Research Article

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Soybean flour induces a greater increase of the antioxidant defenses in rats fed with a normocaloric diet compared with a hypercaloric diet

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Abstract

BACKGROUND: Soybeans, due to their antioxidant properties, present beneficial health effects. The objective was to evaluate if replacing casein with soy flour, modifies antioxidant defenses in rat liver, compared to animals that continued being fed with casein based diets (normocaloric and hypercaloric).

RESULTS: Four groups of rats were used: CC (control casein), CS (control soy), HC (hypercaloric casein) and HS (hypercaloric soy). Malondialdehyde, in serum and liver, did not present differences. In liver, when comparing CS vs. CC: increased superoxide dismutase 1 ($P < 0.001$), catalase ($P < 0.01$) and glutathione reductase ($P < 0.05$) activities, the total glutathione ($P < 0.001$) and reduced glutathione ($P < 0.05$) content and decreased oxidized glutathione content ($P < 0.05$). In HS vs. HC: increased carbonyl groups ($P < 0.01$) and superoxide dismutase 1 activity ($P < 0.05$), and decreased glutathione peroxidase activity ($P < 0.01$), total glutathione ($P < 0.05$) and oxidized glutathione content ($P < 0.001$). In HS vs. CS: decreased glutathione reductase activity ($P < 0.01$), total glutathione ($P < 0.001$) and reduced glutathione ($P < 0.01$) content, and increased oxidized glutathione content ($P < 0.05$).

CONCLUSION: Replacing casein by soybean flour improves antioxidant defenses, mainly in normocaloric diets.

Keywords: soybean flour; hypercaloric diet; reactive oxygen species; antioxidant defenses; rat liver

INTRODUCTION

Oxidative stress is characterized by the excessive production of oxidizing molecules that overwhelm the antioxidant defense systems, which results in oxidative damage.[1] The reactive oxygen species (ROS), are highly reactive ubiquitous molecules, with very short life time, that are derived from molecular oxygen, and their intracellular accumulation can be provoked by exogenous and/or endogenous factors.[2]

Under normal circumstances, the ROS concentrations are closely controlled by the antioxidants. When they are produced in excess, or when the antioxidants are depleted, the ROS can inflict damages to the DNA, lipids, proteins and hydrocarbons.[3]

Due to the possible harmful effects of oxidative stress, even under normal physiological conditions, aerobic organisms have developed an antioxidant system that consist of diverse free radical scavenging enzymes, such as catalase (CAT) and superoxide dismutase (SOD), which act on hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\bullet -}$), respectively, and glutathione peroxidase (GPx), which sweeps away lipid hydroperoxides and H_2O_2.[4]

There are also antioxidant compounds such as glutathione, which plays an important role in its reduced form (GSH). In addition, there is a wide variety of natural antioxidants of vegetable origin, such as carotenoids, vitamins, flavonoids and other vegetable phenolic compounds.[5]

Numerous epidemiological studies have established the interaction between eating habits and oxidative stress. The nutritional or dietary oxidative stress reveals a perturbation of the redox state that results from an excess of oxidative load or the inappropriate supply of nutrients, favoring pro-oxidant reactions.[6]

An overload of nutrients, and in particular, foods high in fats and hydrocarbons, are associated to oxidative stress through diverse processes, such as $O_2^{\bullet -}$ generation from NADPH oxidases (NOX), oxidative phosphorylation, among others.[6,7] In fact, hyperglycemia and hyperlipidemia lead to an increase of the oxidative stress, which has been associated with a higher risk for atherosclerosis and related disorders.[8]

Products of vegetable origin are progressively gaining attention due to their lower toxicity and high efficiency against diseases

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mediated by free radicals. Epidemiological studies have related the intake of whole grains and whole grain products, with a lower incidence of chronic diseases.[9–11] The implementation of ROS in the etiology of these degenerative diseases, has suggested that the phytochemicals, by reducing the oxidative stress, could be an efficient way of preventing the development of these diseases.[12]

Soy, in its leguminous form rich in isoflavones, has recently received considerable attention due to its antioxidant properties. There is a vast number of studies that demonstrate its beneficial effects for coronary disease, cancer prevention, osteoporosis,[13] anti-inflammatory and photoprotective effect on the skin,[14] and many others.

