



The pecan nut (*Carya illinoensis*) and its oil and polyphenolic fractions differentially modulate lipid metabolism and the antioxidant enzyme activities in rats fed high-fat diets



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ABSTRACT

Tree nuts such as pecans (*Carya illinoensis*) contain mostly oil but are also a source of polyphenols. Nut consumption has been linked to a reduction in serum lipid levels and oxidative stress. These effects have been attributed to the oil while overlooking the potential contribution of the polyphenols. Because the evidence regarding each fraction's bioactivity is scarce, we administered high-fat (HF) diets to male Wistar rats, supplementing them with pecan oil (HF + PO), pecan polyphenols (HF + PP) or whole pecans (HF + WP), and analysed the effects of each fraction. The HF diet increased the serum leptin and total cholesterol (TC) with respect to the control levels. The HF + WP diet prevented hyperleptinemia and decreased the TC compared with the control. The HF + WP diet upregulated the hepatic expression of apolipoprotein B and LDL receptor mRNAs with respect to the HF levels. The HF + PO diet reduced the level of triacylglycerols compared with the control. The HF + PP diet stimulated the hepatic expression of liver X receptor alpha mRNA. The HF + WP diet increased the activities of hepatic catalase, glutathione peroxidase and glutathione S transferase compared with the control, and decreased the degree of lipid peroxidation compared with the HF diet. The most bioactive diet was the WP diet.

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1. Introduction

A high consumption of saturated lipids can exert negative health effects by contributing to the development of obesity and obesity-related conditions, such as dyslipidemia and oxidative stress (Yang, Li, Shi, & Le, 2008). Such diets can induce dyslipidemia by increasing the concentration of circulating LDL cholesterol and decreasing the concentration of HDL cholesterol. LDL has apolipoprotein B (APOB) as its main protein component, and its serum concentration is regulated by the LDL receptor (LDLR) (Twisk et al., 2000). It is therefore possible to modify the LDL cholesterol concentration by altering the expression of APOB and or LDLR. Liver X receptor alpha (LXR α) is a transcription factor that is related to cholesterol homeostasis that favours the reversal of

cholesterol transport that is conducted by the HDL particles (Calkin & Tontonoz, 2012). One of its target genes is sterol regulatory-element binding protein 1c (SREBP1c), a transcription factor that can stimulate genes related to *de novo* fatty-acid synthesis (Cagen et al., 2005). Both LXR α and SREBP1c have also been found to be intricately related to the serum triacylglycerol (TAG) concentration (Shearer, Savinova, & Harris, 2012), which makes them likely targets of different dietary components that have an impact on serum lipids.

Oxidative stress can also occur in response to diet, in obese individuals and in many diseases (Hybertson, Gao, Bose, & McCord, 2011). The correlation between fat consumption and oxidative stress is not simple because a number of biochemical mechanisms involved in the catabolism of fatty acids yield H₂O₂ as a byproduct, which in addition to increased oxygen consumption will generate other oxidant molecules, all of which can potentially damage cells if their concentrations are not maintained within a tolerable range (Seifert, Estey, Xuan, & Harper, 2010). The main enzymes

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responsible for the antioxidant response are catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione S transferase (GST), which act in synchrony to protect cells against oxidant molecules.

Pecans (*Carya illinoensis*) are tree nuts that are native to the southern United States and northern Mexico but are also cultivated in other regions of the world, such as Australia, South Africa, Israel and South America (Wakeling, Mason, D'Arcy, & Caffin, 2001). They are rich in unsaturated lipids and their regular consumption can improve the serum lipid profile and the antioxidant status (Ros, 2009). This evidence has been well documented since the Food and Drug Administration (FDA) stated that consumption of tree nuts, including pecans, may reduce the risk of heart disease (FDA, 2003). Pecan oil is thought to be responsible for the health benefits of pecan consumption because it contains mono- and polyunsaturated fatty acids, phytosterols, tocopherols and other micronutrients (Domínguez-Avila et al., 2013). However, the non-lipid fraction is also rich in bioactive compounds, including ellagic and gallic acids, catechin, epicatechin, hydrolysable and condensed tannins, the biological activities of which have been underestimated (de la Rosa et al., 2014).

Therefore, the main goal of this work was to distinguish the effects of different pecan fractions (oil, polyphenols and whole nut) on the control of dyslipidemia and on alterations in the redox equilibrium caused by a high-fat diet.

2. Materials and methods

2.1. Extraction of pecan oil and the polyphenolic fraction

Pecan nuts were kindly donated by producers in three different locations (Ricardo Flores Magón, Delicias and Jiménez) in northern Mexico and were pooled. Pecan oil (PO) was extracted using n-hexane (1:10 w/v ratio) in a commercial blender, and the mixture was vacuum-filtered to separate the hexane-dissolved oil and the defatted matter. The solvent was eliminated by rotary evaporation, and the recovered oil was allowed to stand for 6 h in an open plastic container to eliminate any remaining hexane. The extraction process was repeated until the required amount of oil needed for the diets was obtained (1.4 L). An analysis of the phytochemical composition of the oil was previously reported (Domínguez-Avila et al., 2013). The polyphenols were extracted from the defatted pecan matter using 80% acetone and were included in the diets as a freeze-dried solid after the gallic acid equivalents were quantified using the Folin-Ciocalteu method (de la Rosa et al., 2014).

