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Soybean Flour Improves Fatty Acid Profile and Decreases Hepatic Triglyceride Deposition in Rats Fed with Normocaloric and Hypercaloric Diet

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Abstract

This study investigated the effects of replacing casein with soy flour on the fatty acids profile and triglycerides metabolism in the liver of rats that were previously fed with normocaloric and hypercaloric diets based on casein. *Wistar* male rats were used; one group was fed with control diet (AIN-93) and another with hypercaloric diet (AIN-93 with 34.15% sucrose, 42% fat calories) for 9 weeks. Each group was then divided into two subgroups and casein was replaced with soybean in one of them, obtaining CC (control casein), CS (control soy), HC (hypercaloric casein) and HS (hypercaloric soy), which were fed for 6 weeks. We measured triglycerides in serum, and triglycerides, total lipids, fatty acids profile, the expression of apolipoprotein B (Apo B), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), sterol-regulatory element-binding protein 1c (SREBP-1c), mitochondrial glycerol-3-phosphate acyltransferase (mGPAT), diacylglycerol acyltransferase 2 (DGAT-2), carnitine palmitoyltransferase 1 (CPT-1) and peroxisome proliferator-activated receptors alpha (PPARα) in liver. Histological studies were also performed. When comparing HS vs. HC, a positive effect of soybean flour on hepatic triglycerides deposits was found, possibly through the reduction in DGAT-2 expression ($P < 0.01$) and the increase in Apo B ($P < 0.001$) expression. Soybean flour also decreased fat deposits in control diets when compared with casein, decreasing the DGAT-2 ($P < 0.001$) expression and increasing Apo B ($P < 0.001$), CPT-1 ($P < 0.05$) and PPARα ($P < 0.01$) expressions. Both soy diet subgroups increased unsaturated fatty acids respect to casein diets ($P < 0.01$). Hepatocytes showed few lipid droplets in HS, whereas a fat deposit in HC was ob-

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served. These results suggest that replacing casein with soybean flour in normocaloric and hypercaloric diets reduces triglycerides and improves fatty acids profile in rat liver.

Keywords

Soybean Flour, Normocaloric and Hypercaloric Diets, Fatty Acids, Triglyceride Metabolism, Rat Liver

1. Introduction

It is known that diets with a high caloric content induce the development of obesity, glucose intolerance and hyperlipidemia [1]. These conditions predispose towards cardiovascular diseases, ischemic and cerebral vascular accidents, and peripheral vascular pathologies, which constitute the main cause of death worldwide. On the other hand, the Mediterranean diet, consisting of whole grains, fruits and vegetables, has been associated with lower incidence and prevalence of these diseases [2].

Recently, there has been a growing interest in promoting the use of natural food of vegetable origin due to its nutraceutical effects. Several studies in animals and humans have confirmed that diets based on soybean (*Glycine max*) have a significant impact on health, especially on the prevention of cardiovascular diseases, due to their hypolipemic action. Positive soybean effects have been mainly attributed to its protein composition [3], as well as to its isoflavones content, in particular genistein and daidzein [4].

A high dietary fat intake, in addition to an excessive caloric intake, contributes to the development of hepatic steatosis [5]. This is a common disease and a clear sign of lipotoxicity, attributable in part to an accelerated lipogenesis and the slow oxidation of fatty acids. Hepatic lipid metabolism is partially controlled by the transcription factors known as sterol regulatory element binding proteins (SREBPs) and peroxisome proliferator-activated receptors (PPARs). These transcription factors have different isoforms; in the liver, SREBP-1c and PPARα are the predominant forms.

SREBP-1c stimulates transcription of fatty acid biosynthesis genes such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) [6], and is activated in response to insulin [7]. In contrast, PPARα regulates genes involved in hepatic fatty acid oxidation [8], for example carnitine palmitoyltransferase 1 (CPT-1), an enzyme which regulates the fatty acid β-oxidation in the mitochondria.

Most studies on soybean use it as a protein concentrate or protein isolate. Considering that soy is a very important part of the diet in many cultures and that it is consumed as a whole grain, we decide to work with soybean flour. The aim of this study was to perform a biochemical-molecular and histological study to see if soybean flour modified the triglyceride metabolism and the fatty acids profile in the liver of male *Wistar* rats that were previously fed with normocaloric and hypercaloric casein diets.

2. Materials and Methods

2.1. Experimental Design

Twenty four *Wistar* male rats (Romanelly, Buenos Aires, Argentina), 21 days old and weighting 36.21 ± 2.30 g, were kept in individual cages at 25˚C and exposed to 12 h light: dark cycles, with food and water *ad-libitum*. The animals were subjected to a 10 day adaptation period with a diet prepared according to recommendations of the American Institute of Nutrition 1993 (the AIN-93G diet) [9]. Subsequently, they were separated into two groups: one group was kept with the AIN-93G diet, control casein (CC); and the other group hypercaloric casein (HC) was subjected to an hypercaloric diet, both for 9 weeks. The hypercaloric diets contained 341.4 g·Kg^{-1} sucrose and 42% of calories from fat [10]. After this period, AIN-93G diet was replaced by AIN-93M diet [9], and each group was divided into two subgroups, replacing casein by soy in one of them. Therefore, we ended up with a total of 4 subgroups: control casein (CC), control soy (CS), hypercaloric casein (HC) and hypercaloric soy (HS). Rats were fed with the respective diet for 6 weeks (**Figure 1**). At this time we believe that there is a lipid turnover efficient. The animals were sacrificed 12 h after the last feeding and blood was collected and