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 decided to work with whole grain soybean flour. The goal of this work was to determine if replacing casein with soybean flour modifies, in the liver of male *Wistar* rats, the antioxidant defenses compared to those animals that continued being fed with diets based on casein (normocaloric and hypercaloric).

MATERIALS AND METHODS

Materials and chemicals

Casein was purchased from Milkaut (Santa Fe, Argentina). Casein composition (pasteurized skim milk) was: total protein concentrated by ultra-centrifugation: 800 g kg^{-1}, lipids 300 g kg^{-1}. Soybean flour was acquired from La esquina de las flores group (Buenos Aires, Argentina). Soybean flour composition was: protein 366.6 g kg^{-1}, lipids 236.6 g kg^{-1}, carbohydrates 233.3 g kg^{-1}, fiber 133.3 g kg^{-1} (prior heat treatment).

The protein standard was bovine serum albumin (BSA or fraction V) acquired from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were reagent grade and were purchased from Sigma–Aldrich (Buenos Aires, Argentina).

Animals, diets and experimental design

Twenty-four male *Wistar* rats, purchased from Romanelly (Buenos Aires, Argentina), were used. We followed the general guidelines for the care and use of laboratory animals recommended by the Animal Care Committee of the National University of San Luis. Animals of 21 days of age, weighing 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 1 week adaptation period with a diet prepared according to recommendations of the American Institute of Nutrition 1993, the AIN-93 G diet.[15] They were subsequently separated into two groups: control casein (CC) and hypercaloric casein (HC), for 9 weeks. The hypercaloric diets contained 341.4 g kg^{-1} sucrose and 42% of calories from fat.[16] After this period, the AIN-93G diet was replaced by AIN-93M diet,[15] and each group was divided into two sub-groups, replacing casein by soybeans in one of them. Therefore, we had a total of four sub-groups: control casein (CC), control soy (CS), hypercaloric casein (HC) and hypercaloric soy (HS). Rats were fed with the respective diet during 6 weeks (Fig. 1). The diets ingredients are shown in Table 1.

After an overnight fast, following the 15 weeks dietary period, six rats of each group were anesthetized and sacrificed. Blood samples were collected and serum was stored at −70°C until analysis. Livers were excised immediately, washed with physiological saline solution (0.9%), blotted dry, weighed and stored in liquid nitrogen for analysis. All the determinations were performed in duplicate.

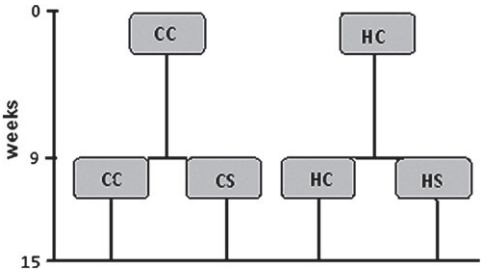

Figure 1. Experimental design. CC, control casein; CS, control soy; HC, hypercaloric casein; HS, hypercaloric soy.

Lipid peroxidation in serum and liver

The livers were homogenized (1:10 p/v) in cold 30 mmol L^{-1} phosphate buffer pH 7.4, 120 mmol L^{-1} KCl, and 1% Triton X-100 (1:20 v/v), in an Ultra Turrax T25 machine (Ramm Doman, Buenos Aires, Argentina). After centrifugation (Beckman Optima TM L-90K Ultracentrifuge, serial N° COL98E50), the supernatants were used to determine malondialdehyde (MDA) and enzymatic activities, in a Beckman DU 600 spectrophotometer (Beckman, Fullerton, CA, USA).

MDA is a marker for lipid peroxidation and was determined according to Draper and Hadley.[17] The method is based on the spectrophotometric measurement of the color produced during the reaction between thiobarbituric acid (TBA) and MDA. In serum and liver, the concentration of MDA was expressed as nmol mL^{-1} and nmol g^{-1} of tissue, respectively.

Protein oxidation

Carbonyl groups constitute the best markers of protein oxidation. The content of protein carbonyls was determined by the 2,4-dinitrophenylhydrazine (DNPH) method, according to Reznick and Packer.[18] Livers were homogenized in 50 mmol L^{-1} Hepes buffer, 125 mmol L^{-1} KCl, pH 7.4, adding protease inhibitors 1:100 (v/v). Centrifugation was performed for 25 min at 10 367×*g*, at 4°C. An aliquot was taken for protein determination. The maximum absorbance of the supernatant was determined in a UV spectral scan from 360 nm to 390 nm. The results are expressed as µmol of carbonyl groups mg^{-1} of protein.

