Carnosine supplementation and retina oxidative parameters in diet-induced obesity model

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Abstract

Purpose: To describe retina oxidative parameters and carnosine influence in diet-induced obesity model.

Methods: Wistar rats were randomly divided into four groups: Standard diet (SD), high sugar-fat diet (HcD), standard diet + carnosine (SD+Carn), and high sugar-fat diet + carnosine (HcD+Car). Evaluation in animals included body weight, adiposity index, plasma glucose, total lipids, high density lipoprotein (HDL), and low-density lipoprotein (LDL), uric acid, creatinine, and triglycerides. The retinas were analyzed for markers of oxidative stress. Hydrogen peroxide production was assessed by oxidation of 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA). The analysis of the antioxidant system included total glutathione (tGSH), total antioxidant capacity (TAC), protein carbonyl, and sulfhydryl type.

Results: The hypercaloric diet induced higher body weight, adiposity index, glucose, and triglyceride. Carnosine supplementation influenced on plasma triglyceride.

Absence of diet effect was verified in retina fluorescent derivative of oxidized DCFH-DA (DCF), TAC, GSH reduced levels and GSH:GSSG ratio. High retina TAC levels were found in rats treated with carnosine. Retina GSH reduced levels and GSH:GSSG ratio improved in carnosine-supplemented rats treated with high sugar-fat diet (vs.SD+Car). Diet was associated with increase in retina carbonyl content and decrease in sulphydryl levels. However, carnosine supplementation had no interference in both retina parameters from animals treated with high sugar-fat diet.

Conclusion: The results suggest that the sugar- and fat-enriched diet fed the rats may trigger an imbalance in the retinal redox state and that carnosine may improve the oxidative parameters.

1. Introduction

Consumption of a hypercaloric diet with an inappropriate ingestion of simple carbohydrates causes metabolic disorders in the body, which result in morphofunctional alterations in various tissues, directly associated with the aging process (1,2). This type of diet induces an excessive production of reactive oxygen species (ROS), mainly the superoxide anion, by means of the mitochondrial electron transport chain, and is associated with obesity, hypertension, hyperglycemia, hyperinsulinemia, or dyslipidemia (3–5). The redox imbalance damages the structural integrity of the biomolecules influencing mechanisms of tissue repair (6–8). In this scenario, the redox imbalance and the inflammatory process, main pathogenic pillars of diseases induced by the metabolic syndrome, are also closely related to the age-related macular degeneration (AMD) pathogenesis (9–10). Age-related macular degeneration is considered the main cause of blindness in the elderly. A systematic review and metanalysis revealed that 8.7% of the world population has developed AMD, and the projected number of AMD-affected people may reach 288 million in 2040 (11). Despite the large amounts of investments on experimental and clinical research, the best results achieved only enabled vision care providers to delay central vision loss. Hence, it is important to acknowledge and act on factors considered modifiable, by either eliminating or attenuating them, with the objective to slow the onset of this disease as much as possible.
Carnosine (L-carnosine) is a cytoplasmic dipeptide (β-alanil-L-histidina) found in high concentrations (millimolar) in the skeletal muscle, liver, intestine and brain of vertebrates and invertebrates (12–14). It may be obtained through a diet; however, it is not absorbed in its whole form, as the carnosinase enzyme present in the digestive system rapidly hydrolyzes it (15–18). This natural product has antioxidative properties, eliminating ROS in the cells (19–23), reducing the lipid hydroperoxides produced by the oxidation of the polyunsaturated fatty acids (PUFAs) of the membrane (24–25). Other effects include its scavenger capacity to sequestrate unsaturated aldehydes and stimulate muscle glucose uptake (26–27). These are biological roles of utmost importance for obese people affected by insulin resistance and/or metabolic syndrome (28–29). Several studies have addressed its properties as an antioxidant, immunomodulator and neuroprotective agent (15). In the ocular globe, high levels of L-carnosine, of approximately 25 µM, were found in human transparent crystalline lenses, whereas a relevant decrease in ripe human cataracts, reaching a level of 5 µM, was observed (30). Studies with bioactive N-acetylcarnosine (NAC) ophthalmic prodrug lubricant eye drops revealed its efficacy not only to prevent cataract but also to treat it (31–32). Carcinine, another peptide that resembles carnosine, is a natural antioxidant with hydroxyl-radical-scavenging activity and promotes neuroprotection of photoreceptor cells after they are exposed to strong light (33). To our knowledge there are no studies addressing the effect of carnosine on the retina of obese subjects.