processed for serum. Liver and, epididymal and perirenal fat were extracted, weighed, and stored at −70˚C for subsequent analyses. Food intake and body weight were recorded every 3 days. The ingredients of each diet are shown in **Table 1**. Casein was purchased from Milkaut (Santa Fe, Argentina). Casein composition (pasteurized skim milk) was: total protein concentrated by ultracentrifugation: 800 $g \cdot Kg^{-1}$, lipids 30 $g \cdot Kg^{-1}$. Soybean flour was adquirid from *La esquina de las flores* group (Buenos Aires, Argentina). Soybean flour composition was: protein 366.6 $g \cdot Kg^{-1}$, lipids 236.6 $g \cdot Kg^{-1}$, carbohydrates 233.3 $g \cdot Kg^{-1}$, fiber 133.3 $g \cdot Kg^{-1}$, (prior heat treatment).

All studies involving experimental animals were conducted in accordance with national and institutional guidelines for the protection of animal welfare. The study was approved by the Animal Care Committee of the National University of San Luis.

2.2. Serum Parameters

Triglycerides were determined by the enzymatic method using commercial kits (Wiener Laboratories S.A.I.C. Rosario—Argentina. A.N.M.A.T. Registered product Cert. No. 3803/00).

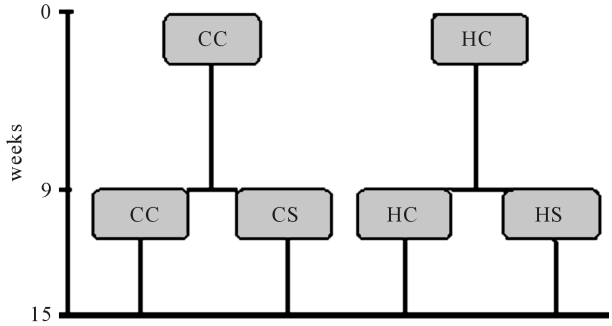

Figure 1. Experimental design. CC, control casein; CS, control soy; HC, hypercaloric casein; HS, hypercaloric soy.

Table 1. Composition of the experimental diets.

Ingredients ($g \cdot Kg^{-1}$)	Groups					
	CC*	HC*	CC†	CS†	HC†	HS†
Cornstarch	397.49	139.00	465.69	389.33	200.00	135.00
Protein sources[a]	212.50	212.50	150.00	327.33	150.00	327.33
Dextrinized cornstarch	119.50	-	145.00	127.67	-	-
Sucrose	100.00	341.46	100.00	100.00	341.46	341.46
Animal fat	-	136.50	-	-	168.38	139.09
Soybean oil	70.00	70.00	40.00	-	40.00	-
Fiber	50.00	50.00	50.00	6.37	50.00	6.37
Mineral mix	35.00	35.00	35.00	35.00	35.00	35.00
Vitamin mix	10.00	10.00	10.00	10.00	10.00	10.00
L-Cystine	3.00	3.00	1.80	1.80	1.80	1.80
Choline bitartrate	2.50	2.50	2.50	2.50	2.50	2.50
Tert-butylhydroquinone	0.014	0.014	0.008	0.008	0.008	0.008

CC, control casein; HC, hypercaloric casein; CS, control soy; HS, hypercaloric soy. *AIN 93G; †AIN 93M; [a]Protein sources: casein, 80 g total protein per 100 g of milk; soybean flour, 36.66 g protein per 100 g of soybean flour.

2.3. Hepatic Lipids Determinations

Lipids were extracted from liver tissue (300 mg) in a hexane/isopropanol mixture (3:2 v/v), containing butylated hydroxytoluene as antioxidant [11]. Total lipids were determined by dry weight [12]. Lipids were resuspended in hexane, and aliquots of the extracts were placed on thin-layer chromatography (TLC) plates coated with silica gel G, using hexane/diethyl ether/acetic acid (80:20:1, v/v/v) as the running solvent, for the separation of the different lipids. Triglycerides were detected by exposing the plates to iodine vapor; they were scraped off and used for the determination of triglycerides [13]. Proteins were determined by the Biuret method [14], with bovine serum albumin as standard (BSA or Fraction V) acquired from Sigma-Aldrich (St. Louis, Missouri, United States).

Capillary gas-liquid chromatography (c-GLC) of the fatty acid methyl esters (FAME) was performed according to Marra and de Alaniz [15]; although in this case, we used a capillary column (Omegawax 250, from Bellefonte, Supelco, PA) mounted on a Hewlett Packard HP 6890 Series GC System Plus (Avondale, PA), equipped with a terminal computer integrator and a data station. A standard procedure for a single-step preparation of dimethyl disulfide adducts of the fatty acids was performed according to Yamamoto *et al.* [16].