Content of total glutathione and reduced glutathione

Livers were homogenized in 0.5 mol L^{-1} $HClO_4$; the acid facilitates the subsequent removal of protein by denaturation. Afterwards, it was centrifuged for 10 min at 1371×*g*, at 4°C. We worked with the supernatant which constitutes the acid extract. Total glutathione was assessed by the enzymatic recycling process according to Griffith.[19] The results are expressed in µmol total glutathione g^{-1} of tissue.

Reduced glutathione (GSH), was determined spectrophotometrically by the technique according to Ball.[20] GSH is oxidized by DTNB producing TNB, which can be spectrophotometrically measured at 412 nm. Results are expressed in µmoles GSH g^{-1} of tissue. The oxidized glutathione (GSSG) was estimated by subtracting GSH from total glutathione.

Liver activities of antioxidant enzymes

Catalase (CAT, E.C. 1.11.1.6) activity was determined by following the decomposition of H_2O_2, which is measured by the decrease in absorbance at 240 nm in a medium containing 50 mmol L^{-1} phosphate buffer (pH 7).[21] One catalase unit is defined as the amount of the enzyme required to decompose 1 µmol L^{-1} of H_2O_2

Table 1. Composition of the experimental diets

Ingredient (g kg^{-1})	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% protein; soybean flour, 36.66% protein.

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

Primer	Sense (5′–3′)	Antisense (5′–3′)	Size (bp)
CAT	CGACCGAGGGATTCCAGATG	ATCCGGGTCTTCCTGTGCAA	175
SOD-1	AGCTGCACCACAGCAAGCAC	TCCACCACCCTTAGGGCTCA	191
GPx	CGGTTTCCCGTGCAATCAGT	ACACCGGGGACCAAATGATG	245
NOX-2	CCAGTGTGTCGGAATCTCCT	ATGTGCAATGGTGTGAATGG	150
Nrf2	AGATTCACAGGCCTTTCTCG	CAGCTCTCCCTACCGTTGAG	201
β-Actin	CGTGGGCCGCCCAGGCACCA	TTGGCCTTAGGGTTCAGAGGG	243

CAT, catalase; SOD-1, superoxide dismutase 1; GPx, glutathione peroxidase; NOX-2, NADPH oxidase 2; Nrf2, nuclear factor E2-related factor 2.

min^{-1} mL^{-1} at pH 7 and 25°C. The CAT activity was expressed as IU mg^{-1} of protein.

The measurement of superoxide dismutase 1 (SOD-1, E.C.1.15.1.1) activity was evaluated by the method of McCord and Fridovich,[22] modified by Flohé and Otting.[23] The SOD-1 activity was determined on the basis of its inhibitory action on the rate of the superoxide-dependent reduction of cytochrome c by xanthine oxidase at 550 nm. One unit of SOD-1 is defined as the amount of enzyme that inhibits cytochrome c reduction by 50%. The enzyme activity was expressed in IU mg^{-1} of protein.

The determination of glutathione peroxidase (GPx, E.C.1.11.1.9) activity was based on the method of Flohé and Günzlere.[24] GPx catalyzes the oxidation of GSH by *tert*-butyl hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the GSSG is immediately converted to the reduced form with the concomitant oxidation of NADPH to NADP$^+$. The decrease in absorbance of NADPH was measured at 340 nm. The enzyme activity was expressed as IU mg^{-1} of protein.

The assay of glutathione reductase (GR, E.C. 1.8.1.7) activity was adapted from the method of Andersen *et al.*[25] GR catalyzes the reduction of GSSG in the presence of NADPH. The determination is based on the spectrophotometric measurement of the decrease of absorbance by the oxidation of NADPH at 340 nm, at 25°C. The enzyme activity was expressed as IU mg^{-1} of protein.