Considering that diet and obesity are modifiable factors of AMD (34), the current study formulated a hypothesis that a high sugar-fat diet is enough to induce retinal oxidative alterations in the rat. Simultaneously, antioxidative effects of carnosine supplementation on the retina of these animals are tested.

### 2. Methods

#### 2.1 Animals and Experimental Protocol

All experiments and protocols were approved by the Animal Ethics Committee of Botucatu Medical School (1292/2019) and were performed in accordance with the Use of Animals in Ophthalmic and Vision Research, and the National Institute of Health Guidelines for the Care and Use of Laboratory Animals. Male Wistar rats (189+/−9 g, 8 weeks of age), were kept in an environmental controlled room (22 °C ± 3 °C; 12 h light-dark cycle and relative humidity of 60 ± 5%). After the tenth week, the animals were randomly distributed into two groups. The group with 19 animals received a normocaloric diet (SD) and the group with 15 animals was fed a hypercaloric diet (HcD), rich in simple carbohydrates, for 20 weeks. In the twentieth week, the two groups (SD and HcD) were subdivided into two new groups. One of the SD groups (SD, n = 9) kept on receiving the normocaloric diet, whereas the other SD group was fed the normocaloric diet and carnosine intraperitoneally (250mg/kg) (SD + Car, n = 10). One of the HcD groups kept on receiving the hypercaloric diet (HcD, n = 7), whereas the other was fed the hypercaloric diet and infusions of carnosine (250mg/kg) (HcD + Car, n = 8). The whole experiment lasted 24 weeks: 20 weeks of induction period and 4 weeks of treatment. HcD groups also received water + sucrose (25%). The diets and water were ad libitum.
2.2. Administration of Carnosine

Carnosine (L-carnosine) was administered intraperitoneally (250mg/kg) (35) for 4 weeks (5 days a week) after the induction period. Infusion was performed with a 30G needle and injected volume was about 500uL.

2.3 Diets

The normocaloric diet consisted of a formulation of adequate macro and micronutrients for rats (36). The hypercaloric diet also respected the micronutrient adequacy, although the main source of energy derived from simple carbohydrates (75 to 80% of the total calorific value of the diet) supplemented by the marginally increased percentage of lipids. To reach the increased percentage values of simple carbohydrates, 25% sucrose solution was also added. Both diets were prepared at the UNIPEX Diet Center, at the School of Medicine of Botucatu. Consumption of water and diet of all animals were measured weekly. The diets used in this study were designed in our laboratory.

The HcD diet contained soybean meal, sorghum, soybean peel, dextrin, sucrose, fructose, lard, vitamins, and minerals, plus 25% sucrose in drinking water. SD contained soybean meal, sorghum, soybean peel, dextrin, soy oil, vitamins, and minerals. The nutrients and nutritional composition of each diet are presented in Table 1.

2.4. Nutritional Analysis

The nutritional profile was evaluated according to the following parameters: food and caloric intake, and body weight. Food consumption was measured daily and body weight weekly. Caloric intake was determined by multiplying the energy value of each diet (g × Kcal) by the daily food consumption. For the HcD group, caloric intake also included calories from water (0.25 × 4 × mL consumed).

2.5. Metabolic Analysis

After 12-h fasting, blood was collected from the tail and the plasma was used to measure biochemical parameters. Glucose concentration was determined by using a glucometer (Accu-Chek Performa; Roche Diagnostics, Indianapolis, IN, USA); triglycerides and uric acid were measured with an automatic enzymatic analyzer system (Chemistry Analyzer BS-200, Mindray Medical International Limited, Shenzhen, China). The rates of basal glucose, total lipids, triglycerides, high density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined by using colorimetric techniques (BT-330 spectrophotometer, Biosystems).