2.4. mRNA Expression by RT-PCR Analyses

The relative mRNA quantities of apolipoprotein B (Apo-B), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), mitochondrial glycerol-3-phosphate acyltransferase (mtGPAT), diacylglycerol acyltransferase 2 (DGAT-2), sterol-regulatory element-binding protein1c (SREBP-1c), carnitine palmitoyltransferase 1 (CPT-1) and peroxisome proliferator-activated receptors alpha (PPARα), were determined by reverse transcription polymerase chain reaction (RT-PCR).

Total RNA from the liver was isolated using TRIZOL Reagent (Invitrogen, Buenos Aires, Argentina), and cDNA synthesis was performed using 200 IU of Moloney Murine Leukemia Virus Reverse Transcriptase (Invitrogen/life Technologies, Buenos Aires, Argentina) and random hexamer primers. cDNA was amplified for 5 min at 94˚C followed by cycles of 1 min of denaturation at 94˚C, 1 min of annealing at primers specific temperatures and 1 min of elongation at 72˚C, followed by a final extension at 72˚C for 5 min.

The sequences of the different primers and sizes of the PCR products are shown in **Table 2**.

The PCR products were analyzed on 2.5% agarose gels containing 0.008% (v/v) of fluorescent nucleic acid gel stains, and photographed under UV transillumination. The intensity of each band was measured using the NIH Image software Scion. The relative abundance of each target band was then normalized according to the housekeeping gene β-actin, calculated as the ratio of the intensity values of each target product to that of β-actin, and reported in arbitrary units (AU).

2.5. Morphological Study

Histological analysis of liver samples was performed with light microscopy. Pieces of liver were excised and immediately fixed in Bouin's liquid. All sections were obtained from the same region of the liver for effective comparison. Sections were stained with hematoxylin-eosin and photographs were taken using a Leitz Dialux optical microscope equipped with a Leica camera (400×).

2.6. Statistical Analysis

The data were expressed as means ± standard deviations. The results were analyzed using one-way analysis of variance (ANOVA), provided by the multiple comparison of Tukey-Kramer means test. A probability of 0.05 or less indicates significant difference [17]. The following comparisons between groups were performed: CS vs.CC, HS vs. HC and HS vs. CS.

3. Results

3.1. Growth Variables and Tissue Weights in Rats

Food intake, body weight gain, liver weight, and epididymal and perirenal fat in rats are shown in **Table 3**. Food intake did not vary among the different groups. Respect to body weight gain, it was observed that CS rats gained less weight than those from the CC group ($P < 0.01$) and similar behavior occurred when comparing HS vs. HC

Table 2. Sequences of the gene-specific primers and sizes of the PCR products.

Primers	Sequences		
	Sense (5'-3')	Antisense (5'-3')	Size (bp)
Apo-B	TACCTCCGGCAGCTCCATTCC	TGCGCTTCCTGCTCTTGCTGTT	340
ACC	ACTCCAGGACAGCACAGATC	TCTGCCAGTCCAATTCTAGC	535
FAS	GTTTGATGGCTCACACACCT	TACACTCACTCGAGGCTCAG	515
SREBP-1c	GGAGCCATGGATTGCACATT	AGGAAGGCTTCCAGAGAGGC	193
mtGPAT	TGATCAGCCAGGAGCAGCTG	AGACAGTATGTGGCACTCTC	508
DGAT-2	GGAGGCCACCGAAGTTAGCAAGAA	AGCCCCCAGGTGTCAGAGGAGAAG	453
CPT-1	TATGTGAGGATGCTGCTTCC	CTCGGAGAGCTAAGCTTGTC	629
PPARα	TCAGGGTACCACTACGGAGTTCA	CCGAATAGTTCGCCGAAAGA	106
β-actin	CGTGGGCCGCCCAGGCACCA	TTGGCCTTAGGGTTCAGAGGG	243

Apo-B, apolipoprotein B; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SREBP-1c, sterol-regulatory element-binding protein 1c; mtGPAT, mitochondrial glycerol-3-phosphate acyltransferase; DGAT-2, diacylglycerol acyltransferase 2; CPT-1, carnitine palmitoyltransferase 1; PPARα, peroxisome proliferator-activated receptors alpha.

Table 3. Effects of diets on growth variables and tissue weights in rats.

Parameters	Groups			
	CC (*n* 6)	CS (*n* 6)	HC (*n* 6)	HS (*n* 6)
Food intake (g/120d)	1504.52 ± 37.13	1594.35 ± 69.86	1535.17 ± 50.28	1605.02 ± 73.21
Body weight, gain g/120d[a]	351.37 ± 10.61	288.78 ± 16.09••	386.12 ± 32.23	342.91 ± 25.91°*
Liver weight (g)	11.07 ± 0.67	9.11 ± 0.47••	11.94 ± 0.80	10.24 ± 0.73°°
Liver (g/100g body wt.)	2.77 ± 0.24	2.92 ± 0.11	2.75 ± 0.05	2.75 ± 0.20
Epididymal fat (g/100g body wt.)	0.72 ± 0.10	0.65 ± 0.10	1.40 ± 0.10	1.10 ± 0.16°**
Perirenal fat (g/100g body wt.)	1.46 ± 0.11	0.87 ± 0.15••	1.90 ± 0.14	1.25 ± 0.30°°

Values are means ± SD (*n* = 6). Analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison test. •CS vs. CC: ••P < 0.01; °HS vs. HC: °P < 0.05, °°P < 0.01; *HS vs. CS: *P < 0.05, **P < 0.01. CC, control casein; CS, control soy; HC, hypercaloric casein; HS, hypercaloric soy. [a]Final weight minus initial weight.