Protein assay

Protein content of liver homogenates was estimated by the method of Biuret, described by Lane,[26] using bovine serum albumin (BSA) as standard. The absorbance was measured at 550 nm.

mRNA expression by RT-PCR analyses

The mRNA expressions of CAT, SOD-1, GPx, NADPH oxidase 2 (NOX-2) and nuclear factor E2-related factor 2 (Nrf2) were

Table 3. Markers of lipid and protein oxidation

Parameter	CC ($n=6$)	CS ($n=6$)	HC ($n=6$)	HS ($n=6$)
MDA (nmol mL^{-1})	1.63 ± 0.20	1.77 ± 0.25	2.28 ± 0.18	1.91 ± 0.26
MDA (nmol g^{-1} of tissue)	1.89 ± 0.27	2.19 ± 0.26	2.53 ± 0.25	2.55 ± 0.25
Carbonyls (μmol mg^{-1} of protein)	2.52 ± 0.35	2.71 ± 0.21	1.45 ± 0.39	2.32 ± 0.20°°

Values are means ± SD ($n=6$), analyzed by one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test. °HS vs. HC, °°$P < 0.01$.
CC, control casein; CS, control soy; HC, hypercaloric casein; HS, hypercaloric soy; MDA, malondialdehyde.

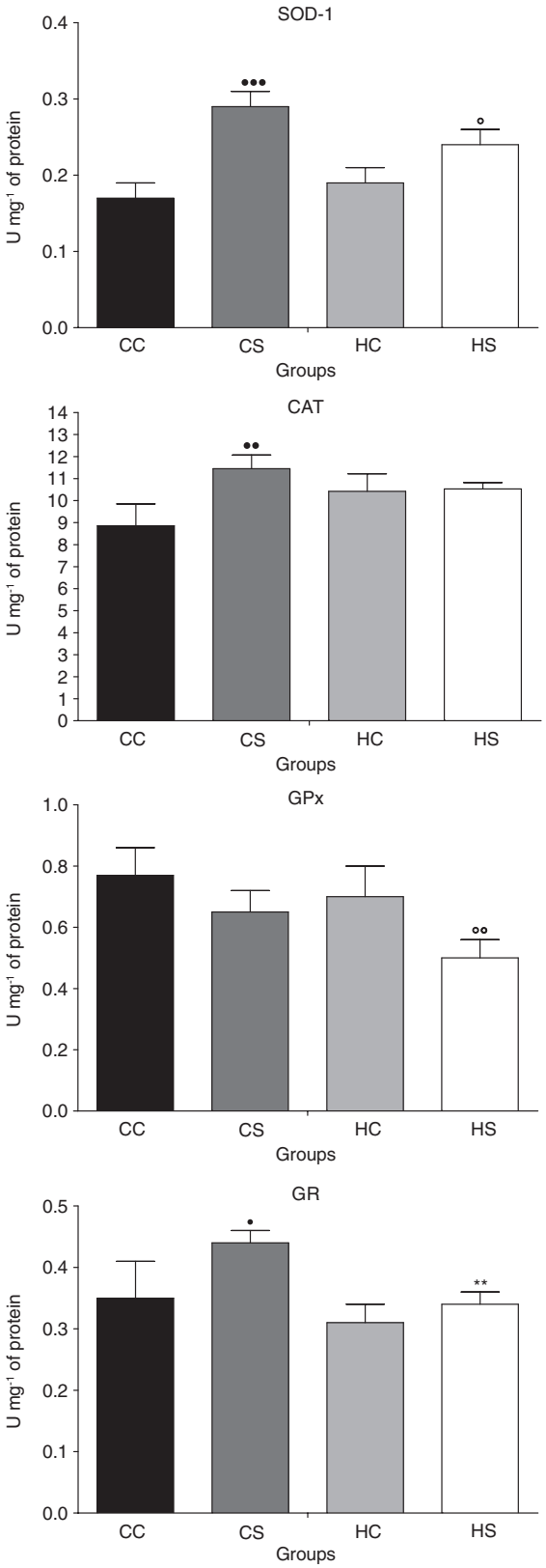

Figure 2. Activities of antioxidant enzymes. Values are means ± SD ($n = 6$). Analyzed by one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test. •CS vs. CC: •$P < 0.05$, ••$P < 0.01$, •••$P < 0.001$; °HS vs. HC: °$P < 0.05$, °°$P < 0.01$; *HS vs. CS: **$P < 0.01$. CC, control casein; CS, control soy; HC, hypercaloric casein; HS, hypercaloric soy; SOD-1, superoxide dismutase 1; CAT, catalase; GPx, glutathione peroxidase; GR, glutathione reductase.