2.2. Animals and diets

The experiment followed the Mexican guideline NOM-062-ZOO-1999 for laboratory animals and was also reviewed and approved by the bioethics committee of the Universidad Autónoma de Ciudad Juárez (UACJ). Thirty male Wistar rats weighing an average of 148 g were randomly divided into five groups (six rats in each group) and were individually housed in polypropylene cages at an average temperature of 23–26 °C and with a 12 h light/dark cycle (07:00–19:00). Five different diets were prepared before beginning the experiment and were frozen in batches of 120 g in plastic bags at –80 °C; batches were immediately before administering the diets to the animals. Five treatments were administered for a 63-day (9 week) period after an initial week of adaptation, as follows: (1) the control group consumed a diet with an optimal concentrations of macro- and micronutrients; (2) the high-fat (HF) group consumed a diet that contained an increased concentration of lipids; (3) the HF + PO group (PO:

pecan oil) group consumed a diet similar to the HF diet except that half of the lipids of the HF diet were substituted with PO while maintaining the same percentage of lipids; (4) the HF + PP group (PP: pecan polyphenols) ingested the same percentage of lipids as the HF group except that it was supplemented with 1.0 mg of pecan polyphenols/g of diet; and (5) the HF + WP group (WP: whole pecan) received a diet that had the percentage of lipids as the HF diet except that half of the lipids were substituted by an amount of ground pecan nuts sufficient to compensate for the necessary amount of lipids. Food grade casein, cellulose, vitamin mix, salt mix and choline chloride were purchased from Bio-Serv (Cat. no. 1100, 3425, F8000, F8505 and 6105, respectively). Starch (Maizena, Mexico), lard (Inca lard, Mexico) and corn oil (Dorasol, Mexico) were obtained from a local grocery store (Table 1). The rats had ad libitum access to water and food. The food that was not consumed each day was weighed and replaced with fresh food. The amounts of ingested food were recorded daily and converted into calories, and the animals' weights were also recorded daily. The rats were anesthetized at the end of the experiment by an intramuscular injection of zoletil 50 (zolazepam and tiletamine 50 mg/mL of each) and the livers were immediately removed, weighed and frozen at –80 °C until analysed.

2.3. Blood biochemistry

Blood was collected at the beginning of the experiment and on the final day after a 4-h fasting period; the initial sample was taken from the saphenous vein and the final one was obtained by cardiac puncture. The samples were centrifuged at 1000×g for 10 min at room temperature to separate the cells from the plasma, which was frozen at –80 °C until analysed. The concentrations of the total cholesterol (TC), HDL cholesterol and triacylglycerols (TAG) were determined using the appropriate kits purchased from Stanbio according to manufacturer's instructions (Cat. no. 1010-430, 0590-040 and 2100-430, respectively). A SER-T-FY I (Stanbio, G427L-005) standard was used for the TC, HDL and TAG assays. The concentration of non-HDL cholesterol (non-HDL) was calculated as the difference between the TC and HDL contents, whereas the concentration of LDL cholesterol was calculated as follows (Friedewald, Levy, & Fredrickson, 1972):

$$\text{LDL} = \text{TC} - \text{HDL} - (\text{TAG}/5)$$

Table 1

Nutrient contents of the diets administered to male Wistar rats during a 9-week period.

Nutrient (w/w%)	Diet				
	Control	HF	HF + PO	HF + PP	HF + WP
Starch	52	30	30	30	30
Sucrose	5	5	5	5	5
Lard	5	13.75	6.88	13.75	6.30
Corn oil	5	13.75	6.88	13.75	6.30
Pecan oil			13.75		
Pecan polyphenols				0.1	
Ground pecans					18
Casein	17	21.5	21.5	21.5	21.5
Vitamin mix	1.8	1.8	1.8	1.8	1.8
Salt mix	6	6	6	6	6
Cellulose	6	6	6	6	4
Choline chloride	0.2	0.2	0.2	0.2	0.2
Water	2	2	2	1.9	0.9
% calories from:					
Protein	15.13	15.04	15.04	15.04	16.48
Fat	25.31	54.77	54.77	54.77	51.48
Carbohydrates	59.57	30.19	30.19	30.19	32.04
Water content	9.08	7.16	7.16	7.06	6.29
Calories per gram	3.58	4.53	4.53	4.53	4.56

Commercial ELISA kits were used to determine the insulin (Cayman Chemical, Cat. 589501), adiponectin (Abcam, Cat. ab108784) and leptin (Cayman Chemical, Cat. 10007609) concentrations according to manufacturer's instructions. The level of blood glucose was measured using a commercial glucose metre (OneTouch Mini™), and this value was used to determine the homeostatic model assessment – insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) together with the quantified insulin concentration. Both the HOMA-IR and QUICKI values are used to determine insulin sensitivity in human and animal models, according to the following equations (Muniyappa, Lee, Chen, & Quon, 2008):

$$\text{HOMA-IR} = (\text{insulin } (\mu\text{IU/mL}) \times \text{glucose (mmol/L)}) \div 22.5$$

$$\text{QUICKI} = 1 \div \log (\text{insulin } (\mu\text{IU/mL})) + \log (\text{glucose (mg/dL)})$$

2.4. Total body fat and liver fat

After the animals were killed, the percentages of their total body fat and liver fat were determined. The stomach and intestines were removed from the carcass to eliminate interference from undigested material; the majority of the liver (9.0 ± 0.3 g for each animal) was also extracted and weighed. The whole carcass and the liver were then dried in an air oven at 105°C ; the livers were dried for 24 h, whereas the whole carcass was dried for 72 h. Afterward, the livers and carcasses were weighed once again and the percentage of water in the liver and body was calculated from the initial and final weights. The dried livers were inserted into previously weighed cellulose thimbles and the lipids were extracted for 4 h in a Soxhlet apparatus using hexane as the solvent. The hexane was removed from the recovered lipids by rotary evaporation and the flask was dried to a constant weight. The amount of lipids recovered was calculated by measuring the difference in weight between the empty flask and its weight after the extraction was completed. To determine the fat content of whole carcasses, the water content was calculated similarly to that of the livers except after the carcass was dried, it was homogenised using a manual grinder. Two gram of this homogenised mixture was used to calculate total body fat using the same Soxhlet extraction procedure described above for the livers; this process was performed in triplicate per animal.