(P < 0.05), furthermore animals fed with HS diet showed a higher increment of body weight gain than those fed with CS diets (P < 0.05). Animals fed with soy flour showed lower liver weight than those fed with casein diets, in both control and hypercaloric diets (P < 0.01). Rats fed with HS diet showed a decrease in the relative epididymal fat weight (per 100 g of body weight) when compared to HC group (P < 0.05), also HS showed an increase when compared to the respective control (P < 0.01). Regarding the perirenal fat, animals fed with soy flour showed decreased relative perirenal fat weights (per 100 g of body weight) compared to those fed with casein diets (P < 0.01).

3.2. Triglycerides in Serum, and Triglycerides and Total Lipids in Liver

Triglycerides in serum, and triglycerides and total lipids in liver, are shown in **Figure 2**. Serum triglycerides showed no differences between groups. Regarding the hepatic triglycerides content, both control and hypercaloric soy flour diets showed significantly lower triglycerides than casein diets, (P < 0.05) and (P < 0.01), respectively, and was observed a significant increased when comparing HS vs. CS (P < 0.05). Variations in the total lipids content followed a relation with the hepatic triglyceride deposits, being lower in soy flour diets compared to casein diets, CS vs. CC (P < 0.001) and HS vs. HC (P < 0.01), with an increase in HS group compared to CS group (P < 0.001).

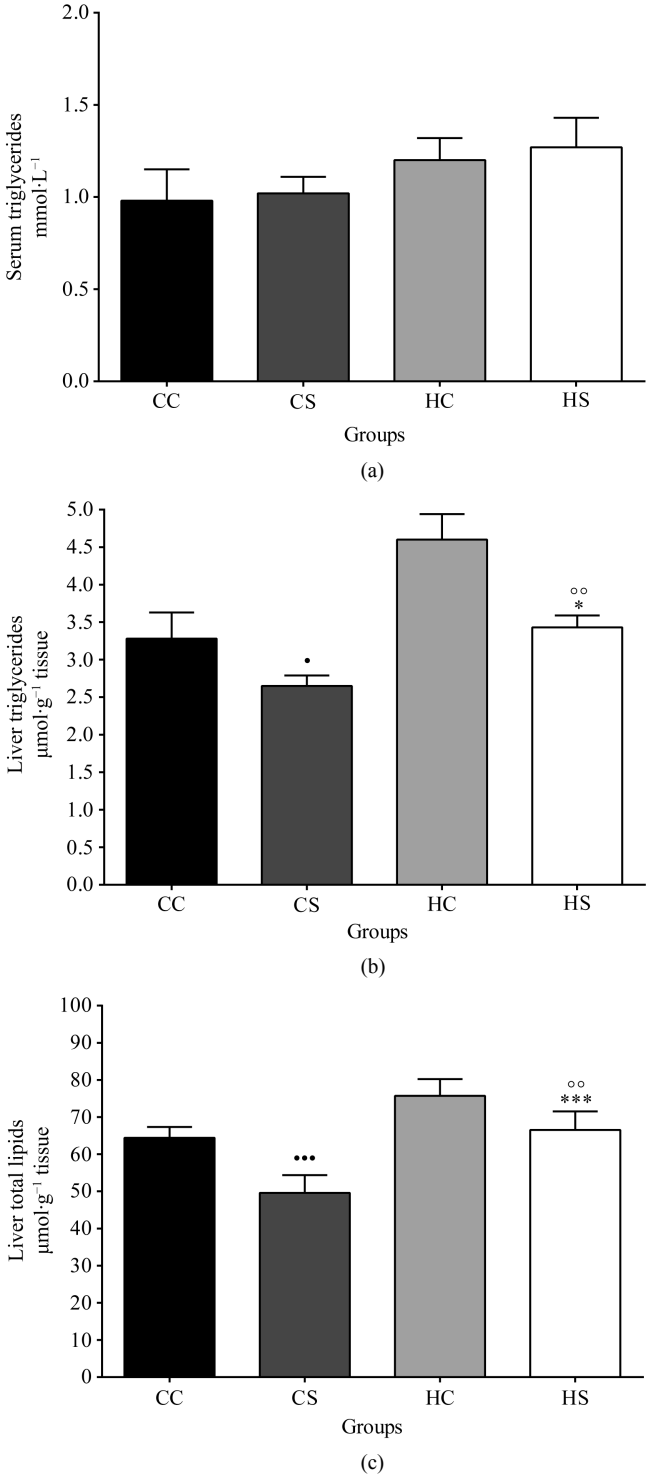

Figure 2. Effects of diets on (a) triglycerides in serum, and (b) triglycerides and (c) total lipids in liver. Values are means ± SD (n = 6). Analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison test. •CS vs. CC: •P < 0.05; •••P < 0.001; °HS vs. HC: °°P < 0.01; *HS vs. CS: *P < 0.05; ***P < 0.001. CC, control casein; CS, control soy; HC, hypercaloric casein; HS, hypercaloric soy.