estimated using RT-PCR (reverse transcription–polymerase chain reaction) analysis. Total RNA was isolated from liver tissues by means of TRIzol (Invitrogen, Buenos Aires, Argentina). The RT reaction was performed using 200 IU of Moloney murine leukemia virus reverse transcriptase (Invitrogen/Life Technologies, Buenos Aires, Argentina) and random hexamer primers. The RT products (cDNA) were then subjected to the PCR at 94°C for 5 min, followed by 35 cycles of three steps each: denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. Finally, after these cycles, a final extension at 72°C for 5 min was performed. All values for each PCR product were normalized and expressed as a ratio with respect to the quantity of β-actin used as internal standard. The intensity of each band was measured with the NIH Image software, and reported in arbitrary units (AU). The sequences of the different primers and sizes of the PCR products are shown in Table 2.

Statistical analysis

The data were expressed as means ± SD, based on the number indicated in the experiment. The results were analyzed using one-way analysis of variance (ANOVA), provided by the multiple comparison of the Tukey–Kramer means test. A probability of 0.05 or less indicates significant difference.[27] The following comparisons between groups were performed: CS vs.CC, HS vs. HC and HS vs. CS.

RESULTS

Analyzing the oxidative stress markers in lipids and proteins (Table 3), it was found that the value of MDA, in serum and liver, did not present differences between CS vs. CC, HS vs. HC and HS vs. CS.

The carbonyl groups did not show significant differences between animals fed with the normocaloric diets (CS vs. CC), but there was an increase in the HS group compared to HC ($P < 0.01$). Both oxidative stress markers did not present significant differences when comparing between the soybean diets.

In this study, we evaluated the activity of some antioxidant enzymes, and the results are shown in Fig. 2. When comparing CS vs. CC, an increase of the SOD-1 ($P < 0.001$), CAT ($P < 0.01$) and GR ($P < 0.05$) activities, was observed. When comparing HS vs. HC, an increase of SOD-1 ($P < 0.05$) and a decrease of GPx ($P < 0.01$), were observed. Finally, the comparison between HS vs. CS showed a decreased of GR ($P < 0.01$).

The mRNA levels of CAT, SOD-1, GPx, NOX-2 and Nrf2 were also determined (Table 4), and only a significant increase was observed in the NOX-2 expression when comparing CS vs. CC ($P < 0.01$).

By analyzing the liver glutathione content (Fig. 3), and comparing CS vs. CC, it was determined that there was an increase in the total glutathione ($P < 0.001$), and GSH ($P < 0.05$), and a decrease of GSSG ($P < 0.05$), which led to a 2.5-fold increase of the GSH/GSSG ratio. When comparing HS vs. HC, a decrease of total glutathione ($P < 0.05$) and GSSG ($P < 0.001$), is observed; and also a 3.3-fold increase of the GSH/GSSG ratio. In addition, a decrease of total glutathione ($P < 0.001$) and GSH ($P < 0.01$), and an increase of GSSH ($P < 0.05$), are observed when comparing HS vs. CS with a 2.9-fold decrease of the GSH/GSSG ratio.

DISCUSSION

Reactive oxygen species cause oxidative damage to biomolecules, resulting in various diseases. Considerable effort has been made to

Table 4. RT-PCR of liver antioxidant enzymes against β-actin as internal standard

Parameter	CC ($n=6$)	CS ($n=6$)	HC ($n=6$)	HS ($n=6$)
CAT	1.05 ± 0.02	0.98 ± 0.05	1.11 ± 0.01	0.99 ± 0.09
SOD-1	1.11 ± 0.10	1.17 ± 0.14	1.12 ± 0.12	1.34 ± 0.13
GPx	1.21 ± 0.04	1.30 ± 0.06	1.12 ± 0.04	1.26 ± 0.04
NOX-2	0.75 ± 0.11	$1.08 \pm 0.11^{\bullet\bullet}$	0.83 ± 0.07	1.01 ± 0.10
Nrf2	0.78 ± 0.05	0.74 ± 0.08	0.85 ± 0.08	0.87 ± 0.05

Values are means $\pm$ SD ($n=6$), analyzed by one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test. $^{\bullet}$CS vs. CC: $^{\bullet\bullet}$ $P < 0.01$.
CC, control casein; CS, control soy; HC, hypercaloric casein; HS, hypercaloric soy; CAT, catalase; SOD-1, superoxide dismutase 1; GPx, glutathione peroxidase; NOX-2, NADPH oxidase 2; Nrf2, nuclear factor E2-related factor 2.

identify antioxidants in foods, and the mechanisms behind their antioxidant activity *in vivo*.[28] We performed an *in vivo* study to examine the potential protective role of soybean flour (vegetable protein) on the oxidative stress.