2.5. Hepatic gene expression

The total RNA was extracted from 0.1 g of hepatic tissue with using TRIreagent (Sigma–Aldrich, T9424) according to the manufacturer's instructions. The recovered RNA was treated with RNase-free DNase (Promega, 9PIM610) and was then reverse-transcribed to cDNA using the GoScript (Promega, A5001) reverse-transcription system. The cDNA was used to determine the expression of several mRNAs using 18S rRNA and cyclophilin as the reference genes. The measurements were conducted in duplicate. A single PCR reaction contained 5 μL of $2 \times$ SYBR Green mastermix (Qiagen, 204074), 1 μL of a forward and reverse primer mix (10 mM each) for the appropriate gene, 1 μL of cDNA (50 ng/reaction) and 3 μL of nuclease-free water, for a final reaction volume of 10 μL . The PCR protocol was 1 cycle at 95°C for 10 min, 40 cycles at 95°C for 10 s, 60°C for 15 s and 72°C for 20 s, with a final melting-curve analytical step (72 – 95°C). Each run included a no-template control (NTC), also in duplicate. The apparatus used was a RotorGene Q series with a 72-tube rotor, and the RotorGene Q series version 1.7 (Qiagen) software was utilised. The results were analysed using the Pfaffl equation (Pfaffl, 2001), incorporating the previously calculated efficiencies of each set of primers. The sequences of the primers used are listed in Supplementary Table 1.

2.6. Hepatic antioxidant-enzyme activities and lipid-peroxidation status

A sample of each liver (0.5 g) was removed and manually homogenised in 1 mL of PBS buffer (pH 7.4) and then centrifuged at $18,000 \times g$ at 4°C for 5 min. The supernatant was used to determine the total protein content, antioxidant-enzyme activity and lipid-peroxidation status. The total protein content was determined using the Bradford reagent (Sigma Aldrich B6916) according to manufacturer's instructions. The CAT activity was assayed by measuring the disappearance of H_2O_2 using a UV spectrophotometer ($\lambda = 240$ nm) (Aebi, 1984); a unit of CAT was defined as the amount of enzyme that catalysed 1 μmol of H_2O_2 /min. The SOD activity was measured the extent of autooxidation of pyrogallol in Tris buffer at pH 8.2 (Marklund & Marklund, 1974); a unit of SOD was defined as the amount of enzyme that inhibited pyrogallol autooxidation by 50%. The GPX activity was measured indirectly (Terevinto, Ramos, Castroman, Cabrera, & Saadoun, 2010), whereby a unit of GPX activity was defined as the amount of enzyme that catalysed the oxidation of 1 nmol of NADPH/min. The extent of lipid peroxidation was determined using the TBARS (thiobarbituric acid-reactive substances) assay (Lee et al., 2009). Lipid peroxidation was expressed as nmol MDA/mg protein. Glutathione S transferase (GST) activity was determined from the extent of conjugation of 1-chloro, 2-4, dinitrobenzene (CDNB) and glutathione (GSH) (Habig & Jakoby, 1981); one unit of GST was defined as the amount of enzyme that synthesized 1 μmol of GS-CDNB adduct/min.

2.7. Statistical analysis

The statistical analyses were performed using Minitab statistical 16.0 software. The normality of the data was determined using the Anderson–Darling test. The normally distributed data were subjected to ANOVA and Tukey's tests, whereas the non-normal data were analysed using the Kruskal–Wallis test. The correlations between variables were determined using Pearson's correlation coefficient (R). A p value of <0.05 was considered statistically significant.

3. Results

3.1. Diet and adiposity

The daily food consumption was evaluated and transformed into kcal/day (Table 2). The control diet was the most ingested diet, whereas the HF + PP and HF + PO diets were the least consumed diets, which was reflected in the lower energy intake of the rats fed the latter diets compared with the control diet. Although the energetic consumption of the groups differed, this was not reflected in the final weight, which was the same in all of the groups. In contrast, the percentage of total body fat (TBF) was significantly increased in the HF, HF + PO and HF + PP groups compared with that of the control group, whereas that of the HF + WP group was similar to the control value. The percentage of liver fat (LF) was significantly increased in all of the HF groups compared with that of the control.

3.2. Blood biochemistry

The variations observed in the values for the blood biochemical parameters indicated a change in the metabolic pathways. The HF group presented the highest values for most of the variables that were measured (except TAG), indicating the tendency of this diet to increase the level of plasma lipids. The HF + PP group had lower

Table 2

Diet/energy intake, weight and percentage of adiposity of male Wistar rats after a 9-week treatment with HF diets.

	Control	HF	HF + PO	HF + PP	HF + WP
DC ^{1**}	21.8 ± 0.2 ^a	17.2 ± 0.2 ^b	16.5 ± 0.2 ^{cd}	16.1 ± 0.2 ^d	17.1 ± 0.1 ^b
EI ^{2*}	77.9 ± 0.6 ^a	77.6 ± 0.8 ^a	74.4 ± 0.9 ^b	72.7 ± 0.8 ^b	77.7 ± 0.4 ^a
FW ^{3*}	421.3 ± 14.7 ^a	419.6 ± 15.2 ^a	431.1 ± 15.3 ^a	429.4 ± 3.5 ^a	426.8 ± 16.5 ^a
TBF ^{4**}	16.4 ± 1.0 ^b	22.9 ± 2.9 ^a	20.8 ± 2.0 ^a	21.2 ± 1.3 ^a	19.2 ± 1.7 ^b
LF ^{4**}	1.7 ± 0.2 ^b	4.1 ± 0.5 ^a	4.0 ± 0.7 ^a	6.6 ± 2.0 ^a	4.9 ± 1.2 ^a

DC: diet consumed ¹ (g/day); EI: energy intake ² (kcal/day); FW: final weight ³ (g) of the rats; TBF: total body fat ⁴ (%) and LF: liver fat ⁴ (%). The values in a row that do not share the same superscripted letter are significantly different ($p < 0.05$) as determined using an * ANOVA and Tukey's test or ** the Kruskal–Wallis test.

