and related disorders, the present study provides insights that may contribute to the design of preventive and therapeutic strategies for mitigating the impact of obesogenic diets on human health.

Author's Contributions

J.M.G-O., A.A-M. and N. M-C: Data curation, methodology, formal analysis and writing—original draft. N. M-C: Funding acquisition, project administration and supervision. R.E-O: Writing—original draft. All authors have read and agreed to the published version of the manuscript.

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Ethical considerations

The ethics committee at the University Industrial de Santander approved this study (code: 4110) on June 29, 2023.

Competing interests

The authors declare no conflicts of interest.

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