MDA is known as a marker of oxidative stress and the antioxidant status.[29] Our results indicate that MDA those not show significant differences between the compared groups, both in serum as well as in tissue, indicating that the replacement of casein by soybean flour in our experimental model does not modify lipid oxidation, even with hypercaloric diets. Madani *et al.*[30] found that rats fed with highly purified soybean protein, have lower plasma MDA concentrations with respect to rats fed with casein. Yang *et al.*[31] reported that the concentrations of MDA and 4-hydroxyalkenals were lower in rats consuming soybean protein. The differences between our findings and that of other authors can be due to the use of soybean flour instead of protein isolate or protein concentrate. In the plasma, however, although that there is no significant difference, a decreasing trend of 16% is observed when comparing HS vs. HC. This could be due to the antioxidant compounds that are present in the soybean flour, such as isoflavones[32–34] and vitamin E,[35] which can protect against lipid peroxidation.[36]

On the other hand, there is increasing scientific evidence supporting that the diet is a factor of great importance in the modulation of the oxidative stress; however, the effects of the dietary factors on the oxidative stress markers are still inconclusive,[37] and there are many authors that question the capacity of the antioxidants present in the diet.[38]

Highly reactive oxygen species that are formed during normal metabolism, and under oxidative stress conditions, are able to oxidize proteins or convert lipids and carbohydrate derivatives into compounds that react with proteins functional groups. Among other changes, these ROS-mediated reactions lead to the formation of protein carbonyl derivatives, which serve as a marker of ROS-mediated protein damage.[39]

The analysis of carbonyl groups suggests that in the presence of a caloric overload, there is a greater damage on the vegetable origin protein, probably due to the increased content of amino acid susceptible to oxidation.

Regarding the activities of SOD-1, CAT and GR, our results show that the soybean flour in the normal diet stimulates these antioxidant enzymes, while this favorable behavior is only observed in the SOD-1 activity for the hypercaloric diet, suggesting that the protective effect is higher when the soybean diet is normocaloric. Our data agree with those of Yang *et al.*,[31] in which the activities of these enzymes were higher in the groups were soybean protein was incorporated.

When observing the behavior of the GPx activity, there are no differences between the animals fed with normocaloric diet, therefore, it is likely that mainly the catalase enzyme is the one in charge of sweeping the H_2O_2. On the other hand, the caloric overload decreases the GPx activity in the diet base on soybeans, which would not be compensated by CAT.

The lack of correlation between the genetic expressions and the enzymatic activities, may indicate that one or more mechanisms of post-transcriptional control might be involved.[40]

The nature and the level of dietary protein (especially their sulfur-containing amino acid contents), influence the glutathione cellular concentration,[41,42] which plays a critical role in the detoxification of reactive intermediates involved in oxidative metabolism.[43] When hepatic glutathione is depleted, animals are more susceptible to tissue damage caused by free radical generating compounds.[44]

In our experimental model, total glutathione levels are increased in the CS group compared to CC group, which can be attributed to the increase of the GSH levels. When comparing HS vs. HC, the decrease of total glutathione is attributed to a decrease of GSSG. If we take into account the GSH/GSSG ratio in the cells, which is often used as a measurement of cellular toxicity,[45] it may be concluded that both soybean flour diets produce an increase in the GSH/GSSG respect to casein diets, and contribute to improving the animals redox state. The favorable effects of soybean isoflavones and soybean protein on the activity of glutathione metabolizing enzymes, have also been reported,[46,47] supporting our results.

The significant increase of NOX-2 in the CS group compared with the CC group, and the increasing tendency of HS vs. HC (18%), is reflected in the increase of the SOD-1 activity needed to sweep away excess $O_2^{\bullet-}$ radicals.

Nrf2 is a transcription factor that activates genes that regulate and increase cytoprotection.[48] No significant differences were observed in our model when comparing the soybean flour effect with respect to casein; however, there is an increasing tendency of this factor in both hypercaloric diets, which agrees with chemical-induced hepatotoxicity,[49–51] where an increase of that factor is observed.