Table 3

Blood biochemistry of male Wistar rats after a 9-week treatment with HF diets.

Type of lipid	Control	HF	HF + PO	HF + PP	HF + WP
TC	96.2 ± 3.4 ^b	110.9 ± 5.0 ^a	108.6 ± 4.3 ^a	96.1 ± 4.8 ^b	83.7 ± 4.7 ^c
Non HDL	73.7 ± 2.4 ^{abc}	82.4 ± 4.8 ^a	80.4 ± 2.2 ^{ab}	69.6 ± 5.2 ^{bc}	63.6 ± 3.7 ^c
LDL	50.1 ± 1.2 ^{ab}	60.9 ± 4.8 ^a	59.6 ± 2.8 ^a	48.3 ± 4.8 ^b	44.6 ± 2.8 ^b
HDL	22.5 ± 3.0 ^{ab}	28.4 ± 1.4 ^a	28.2 ± 2.8 ^a	26.4 ± 3.0 ^{ab}	20.2 ± 1.7 ^b
TAG	186.0 ± 13.0 ^a	169.6 ± 6.9 ^{ab}	163.8 ± 10.8 ^b	168.3 ± 6.3 ^{ab}	149.2 ± 9.0 ^b

All units are expressed in mg/dL. TC: total cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol and TAG: triacylglycerols. The values in a row that do not share the same superscripted letter are significantly different ($p < 0.05$) as determined using ANOVA and Tukey's test.

Table 4

Circulating hormone concentrations and values for the insulin-resistance parameters of male Wistar rats after a 9-week treatment with HF diets.

	Control	HF	HF + PO	HF + PP	HF + WP
Insulin ^{1**}	3.2 ± 0.6 ^a	3.2 ± 0.5 ^a	3.0 ± 0.4 ^a	3.6 ± 0.7 ^a	2.7 ± 0.3 ^a
HOMA-IR [*]	38.0 ± 7.8 ^a	41.8 ± 4.5 ^a	42.0 ± 6.7 ^a	47.1 ± 11.4 ^a	39.4 ± 6.6 ^a
QUICKI [*]	0.24 ± 0.00 ^a	0.23 ± 0.00 ^a	0.23 ± 0.00 ^a	0.23 ± 0.00 ^a	0.24 ± 0.00 ^a
Adiponectin ^{2*}	26.7 ± 1.9 ^a	20.8 ± 1.5 ^{ab}	19.3 ± 1.2 ^b	19.8 ± 2.1 ^b	14.6 ± 1.3 ^b
Leptin ^{1**}	7.1 ± 0.9 ^b	14.5 ± 2.2 ^a	13.0 ± 1.9 ^a	10.5 ± 1.9 ^{ab}	8.6 ± 1.8 ^b
Ad/Lp ^{**}	4.2 ± 0.5 ^a	1.7 ± 0.2 ^b	1.6 ± 0.1 ^b	2.2 ± 0.3 ^b	2.1 ± 0.3 ^b

¹ ng/mL; ² µg/mL, HOMA-IR: homeostatic model assessment-insulin resistance; QUICKI: quantitative insulin-sensitivity check index and Ad/Lp: adiponectin/leptin ratio. The values in a row that do not share the same the same superscript letter are significantly different ($p < 0.05$) as determined using an *ANOVA and Tukey's test or **Kruskal–Wallis test.

TC, non-HDL, and LDL concentrations compared with those of the HF group. The values for these variables were even lower in the HF + WP group than in the control group, consistent with the hypolipidemic effects of pecans. The HF + PO and HF + WP diets also decreased the TAG concentration to values below that of the control group (Table 3).

Determination of the serum hormone concentrations revealed that the treatment diets had no significant effect on insulin secretion (Table 4). Insulin sensitivity was also unaffected by the treatment, which was corroborated by values for the HOMA-IR and QUICKI parameters. The adiponectin concentration was not affected by the HF diet, whereas the HF + PO, HF + PP and HF + WP groups showed a significant decrease with respect to the control value. The leptin concentration was significantly increased in the HF and HF + PO groups with respect to the control value. The HF + PP diet resulted a leptin concentration similar to that of the HF and HF + PO groups and that of the HF + WP group, although the latter demonstrated mitigated leptin increase by presenting a leptin concentration similar to that of the control group. The decrease in the adiponectin content and an increase in the leptin content modified the adiponectin/leptin (Ad/Lp) ratio in all of the HF groups, which was significantly lower than that of the control group.

3.3. Expression of lipid metabolism and antioxidant-stress response genes in the liver

To understand the metabolic changes induced by the diets, the relative level of mRNA expression (with respect to that of the control group) of hepatic transcription factors and proteins related to

lipid metabolism was analysed using real time PCR (Fig. 1). The level of APOB expression in the HF group was decreased with respect to those of the control and HF + WP groups and the HF + PO and HF + PP groups had intermediate values similar to those of the remaining groups. This result suggested that the HF + WP diet reversed the effect of the HF diet on the expression of this gene. The level of LDLR expression in the HF + WP group was significantly increased with respect to those of the control and HF groups, which were similar. The level of LXR α expression was significantly increased in the HF + PP group with respect to those of the other groups, except the HF group. The HF + WP group had level of LXR α expression that was lower than that of the HF group and similar to that of the control group. The level of SREBP1c expression was significantly increased in the HF + WP group with respect to that of the control group and the level of its expression in the HF + PP group was intermediate between that of the HF + WP group and the other groups. The level of Nrf2 expression was lowest in the control group and was significantly increased in the HF, HF + PP and HF + WP groups, whereas the HF + PO group showed an intermediate expression level.