3.3. mRNA Levels of Enzymes and Transcription Factors Involved in Lipogenesis and Fatty Acid Oxidation in Liver Rat

Table 4 shows the mRNA levels of Apo-B, lipogenic enzymes and SREBP-1c. Soy flour significantly increased the expression of Apo-B gene in soy diets when compared to casein, CS vs. CC and HS vs. HC ($P < 0.001$). In order to find out the variations in the lipogenic enzymes expression and its regulation, we determined the expression of ACC, FAS, mtGPAT, DGAT-2 and SREBP-1c. There were no significant differences in the expression of ACC, FAS and SREBP-1c among the groups. The mtGPAT expression was decreased in HS group when compared to CS ($P < 0.001$). Soy flour significantly decreased DGAT-2 gene expression in soy diets groups when compared to casein, CS vs. CC ($P < 0.001$) and HS vs. HC ($P < 0.01$). mRNA levels of genes involved in fatty acid oxidation are also shown in Table 4. The expressions of CPT-1 and PPARα in rats fed with CS diet were significantly higher than those in rats fed with CC diet, with ($P < 0.05$) and ($P < 0.01$), respectively. The mRNA abundance of both transcripts was significantly lower in rats fed with HS diet than in animals fed with CS diet, ($P < 0.05$) and ($P < 0.001$), respectively.

3.4. Fatty Acids Composition in Rat Liver

The analysis of the fatty acids profiles in rat liver (Table 5), showed that replacing the casein diets by a vegetable protein, such as soybean flour, induces an increase of unsaturated fatty acids and a decrease of saturated ones ($P < 0.01$), even with the hypercaloric diets. There was a clear decrease in lauric, myristic and palmitic acids in liver of rats fed with soy. The unsaturated: saturated fatty acids ratio (unsat: sat), increased in both soy diets compared with casein. The total omega-6 polyunsaturated fatty acids (PUFA n-6) was significantly higher in the CS and HS groups compared to the casein groups ($P < 0.01$). Similar behavior was observed in total omega-3 polyunsaturated fatty acids (PUFA *n*-3), and a lower *n*-6:*n*-3 ratio was observed in soy groups compared to casein groups.

3.5. Histological Studies

Liver histological studies showed that the parenchyma of the CC group rats, had hepatocytes infiltrated by fatty deposits; while in the parenchyma of the CS group rats, fatty deposits were rare and localized, and the hepatocytes had a normal appearance with lipid droplets in the cytoplasm. The parenchyma of the HC group rats showed widely distributed fatty deposits, occupying most of the cytoplasm, which had a spongy appearance; however in HS group, the hepatocytes had a normal histoarchitecture with few lipid droplets (Figure 3).

Table 4. Effects of diets on mRNA levels of enzymes and transcription factors involved in lipogenesis and fatty acid oxidation in liver rat.

Parameters (AU)	Groups			
	CC (*n* 6)	CS (*n* 6)	HC (*n* 6)	HS (*n* 6)
Apo B	0.75 ± 0.04	1.04 ± 0.05•••	0.80 ± 0.03	1.09 ± 0.04°°°
ACC	0.34 ± 0.03	0.35 ± 0.04	0.32 ± 0.02	0.34 ± 0.04
FAS	0.85 ± 0.05	0.99 ± 0.08	0.82 ± 0.07	0.82 ± 0.07
SREBP-1c	1.49 ± 0.14	1.67 ± 0.10	1.57 ± 0.12	1.59 ± 0.18
mtGPAT	1.00 ± 0.08	0.93 ± 0.02	0.79 ± 0.01	0.74 ± 0.04***
DGAT-2	1.06 ± 0.04	0.80 ± 0.03•••	0.97 ± 0.02	0.86 ± 0.02°°
CPT-1	0.71 ± 0.06	0.87 ± 0.09•	0.65 ± 0.03	0.65 ± 0.06*
PPARα	0.83 ± 0.04	0.97 ± 0.01••	0.83 ± 0.03	0.77 ± 0.03***

Values are means ± SD (*n* = 6). Analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison test. •CS vs. CC: •P < 0.05; ••P < 0.01, •••P < 0.001; °HS vs. HC: °°P < 0.01, °°°P < 0.001; *HS vs. CS: *P < 0.05; ***P < 0.001. CC, control casein; CS, control soy; HC, hypercaloric casein; HS, hypercaloric soy. AU, arbitrary units. Apo-B, apolipoprotein B; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SREBP-1c, sterol-regulatory element-binding protein 1c; mtGPAT, mitochondrial glycerol-3-phosphate acyltransferase; DGAT-2, diacylglycerol acyltransferase 2; CPT-1, carnitine palmitoyltrans ferase 1; PPARα, peroxisome proliferator-activated receptors alpha.

Table 5. Effects of diets on fatty acid composition in rat liver.