CONCLUSION

The results of this work allowed to conclude that: (1) the substitution of casein by soybean flour, in a normocaloric diet, is capable of improving the antioxidant defense system in the liver of *Wistar* male rats, in comparison to the animals that continued being fed with diets based in casein; (2) this behavior of the soybean flour is also observed in the hypercaloric diets, although in less extent, so that a greater number of studies will be necessary to clarify this

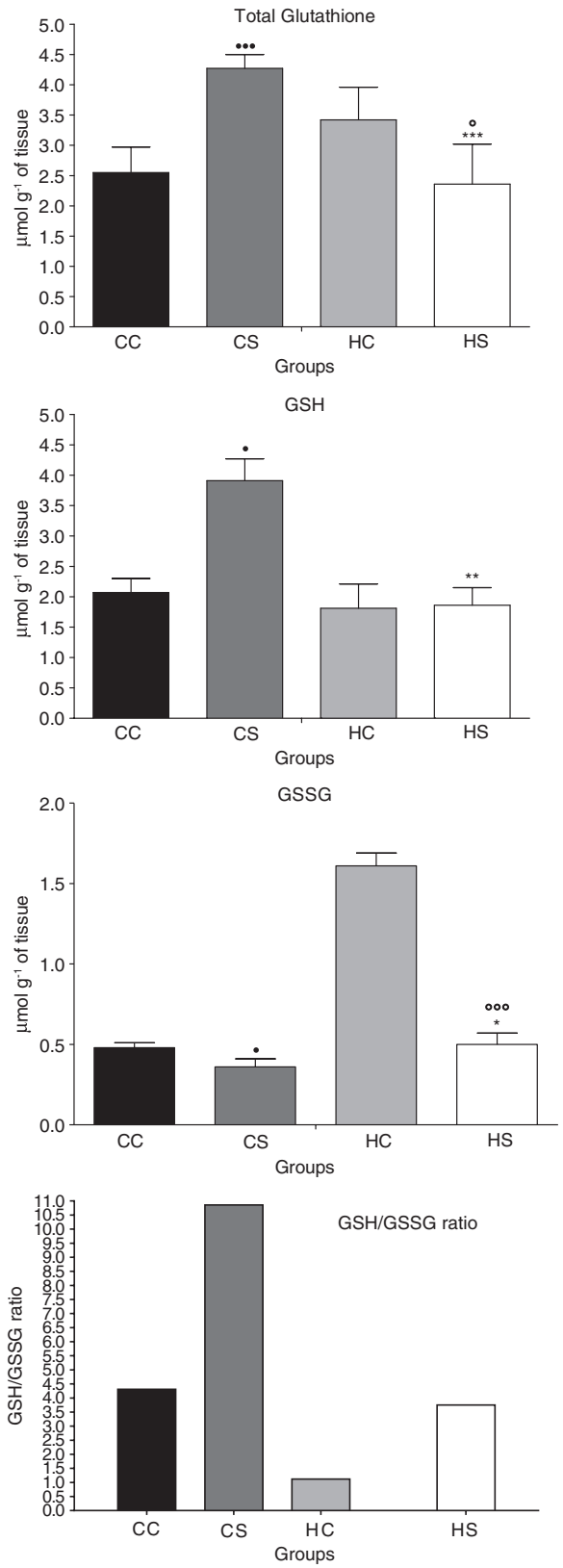

Figure 3. Content of glutathione in liver. Values are means ± SD ($n = 6$). Analyzed by one-way analysis of variance (ANOVA) followed by the Tukey multiple comparison test. •CS vs. CC: •$P < 0.05$, •••$P < 0.001$; °HS vs. HC: °$P < 0.05$, °°°$P < 0.001$; *HS vs. CS: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. CC, control casein; CS, control soy; HC, hypercaloric casein; HS, hypercaloric soy. GSH, reduced glutathione; GSSG, oxidized glutathione.

action; (3) soybean flour improves the cellular redox state at hepatic level, being this effect more marked in normocaloric diets; and (4) the mechanisms by which soybean flour stimulate the antioxidant defenses could be due to the composition of amino acids of its protein, to its elevated content of polyunsaturated fatty acids, and to the presence of isoflavones, which, as a whole, contribute to the soy antioxidant properties. All the above suggest that the inclusion of soybean flour to the diet can offer benefits in comparison to casein.

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