3.4. Hepatic antioxidant-enzyme activities and lipid peroxidation status

The antioxidant-enzyme activities were measured to analyse the impact of the HF diet on the antioxidant status of the liver (Fig. 2). The level of CAT activity was significantly increased in the HF + WP group compared with those of the control and HF + PO groups, whereas the levels in the remaining groups were similar among them. The level of SOD activity was decreased in the

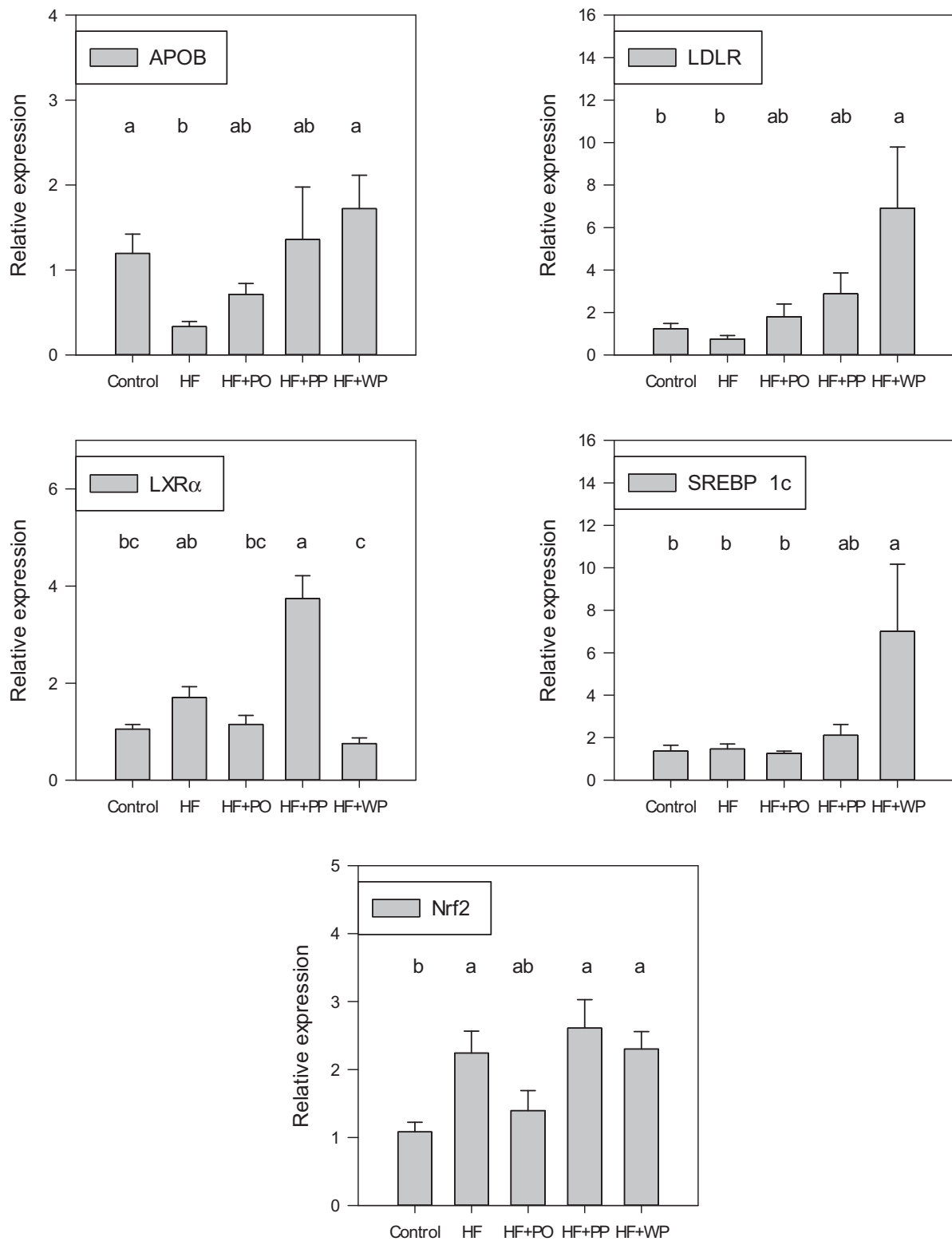


Fig. 1. Effect of high fat diets with and without pecan fractions on the relative levels of gene expression in rat livers. The values were calculated by the Pfaffl equation respect to the control group, normalizing genes were rRNA18S and cyclophilin. The values that do not share the same letter are significantly different ($p < 0.05$) as determined using the Kruskal–Wallis test.