2.6. Obesity Characterization

The presence of obesity was established based on weight gain and adiposity index, by measuring body weight weekly. The weight gain was calculated by subtracting the initial weight from the final weight of the animals [weight gain (g) = final weight (g) – initial weight (g)]. The adiposity index represents the ratio
of the sum of the epididymal, visceral, and retroperitoneal fat deposits by the final weight multiplied by 100 [adiposity index (%) = (epididymal (g) + visceral (g) + retroperitoneal (g))/final weight (g) × 100].

2.7 Sample preparation

After the 24th week, the rats were killed via decapitation under deep isoflurane anesthesia and the eyes were slid open with a sharp surgical knife. With the aid of a dissecting microscope, the neural retina layer was carefully peeled off from the posterior section of the eye and placed on a weighing paper. The retinas were then scraped out, put in a plastic vial, and homogenized with 800uL of phosphate-buffered saline (PBS) and stocked in a -80˚C freezer for further oxidative stress analysis.

2.8 Oxidative stress assays

ROS production. Hydrogen peroxide production was assessed by oxidation of 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA) and measured by flow cytometry. The samples were homogenized 1:1 (v/v) of PBS at room temperature. After, 20 µL of the sample was incubated in 50 µL of DCFH-DA (10 µM) at 37°C for 30 minutes, and the formation was stopped at 4 ° C. The formation of the fluorescent derivative of oxidized DCFH-DA (DCF) was monitored with excitation and emission of wavelengths of 488 and 525 nm, respectively, using the BD Accuri C6 Cytometer.

Antioxidant system. The total glutathione (tGSH) levels were assessed based on the reaction of GSH with 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB; Ellman's Reagent, Sigma Aldrich Corporation, St. Louis, MO, USA), which generated an oxidized glutathione-TNB product that was later reduced by glutathione reductase in the presence of NADPH, consequently generating GSH. The oxidized GSH (GSSG) was measured using the recycling of GSSG through spectrophotometric monitoring of NADPH in the presence of 2-vinylpyridine. The GSH and GSSG concentrations were determined using a regression curve from various GSH or GSSG standards (37). According to the manufacturer's instructions, the antioxidant equivalent concentrations were measured at 570 nm as a function of Trolox concentration. Total antioxidant capacity (TAC) was measured using the colorimetric assay kit (Sigma Aldrich Corporation, St. Louis, MO, USA). The antioxidant equivalent concentrations were measured at 570 nm as a function of the Trolox concentration described above. Sulphydryl groups were measured as described previously (38). Samples were diluted at a 1:6 ratio in 0.1 M sodium phosphate containing 1 mM EDTA (pH 8.0), and 100 µL of this dilution was used to react with 50 µL (4 mg/mL) of DTNB. After an incubation period of 15 min at room temperature, sample absorbance was measured at 412 nm using a microplate reader (Versamax, Molecular Devices, EUA). Concentrations of sulphydryl groups were determined by parallel measurements of an L-cysteine standard curve. Protein carbonylation was determined by the reaction of 2,4-dinitrophenylhydrazine (DNPH) with carbonyl, generating an adduct absorbed at 366 nm (39). Oxidative stress was also expressed by GSH/GSSG ratio.

2.9 Statistical analysis

Results are displayed as the mean ± standard error of the mean (SEM). Differences among groups were determined by one-way analysis of variance (ANOVA) followed by the Newman-Keuls post hoc test when
appropriate. Differences with $p < 0.05$ were considered statistically significant. All statistical analyses were performed using Graphpad Prism 8 software (version 8).

3. Results

3.1 Body weight, adiposity index, and serum metabolic parameters

Considering the initial average body weight of 189±9 grams, the four groups displayed important weight increase as shown in Table 2. At the end of the experiment, HcD animals displayed a higher body weight than SD + Car group. Statistical significance was not observed among other groups (Table 2). The highest adiposity index was observed in the groups fed the hypercaloric diet, revealing no carnosine effect (Table 2). Glucose plasma levels were higher in HcD and HcD + Car when compared to SD group. No significant statistical differences were identified among other groups (Table 2). Our results also showed that HcD group presented significantly increased plasma triglycerides levels when compared to SD and SD + Car, whereas HcD + Car had significantly higher serum levels than SD and SD + Car (Table 2).