Fatty acids ($\mu mol \cdot mg^{-1}$ of total lipids)	Groups			
	CC (*n* 6)	CS (*n* 6)	HC (*n* 6)	HS (*n* 6)
12:0	0.8 ± 0.1^{a}	0.2 ± 0.1^{b}	0.9 ± 0.2^{a}	0.3 ± 0.03^{b}
14:0	2.5 ± 0.1^{a}	0.5 ± 0.1^{b}	3.1 ± 0.2^{c}	0.4 ± 0.02^{b}
15:0	0.1 ± 0.03^{a}	0.1 ± 0.01^{a}	0.2 ± 0.07^{a}	0.1 ± 0.06^{a}
16:0	18.8 ± 0.7^{a}	13.2 ± 0.3^{b}	19.5 ± 0.5^{a}	16.1 ± 0.3^{c}
16:1 *n*-7	2.0 ± 0.1^{a}	2.5 ± 0.1^{a}	2.2 ± 0.2^{a}	2.7 ± 0.2^{a}
16:1 *n*-4	0.2 ± 0.01^{a}	0.2 ± 0.05^{a}	0.3 ± 0.03	0.1 ± 0.04
17:0	0.1 ± 0.02^{a}	0.2 ± 0.02^{a}	0.1 ± 0.04^{a}	0.2 ± 0.03^{a}
18:0	29.2 ± 0.8^{a}	24.1 ± 0.8^{b}	28.5 ± 0.4^{a}	23.5 ± 0.5^{b}
18:1 *n*-9	5.3 ± 0.3^{a}	6.0 ± 0.2	5.1 ± 0.2^{a}	7.9 ± 0.3^{b}
18:1 *n*-7	0.5 ± 0.2^{a}	0.8 ± 0.2^{a}	0.7 ± 0.2^{a}	0.9 ± 0.2^{a}
18:2 *n*-6	17.2 ± 0.3^{a}	23.9 ± 0.4^{b}	18.0 ± 0.3^{a}	27.2 ± 0.5^{c}
18:3 *n*-6	0.3 ± 0.03^{a}	0.2 ± 0.01^{a}	0.2 ± 0.04^{a}	0.2 ± 0.02^{a}
18:3 *n*-3	0.1 ± 0.03^{a}	3.1 ± 0.2^{b}	0.2 ± 0.06^{a}	3.2 ± 0.1^{b}
20:0	2.5 ± 0.2^{a}	1.1 ± 0.1^{b}	2.9 ± 0.2^{a}	1.0 ± 0.2^{b}
20:1 *n*-9	1.5 ± 0.4^{a}	1.9 ± 0.2^{a}	1.6 ± 0.3^{a}	2.0 ± 0.2^{a}
20:2 *n*-6	1.6 ± 0.1^{a}	0.5 ± 0.1^{b}	1.7 ± 0.2^{a}	0.7 ± 0.1^{b}
20:3 *n*-6	1.5 ± 0.1^{a}	3.5 ± 0.1^{b}	1.9 ± 0.2^{a}	3.4 ± 0.1^{b}
20:3 *n*-9	0.8 ± 0.1^{a}	Traces^{b}	2.2 ± 0.2^{c}	0.2 ± 0.05^{d}
20:4 *n*-6	24.3 ± 0.4^{a}	33.1 ± 0.6^{b}	26.1 ± 0.5^{a}	31.0 ± 0.5^{b}
20:5 *n*-3	0.5 ± 0.1^{a}	1.5 ± 0.1^{b}	0.4 ± 0.1^{a}	1.8 ± 0.1^{b}
22:4 *n*-3	0.7 ± 0.1^{a}	0.8 ± 0.1^{a}	1.3 ± 0.1^{b}	2.0 ± 0.2^{b}
22:4 *n*-6	1.1 ± 0.1^{a}	1.5 ± 0.1^{a}	1.2 ± 0.1^{a}	1.6 ± 0.1^{a}
22:5 *n*-3	2.2 ± 0.1^{a}	3.4 ± 0.2^{b}	2.5 ± 0.2^{a}	3.5 ± 0.1^{b}
22:5 *n*-6	0.2 ± 0.01^{a}	0.3 ± 0.01^{a}	0.3 ± 0.1^{a}	0.2 ± 0.03^{a}
22:6 *n*-3	5.3 ± 0.1^{a}	7.9 ± 0.2^{b}	4.9 ± 0.1^{a}	7.5 ± 0.2^{b}
Total unsaturated fatty acid	65.3 ± 3.0^{a}	91.1 ± 2.9^{b}	70.8 ± 2.6^{a}	96.1 ± 3.0^{b}
Total saturated fatty acid	54 ± 2.0^{a}	39.48 ± 1.4^{b}	55.2 ± 2.0^{a}	41.6 ± 1.4^{b}
Unsatured:satured	1.20	2.30	1.28	2.31
Total PUFA *n*-6	46.2 ± 1.0^{a}	63.0 ± 1.3^{b}	49.4 ± 1.4^{a}	64.3 ± 1.3^{b}
Total PUFA *n*-3	8.8 ± 0.4^{a}	16.7 ± 0.8^{b}	9.3 ± 0.7^{a}	18.0 ± 0.7^{b}
n-6 : *n*-3 ratio	5.25	3.77	5.31	3.57

Values are means ± SD ($n = 6$). Analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparison test. Means in row with different letter differ significantly ($P < 0.01$). CC, control casein; CS, control soy; HC, hypercaloric casein; HS, hypercaloric soy. PUFA *n*-6, omega-6 polyunsaturated fatty acids; PUFA *n*-3, omega-3 polyunsaturated fatty acids.