HF + PP group compared with those of the control and HF groups and the activity of the HF + WP group was lower than that of the HF group and similar to that of the control group. The level of GPX activity was highest in the HF, HF + PO and HF + PP groups

(which were all similar); the HF + WP group also presented an increased level with respect to that of the control group although the values were significantly lower than those of HF, HF + PO and HF + PP groups. The level of GST activity in the HF group

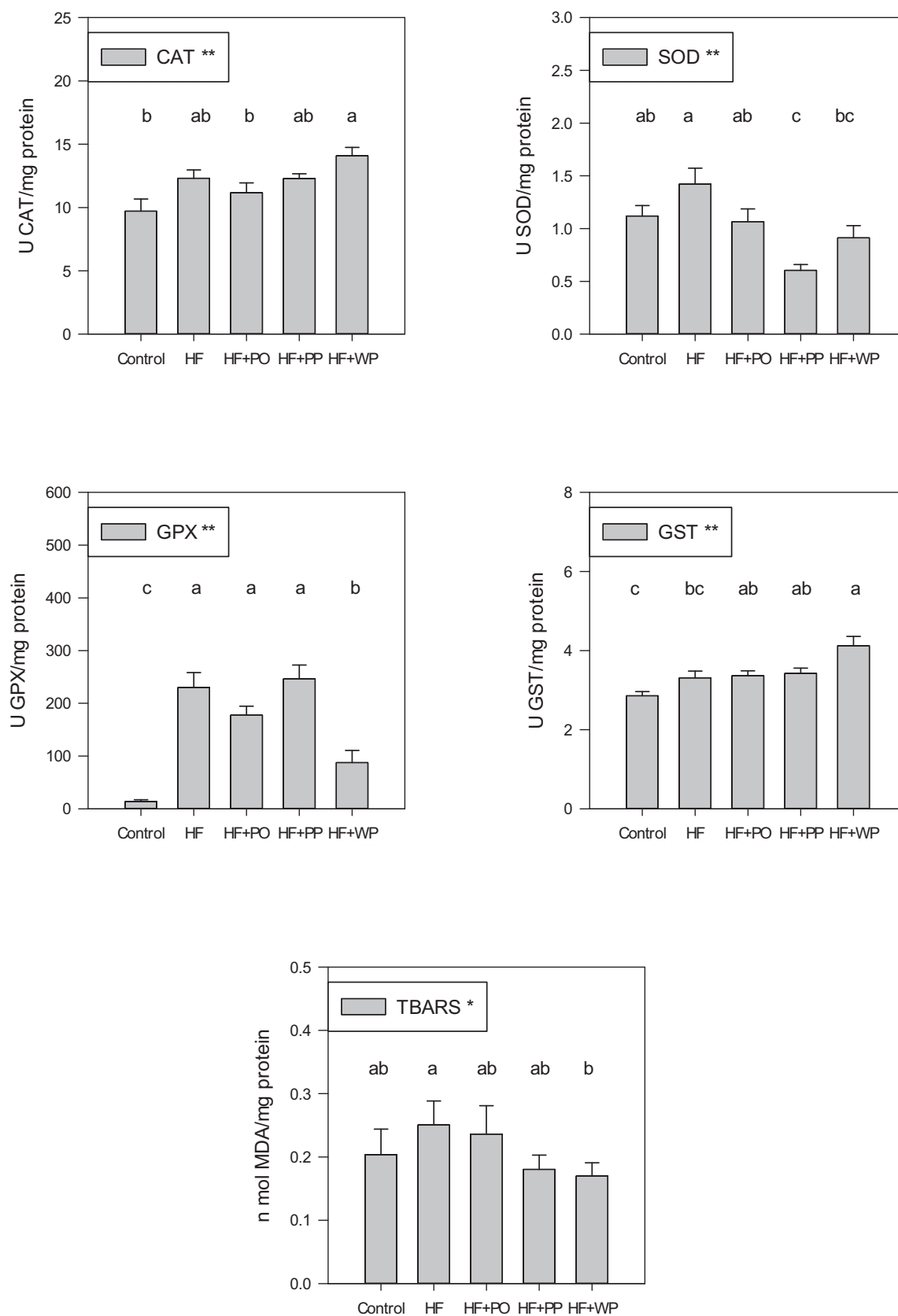


Fig. 2. Effect of high fat diets with and without pecan fractions on the antioxidant-enzyme activities and lipid peroxidation status of the rat livers. The values that do not share the same letter are significantly different ($p < 0.05$) as determined using *ANOVA and Tukey's test or **the Kruskal–Wallis test.

was similar to that of the control group, whereas those of the HF + PO, HF + PP and HF + WP groups were significantly increased with respect to the control value.

The extent of lipid peroxidation in the liver, as determined using the TBARS method, was also used to evaluate the redox equilibrium in the rats fed HF diets. The HF + WP group had the lowest TBARS value, which was significantly different from that of the HF group, and the control, HF + PO, and HF + PP groups had TBARS values intermediate between those of the HF and HF + WP groups.

4. Discussion

The control diet was the most consumed diet because it had the least amount of energy per gram, resulting in the animals ingesting a larger amount of the control food to satisfy their energy requirements. Evaluation of the energy intakes showed that the animals fed the HF + PO and HF + PP diets consumed significantly less energy than did the remaining groups, which had similar amounts of energy. This result indicated that these two diets increased the sensation of satiety. It has been demonstrated that unsaturated fatty acids (Jones, Jew, & AbuMweis, 2008), polyphenols, and other phytochemicals (Molan, Lila, & Mawson, 2008) can cause satiety in humans and in animal models. However, these components did not have the same effect when administered as part of the whole food matrix (HF + WP).

The HF diets (except the HF + WP diet) increased the TBF level despite having negligible effects on the body weight (Table 2). This finding indicated that similar a caloric intake from different nutrient sources (complex carbohydrates in the control diet or lipids in the HF diets) is not equivalent because lipids are preferentially directed toward storage in adipose tissue, whereas carbohydrates are readily oxidised. Nevertheless, the lipid sources and the potentially functional ingredients, such as polyphenols, may also alter the metabolic fate of the ingested nutrients. The HF + WP diet did not induce an increase in the TBF compared with that in the HF group even though it contained the same amount of lipids and carbohydrates as the HF diet, suggesting the occurrence of different metabolic processes of the orally ingested lipids due to the nature of those lipids and the effect of the other micronutrients present. To explore some of the pathways involved in these differences, we analysed the expression of several proteins and transcription factors that regulate transport and metabolism of lipids.