3.2 Levels of 2', 7'-dichlorodihydrofluorescein diacetate (DCFH-DA)

The production of oxidants is shown in Fig. 1 (DCF levels). There are no differences among the groups; administration of diet and carnosine produced no effect.

3.3 Antioxidant system in the retina

Absence of diet (HcD) effect was verified in retina TAC levels. However, carnosine supplementation increased TAC in SD + Car when compared to control (SD), as well as in HcD + Carn when compared to HcD group (Fig. 2a.). Lack of diet (HcD) influence was observed in retina GSH reduced levels. Carnosine supplementation rose GSH reduced levels in HcD + Carn when compared to SD + Car group (Fig. 2b.). Absence of diet effect was found in the GSH:GSSG ratio. Carnosine supplementation increased the ratio in HcD + Carn as compared to SD + Carn group (Fig. 2c.).

Diet (HcD) was associated with an increase in retina carbonyl content (HcD > SD). Carnosine supplementation was related to parameter enhancement in carnosine-supplemented rats treated with high sugar-fat diet when compared to SD + Carn group (HcD + Carn > SD + Car) (Fig. 3a.). Diet (HcD) was also associated with a decrease in retina sulphhydryl type levels. Absence of carnosine supplementation effect was verified in this parameter (Fig. 3b.).

4. Discussion

The aim of this study was to describe the retina oxidative parameters and the carnosine influence on diet-induced obesity model. The experimental model proposed in this study promoted metabolic changes,
represented by increased levels of fasting blood glucose and of plasmatic triglycerides. Carnosine supplementation influenced on plasma triglyceride. A greater weight gain and a different body composition represented by the adiposity index were also observed. In the retinal tissue, the hypercaloric diet did not influence the redox state. Nevertheless, carnosine exerted a reducer effect, with higher concentrations of TAC, GSH, and GSH:GSSG ratio. Oxidative damages associated with the hypocaloric diet, characterized by a higher concentration of carbonylated proteins and lower concentrations of the sulfhydryl groups, were observed. However, treatment with carnosine only induced a non-significant increase in the sulfhydryl type levels. Figure 4 summarizes these findings.

Simple carbohydrate and saturated fatty acid are associated with the development of the metabolic syndrome and multiple comorbidities (40–42). In relation to vision, the Beaver Dam Eye Study associated high intake of saturated fat and cholesterol with an increased risk for early age-related maculopathy (43). Another study corroborated these findings, observing that ingestion of high glycemic index foods also contributed to the development of AMD 2.71 times more than the ingestion of low glycemic index foods (fruits, cereals, vegetables, whole wheat bread) (44). The present study used a hypercaloric diet enriched with simple carbohydrates with 25% sucrose solution. It is an innovative model that mimics the development of obesity with metabolic complications, according to a protocol previously adopted by our group (36). This diet can induce hyperadiposity, insulin resistance with hyperglycemia, dyslipidemia, and even arterial hypertension, as Wistar rats hardly develop hypertension via diet models (36). In this model, echocardiographic and renal function alterations were observed (36). For clarification purposes, it is important to point out that the “control” animals (SD) are usually fed ad libitum, which frequently leads to over-eating and excessive gain of body weight (45–46). This fact was confirmed at the end of the study when weight gain was observed in the animals of the four groups in relation to the start of the experiment. The amount of energy intake by the animals fed ad libitum many times significantly exceeds their energy expenditure, resulting in a substantial gain of body weight or positive energy balance, frequently associated with early diseases (47). Consequently, the group that ingests food ad-libitum may be subject to harmful factors induced by the intake above the body requirements (48). For this reason, our study comprised four groups: SD group; HcD group; SD + Car group; and HcD + Car group. As expected, the hypercaloric diet induced a significant increase of glucose in relation to SD group, corroborating literature findings (49). However, the addition of carnosine (HcD + Car) was not able to revert this condition. The hypercaloric diet also induced a significant increase in serum triglyceride (TG) levels. Similar results have been shown in studies of diets high in refined sugar (3,50). Carnosine supplementation led to a decrease in triglycerides in relation to HcD, although with no statistical significance.