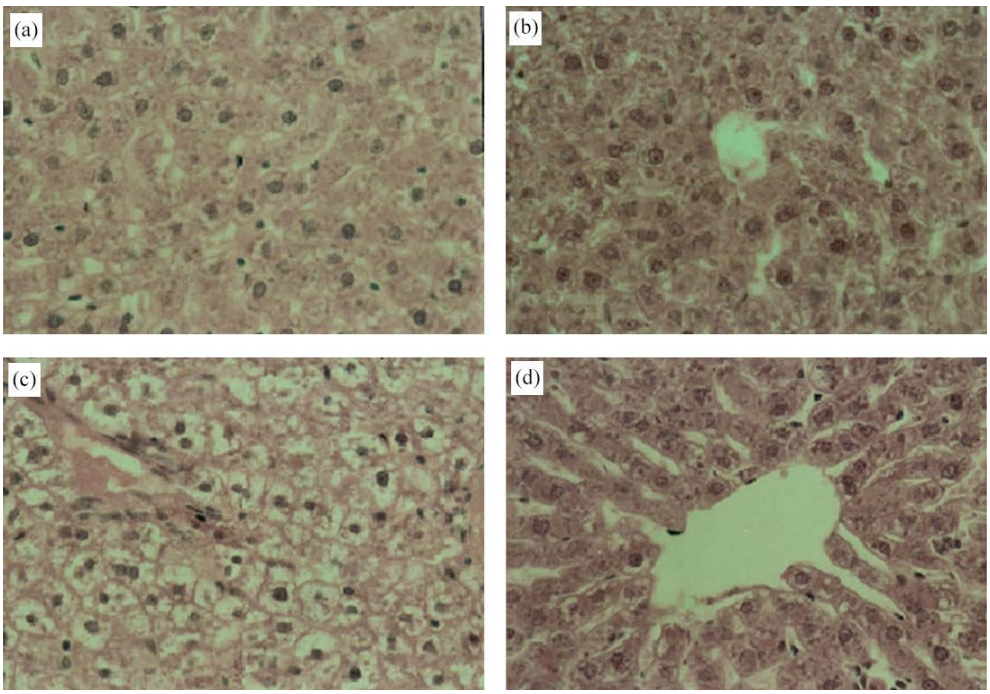

Figure 3. Photomicrographs of the effects of diets on hepatic parenchyma in rats. (a) Parenchyma of rats CC (control casein) group. (b) Parenchyma of rats CS (control soy) group. (c) Parenchyma of rats HC (hypercaloric casein) group. (d) Parenchyma of rats HS (hypercaloric soy) group.

4. Discussion

It is known that dietary composition significantly influences the development of certain chronic diseases [18], and that the long term consumption of a diet high in fat accelerates the development of obesity [19]. Numerous investigations indicate that soybean has many nutritional attributes, given by its content of isoflavones, essential amino acids, fibers, poly-unsaturated fatty acids, vitamins and minerals. There are multiple studies on the different mechanisms of action of soy protein and its isoflavones, but there is little literature on the potential effects of soy flour (whole grain) as a possible treatment for to improve lipid metabolism. Considering that soy is consumed essentially in the form of flour or whole grain, we decided to investigate if soybean flour is capable of modifying triglyceride content and fatty acid profile in the rats liver, after were subjected to a casein diet for 9 weeks.

Rats fed with soy diets gained significantly less weight than those fed with casein diets. This difference could be mainly due to the lower body fat content observed, in particular in the epididymal and perirenal fat, as well as a lower liver weight. Al-Dwairi *et al.* [20] reported that inclusion of soy protein isolate in the diet lowered body fat content, and found that wild type mice fed with soy protein isolate showed significantly reduced weight gains compared to those fed with casein hypercaloric diet. Our results also agree with those obtained by Torre-Villalvazo *et al.* [21]. On the other hand other components of soybean flour like flavonoids could contribute to less body weight [22]. Furthermore, our results show that soy flour provides more amount of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) than casein. It is know that these fatty acids have anti-obesity effects and reduce body weight [23]. These results suggest that soybean began to decrease fat accumulation caused by the previously administered casein diets.

We found no differences in serum triglycerides levels between rats fed with soy and those fed with casein. The results reported in the literature are controversial, some authors have reported a decrease [24] and others an increase [25], and there are also studies reporting no differences in this parameter [26]. Regarding triglycerides in the liver, we observed less accumulation in rats fed with soy-based diets, and this difference was more significant in the hypercaloric groups. Ascencio *et al.* [27] found that hepatic triglycerides were reduced by consumption of soy protein or soy protein high fat diets compared with rats fed with casein or casein high fat diets. Thus, we conclude that soybean flour with a protein concentration of 36.66% protects against hepatic triglyceride de-

positionin this model, being this effect more marked in the case of hypercaloric groups.

Apo B, an essential structural component of very low density lipoprotein (VLDL) and low density lipoprotein (LDL), must be assembled into lipoprotein particles before being secreted from hepatocytes [28]. The expression of Apo-B increased in soybean diets compared to casein diets, this behavior correlate with hepatic triglycerides levels.