The percentage of LF was significantly increased in all of the HF groups compared with the control value (Table 2). This increase correlated with level of LXR α expression in the liver ($p = 0.01$). LXR α protects the liver against an excess of cholesterol through several mechanisms, including stimulating reverse cholesterol transport and promoting the efflux of cholesterol (Calkin & Tontonoz, 2012). For these reasons, the stimulation of LXR α expression by agonists such as GW3965 (Donkin et al., 2010) has been used as an anti-atherosclerotic therapeutic strategy; however, this stimulation can induce hypertriglycerolemia, an increase in hepatic lipogenesis and consequent hepatosteatosis as negative side effects (Fievet & Staels, 2009). In the present study, the level of circulating TAG was not found to be increased in any of the HF groups (actually the concentration of blood TAG was reduced in the HF + PO and HF + WP groups) nor was there a significant correlation between the LXR α expression and the TAG concentration ($p = 0.25$). However, the positive and significant correlation found between the percentage of liver fat and the level of LXR α expression suggested a possible role of this transcription factor in the accumulation of liver lipids. The level of LXR α expression was highest in the HF + PP group. Because LXR α is an autoinducible gene (Li et al., 2002), our results suggested that pecan polyphenols may activate the expression LXR α and that they

therefore may possess a potential antiatherosclerotic activity; however, their effect on the accumulation of fat in the liver should be taken in consideration and further studies should be conducted to determine the mechanism(s) underlying this effect.

The lipid-lowering effects of pecan consumption that were previously observed (Mukuddem-Petersen, Oosthuizen, & Jerling, 2005) were also observed in the present study but could not be fully replicated by consumption of either the oil fraction or the polyphenol fraction of pecans (Table 3). A review of various almond-based studies established that almonds can lower level of LDL particles in the blood due to their fatty acid profile and “possibly other constituents” (Berryman, Preston, Karmally, Deckelbaum, & Kris-Etherton, 2011). In the case of *C. illinoensis*, the other constituents with cholesterol-reduction ability might be polyphenols (Ngamukote, Makynen, Thilawech, & Adisakwattana, 2011) because these polyphenols reduced the levels of plasma TC, non HDL, and LDL compared to those of the HF diet but without having the additional lowering effect of the HF + WP diet (Table 3). The mechanism of action of the pecan polyphenols may involve the stimulation of expression of hepatic LXR α (as previously mentioned). Other mechanistic routes are also possible, for example, a recent experiment showed that black-tea polyphenols reduced the solubility of cholesterol micelles *in vitro* while also reducing their intestinal absorption in rats (Ikeda, Yamahira, Kato, & Ishikawa, 2010). Because the effect on the level of serum cholesterol and that of its fractions was greater in the HF + WP group, it could be argued that other nut components (fibre, protein or micronutrients) may also have an important lipid-lowering effect and that a synergistic effect of the bioactive components may occur when whole foods are consumed.

The lipid-lowering effect of WP and PP may also be related to changes in the metabolism of APOB, which is the main protein present in the non-HDL cholesterol particles. Contrary to what might be expected, the level of APOB mRNA expression was inversely correlated with the concentrations of TC ($p = -0.03$), LDL cholesterol ($p = -0.03$) and non-HDL cholesterol ($p = -0.02$), suggesting that the increase in APOB mRNA expression may be linked to the increased catabolism of the circulating non-HDL cholesterol particles that contain APOB protein. The observed upregulation of the hepatic LDLR expression supports this notion and appears to be key in the reduction of serum cholesterol because the level of LDLR expression was positively correlated with the level of APOB expression ($p = 0.007$), indicating that an increase in the LDLR content may increase the level of catabolism of APOB-containing particles. It has been demonstrated that the secretion of APOB is regulated by the LDLR, in the intracellular phase and after its secretion (Twisk et al., 2000), and some authors have referred to the LDLR as a “gatekeeper” that targets APOB-containing particles (Larsson, Skogsberg, & Björkegren, 2004). The most potent dietary inducer of LDLR expression was the HF + WP diet; the level of LDLR expression in this group was significantly higher than that of the control and HF groups. The HF + WP diet also induced the lowest levels of circulating total cholesterol, and the LDL and non-HDL values in the HF + WP diet group were lower than those of the HF group. The level of HDL-cholesterol was also lowered by this diet, suggesting the action of additional regulatory mechanisms.

The strongest effect of the PO fraction was observed in the concentration of plasma TAG. The level of TAG was significantly lower in the HF + PO and HF + WP groups than in the control group. The mechanisms through which an edible oil can lower the TAG concentration have been studied mainly using fish oils; it has been shown that these oils act upon SREBP1c by reducing its mRNA expression and by inhibiting the maturation of SREBP1c protein (Shearer et al., 2012). In the present study, the level of SREBP1c expression was increased in the HF + WP group but not in the

HF + PO group, which indicated that the TAG-lowering effect of the HF + PO and HF + WP diets was not mediated through SREBP1c signalling. A number of other possible mechanisms for this effect exist, which may be hepatically or peripherally based.