The high sugar-fat diet group received carnosine supplementation with the objective to test its antioxidant effects on the retina. The intake of hypercaloric diets associated with a western lifestyle has been associated with an excessive generation of ROS (3,51–52). It is known that a sucrose-based diet interferes in the performance of the antioxidant system, overloading the organism's defense system, leading to oxidative stress (53–55). It has also been demonstrated that the high fructose and high-fat diets affect the function and structure of the rat retina (56). In our study, the hypercaloric diet did not
interfere in the production of hydrogen peroxide in the retina, as shown in Fig. 1, with no significant statistical difference among groups.

The levels of total antioxidant activity (TAC), expressed in mM Trolox equivalent, were higher in the retina of the groups that received carnosine supplementation when compared to the SD groups (Fig. 2a). It is important to point out that a low TAC value directly indicates the deficit of its specific composing substances (57), considered an important biomarker of tissue antioxidant system (58). Patients with AMD had lower plasma TAC levels when compared to the control group (57, 59–64). It is a likely indication that the oxidoreduction disturbance may be involved in the pathogenesis of AMD and that the increase in TAC levels may be associated with a protective factor of the retina. In this regard, carnosine may have played a protective role in the retina of the rats fed the hypercaloric diet.

The hypercaloric diet group (HcD) presented a decrease in reduced GSH level in relation to SD group, however, with no statistical relevance. It has been observed that a high sucrose diet (545 g/Kg of sucrose) administered for more than three months, induced a significant decrease of GSH in the rat brain (55). Probably, the smaller amount of sucrose (80 g/kg) offered to HcD animals in the present study may account for the discreet reduction of GSH, corroborating the findings of another study (65). Of great importance is the finding that glutathione deficiency contributes to oxidative stress and, therefore, may play a key role in the pathogenesis of many diseases (66). Conversely, HcD + Car group had a significant increase in the GSH levels in relation to SD + Car group (Fig. 2b). It is important to remember that glutathione has several major physiological functions, such as: protection of cells against destructive effects of reactive oxygen intermediates and free radicals, detoxication of external substances such as drugs and environmental pollutants, maintenance of red cell membrane stability, and enhancement of immunological function through its effects on lymphocytes (67). It was shown that oxidation induces apoptosis of RPE cells may be protected by GSH (68). Another study reported a significantly lower plasma GSH in older individuals affected by AMD, diabetes, and controls (elderly with no diabetes or AMD) than in younger individuals (69). Considering the increase of GSH in the HcD + Car group, it is possible to suggest that carnosine played a protective role in the retina.

A decrease of the GSH:GSSG ratio in the HcD group was observed when compared with HcD + Car group, although with no significant differences. Aging, chronic diseases, hypercaloric diet, and even AMD may potentially reduce the GSH:GSSG ratio (69,70–72). It is a known fact that the activity of GSH as an antioxidant can be expressed in two ways: as a function of GSH concentration and as a function of the redox state of the GSH:GSSG ratio (69). In the present study, the hypercaloric diet did not influence the retinal redox state. Nevertheless, the treatment with carnosine revealed higher GSH concentrations, observed in the GSH:GSSG ratio and, consequently, in a higher antioxidant power.