The fact that there were no changes in the expression of the ACC and FAS genes, and the SREBP-1c involved in the fatty acid synthesis, suggests that they probably suffer post transcriptional modifications. However Xiao *et al*. [29] found that ingestion soy protein isolate decreases plasma TAG level and down-regulates ACC gene expression in the liver. Besides, Shukla *et al.* [30] observed that isoflavone-poor soy protein isolate alters lipid metabolism by the down-regulation of SREBPs and its target genes in the liver.

Hepatic triglyceride deposits are lower in animals fed with soybean flour. In order to better understand these results, we determined the expression of genes of enzymes involved in the synthesis of triglycerides: mtGPAT and DGAT-2. The decrease of the mtGPAT expression when comparing HS vs. CS, suggests a possible down-regulation of the enzyme due to excess fat. Furthermore, an interesting behavior was observed in the expression of DGAT-2 gene, enzyme that catalyzes the final step in the main pathway for hepatic triglycerides synthesis. The lower expression in soybean diets compared to casein diets may be due to the higher content of polyunsaturated fatty acids present in the soy flour. This is in accord with Rustan *et al.* [31], who demonstrated that diets with omega-3 fatty acids can inhibit the activity of DGAT-2. A significant increase of the total PUFA *n*-3 in both soy diets, compared to casein, is observed in our model.

CPT-1 is an enzyme that catalyzes the ingress of fatty acids into the mitochondria for subsequent oxidation, and can be regulated by PPARα. The regulatory action of PPARα is on lipid metabolism that acts on the uptake, the activation and the mitochondrial/peroxisomal β-oxidation of fatty acids [32] [33]. Considering these two genes (CPT-1 and PPARα), we observed that in normocaloric diets, soy flour compared to casein exerts a positive control on fatty acids transport into the mitochondria and could favor their catabolism. This effect is supported by the higher expression of PPARα. The bigger expression of both genes could be attributed to the higher content of unsaturated fatty acids supplied by the soy diet. On the other hand, the lower expression of CPT-1 and PPARα in HS compared to CS can be attributed to the higher content of saturated fatty acids in the HS diet, which exerts an inhibitory effect on the mentioned genes. Sampath and Ntambi [34] reported that the changes in the fatty acids saturation status were implicated in the process of down-regulation of genes involved in fatty acids oxidation.

The diet fatty acids composition is known to influence the fatty acid composition of stored and structural lipids in the body [35] [36].

In the analysis of the fatty acids profile in liver of rats fed with soybean flour, compared to those fed with casein, we found a favorable unsat: sat ratio. This allows us to assume that soybean flour, even in hypercaloric diets, would have a positive effect on the prevention of simple steatosis or non-alcoholic steatohepatitis [37]. In addition, respect to polyunsaturated fatty acids a lower omega-6:omega-3 ratio was observed in the soy diet groups compared to the casein groups; thus, exerting a positive effect in preventing many diseases, including: cardiovascular disease, inflammatory and autoimmune diseases [38]. On the other hand, the flavonoids present in soy flour act synergistically with omega-3 fatty acids exert an anti inflammatory effect [39] [40].

In a liver histological study, Ascencio *et al.* [27] observed that rats fed with casein or casein high fat diets presented an increase in the number of oil droplets in the liver compared with rats fed with soy protein or soy protein high fat diets. We saw that soybean flour decreased fat deposits when compared to casein; and if we consider that the major portion of those deposits is due to the hepatic content of triglycerides, these data are consistent with the biochemical and molecular results showed in this study.

5. Conclusions

Under our experimental conditions, we can conclude that in animals that are being fed with a casein based normocaloric diet, replacing it with whole grain soybean flour exerts a protective effect on hepatic triglyceride deposition and improves the fatty acid profile. This behavior was also observed in hypercaloric diets, although in less extent.

We believe that consumption of soybean flour (whole grain) is a very good dietary resource for improving hepatic triglyceride deposits and fatty acid profile, and therefore, it has a beneficial health effect.

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List of Abbreviations

ACC	Acetyl-CoA Carboxylase
AIN-93	American Institute of Nutrition 1993
ANOVA	One-Way Analysis of Variance
Apo B	Apolipoprotein B
AU	Arbitrary Units
CC	Control Casein
c-GLC	Capillary Gas-Liquid Chromatography
CPT-1	Carnitine Palmitoyltransferase 1
CS	Control Soy
DGAT-2	Diacylglycerol Acyltransferase 2
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
FAME	Fatty Acid Methyl Esters
FAS	Fatty Acid Synthase
HC	Hypercaloric Casein
HS	Hypercaloric Soy
LDL	Low Density Lipoprotein
mGPAT	Mitochondrial Glycerol-3-Phosphate Acyltransferase
PPARα	Peroxisome Proliferator-Activated Receptors Alpha
PUFA *n*-3	Omega-3 Polyunsaturated Fatty Acids
PUFA *n*-6	Omega-6 Polyunsaturated Fatty Acids
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SREBP-1c	Sterol-Regulatory Element-Binding Protein 1c
TG	Triglycerides
TLC	Thin-Layer Chromatography
VLDL	Very Low Density Lipoprotein