The hormones adiponectin and leptin are secreted by white adipose tissue (WAT) and the level of serum adiponectin has been found to inversely correlate with the WAT mass. This hormone has many functions, including a role in insulin-sensitization, which favours the oxidation of fatty acids (via the PPAR α -signalling pathway) and other antiatherosclerotic effects (Kadowaki & Yamauchi, 2005). Because the content of adipose tissue was increased by our treatment, the concentration of adiponectin was in turn decreased, which was confirmed by the correlation between the TBF and the adiponectin concentration ($p = -0.02$); the level of secreted adiponectin was also negatively correlated with the level of LDLR expression ($p = -0.03$). The leptin concentration was found to have a highly significant positive correlation with the TBF ($p < 0.001$). One of the most important roles of leptin is to prevent obesity by decreasing the energy intake and favouring energy expenditure, mainly by acting on the centres of the brain that are responsible for the behaviours in which leptin receptors are highly expressed (Sweeney, 2002). A number of negative effects of hyperleptinemia occur, such as obesity and leptin resistance, which can contribute to atherogenesis via different routes (Scarpace & Zhang, 2009). An increased leptin concentration in addition to an increased adipose tissue mass and an increased cholesterol concentration is therefore an indicator of an increased atherogenic risk in our groups. Because the HF and HF + PO groups had the highest leptin concentration and the HF + WP diet prevented its increase, we have demonstrated that the WP diet mitigated the level of HF-induced hyperleptinemia. This study also showed that PO, PP and possibly other minor compounds might act in synergy to elicit this effect because none of the pecan fractions replicated the effect of WP by itself. The decrease in the level of adiponectin combined with the increase in leptin levels decreased the Ad/Lp ratio in all of the HF groups despite the pecan-elicited mitigation of hyperleptinemia. The decrease in this ratio has been linked to metabolic syndrome in humans (Vega & Grundy, 2013).

In addition to altering lipid metabolism, HF diets can also affect the redox balance in an organism through different pathways, the first of which is a diet that limits carbohydrate intake in favour of lipids, which directs metabolism toward the oxidation of the lipids, which is considered to be a strong generator of reactive oxygen species (ROS) (Vial et al., 2011). Second, because ROS are being produced at an increased rate, a healthy organism increases its endogenous antioxidant response. A previous study conducted in mice fed HF diets suggested that the activity of the antioxidant system of the liver was increased as an adaptive response (Do et al., 2011). This response would involve Nrf2 stimulation because this transcription factor regulates the cellular antioxidant response and the expression of antioxidant enzymes as well as other cellular processes related to cell survival (Kensler, Wakabayashi, & Biswal, 2007). The HF, HF + PP and HF + WP diets significantly increased the expression of Nrf2 to levels higher than the control, most likely due to the greater lipid intake that stimulated the oxidative pathways, although further mechanisms may be involved because the HF + PO diet, which had a lipid content similar to all HF diets, did not increase the level of Nrf2 expression. It has also been reported that polyphenols in food may modulate the expression of various enzymes through activating the Nrf2 pathway (Scapagnini et al., 2011).

The level of Nrf2 expression was not significantly correlated with the increased activity of any of the antioxidant enzymes that were assessed in the present study, which may indicate the following: (a) that the enzyme activity of CAT, SOD, GPX and GST is regulated by mechanisms different or additional to Nrf2-mediated

transcriptional regulation and (b) that Nrf2 activation is most likely involved in the regulation of additional signalling pathways, as previously reported (Kensler et al., 2007). The levels of enzyme activities and lipid peroxidation in the liver were affected by the different dietary treatments.

The HF group had a significantly higher liver lipid peroxidation compared with that of the HF + WP group, suggesting that the HF + WP diet was able to regulate oxidative changes due to the high level of lipid metabolism. It is tempting to speculate that GST activation may be the major mechanism that led to the prevention of lipid peroxidation in the HF + WP group because this enzyme was the only one that was significantly increased with respect to the levels in the HF group and GST activity has been considered highly protective in previous studies conducted in HF-fed rodents (Hsu & Yen, 2007; Kirpich et al., 2011).

The reduction of SOD activity in the HF + WP and HF + PP groups may be due the suppression of superoxide-anion (or other ROS) generation. This effect on ROS generation was most likely mediated by the polyphenolic fraction because the HF + PP diet had the strongest effect on the level of SOD activity. Other authors have reported that polyphenol-containing extracts suppressed the *in vivo* generation of superoxide anions in healthy human subjects (Ghanim et al., 2011).

The HF + PO diet showed no effects in any of the measured antioxidant responses compared with those of the HF diet, suggesting that the effects of the WP diet were mainly due to the PP fraction. The oil fraction might act as a regulator of the PP activity, which could ultimately have positive overall effects. For example, the level of SOD activity was strongly reduced in the HF + PP group and was only mildly reduced in the HF + WP group, which had the lowest TBARS level.

The overall changes in the level of antioxidant enzyme activities, lipid TBARS levels and the level of Nrf2 expression indicated that the antioxidant response of the rats was stimulated by a greater than normal generation of oxidant molecules and that the polyphenolic compounds present in the WP diet further regulated this response.

5. Conclusion

A HF diet altered the lipid metabolism and redox state in rats without having a measurable effect on their body weight. WP and pecan fractions were able to mitigate or reverse some of the HF diet-induced alterations. The HF diets decreased the concentration of serum adiponectin and increased the concentration of leptin, whereas the HF + WP diet was effective in preventing hyperleptinemia but not in preventing hypo adiponectinemia. The HF + WP diet also reduced the concentration of blood lipids (in some cases, to values lower than those of the control group) through the upregulation of the levels of APOB and LDLR mRNA. The HF + PP diet prevented decreased blood lipids (TC, non HDL and LDL) compared with HF diet but not with control diet. The HF group showed increased LF, Nrf2 expression and increased activity of antioxidant enzymes, suggesting increased oxidative stress. This condition was prevented by dietary WP, possibly through an increase in the GST activity. The effects of WP consumption could not be fully replicated by consumption of either of its isolated fractions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2014.07.092>.

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