The present research also establishes a mapping of protein carbonylation of the Wistar rat retina fed the hypercaloric diets, as high values of protein carbonyl groups have been found in patients with AMD (59,73). The reactive carbonyl species are important cytotoxic mediators produced due to the oxidative damage of biomolecules (lipids and sugars), leading to alterations in the cell-signaling mechanisms to
the nucleus, positively regulating the redox-sensitive transcription factors, and inducing the irreversible structural modification in important molecules [proteins, peptides (cysteine, lysine, histidine), lipids, DNA] (74). Protein carbonyls are the most widely studied markers of protein oxidation and are frequently used as markers of oxidative stress, being indicators of the amount of protein that has been oxidized by highly reactive free radicals (75–76). Due to this ROS overproduction, increased protein carbonylation levels have been described along with these diet-induced disorders (77–78). Our results show that long-term intake of an HcD diet was associated with formation of carbonyl functional groups in relation to groups SD groups (Fig. 3a). It has been demonstrated that a high-caloric diet promotes similar results in the plasma and liver (73). Studies indicate that carnosine acts by a direct antioxidant mechanism and by sequestering reactive carbonyls (RCS), the byproducts of lipid and glucose oxidation, thus inhibiting AGE and ALE which are the reaction products of RCS with proteins (79). Although the in vitro capacity of carnosine to scavenge acrolein and form 3-methylpyridinium carnosine adduct has been demonstrated (8,27), in the present study, the dietary intervention of rats supplemented with carnosine did not show specificity by down-regulating carbonylation in the retina. On the other hand, an increase in carbonyl protein levels was observed in the HcD + Carn group. This does not mean that carnosine induced higher levels of carbonyl in HcD, as there was no increase in the level of SD + Car in relation to SD.

Besides the oxidative damage to proteins that a hypercaloric diet causes, it was observed that this diet also leads to a significant loss of the sulfhydryl group (Fig. 3b). Supplementation of carnosine to hypercaloric diet group restored values of sulfhydryl levels in the retina similar to SD (Fig. 3b). This is possible due to the increased GSH concentrations (80). It is important to remember that sulfhydryl groups, also biomarkers of oxidative stress, are considered the most powerful and most frequent antioxidants in the plasma (81), and their expression is reduced in AMD patients (82).

The diet prepared for this study, which mimics modern eating habits, induced oxidative changes in the retina. Carnosine seems likely to induce a potential antioxidant effect, elevating TAC and GSH concentrations in retinal tissue. Although some studies associate carnosine with the prevention and even the cure of cataract, the action of this antioxidant was not tested in oxidative and inflammatory markers of age-related macular degeneration. Further studies are required to determine the antioxidative effect of carnosine on AMD.

5. Conclusion

The high sugar-fat diet was associated with a significant decrease sulphydryl type levels and increase protein carbonylation in animal retina, suggesting an oxidant role in the retina. The retina of carnosine-treated animals showed an increase in TAC, GSH reduced levels and GSH:GSSG ratio. These results suggest that a sugar- and fat-enriched diet administered to rats may trigger an imbalance in the retinal redox state and that carnosine may improve the oxidative parameters.

Declarations
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Competing Interests

The author and all co-authors declare that they have no conflicts of interest regarding any support and financial involvement which could have influenced the writing of the article.

Author's and Co-Authors Contributions

Rogil Jose de Almeida Torres, the main and corresponding author, designed the study, collected, analyzed, and interpreted data, wrote the manuscript, approved the final version of the manuscript, and has agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Fernando Moreto, Sofia Pimentel Longo, Ricardo A Pinho, Seigo Nagashima, Lucia de Noronha, Artur Junio Togneri Ferron, Carol Cristina Vagula de Almeida Silva, and Camila Renata Correa designed the study, collected, analyzed, and interpreted the manuscript data. Andrea Luchini, Rogerio Joao de Almeida Torres, Giancarlo Aldini, and Ana Lucia Anjos Ferreira wrote the main manuscript text. All authors reviewed the manuscript and approved its final version to be published.

Ethics Approval

The author and co-authors declare that the research complies with internationally accepted standards for research practice and reporting and has been carried out within an appropriate ethical framework. Additionally, the authors declare that article they are submitting is their own original work, has not been previously published elsewhere, and is not currently being considered for publication elsewhere. The study reflects the authors’ own research and analysis in a truthful and complete manner and the experiments and protocols have been approved by the Animal Ethics Committee of Botucatu Medical School (1292/2019) and were performed in accordance with the Use of Animals in Ophthalmic and Vision Research, and the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

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Tables

Tables 1-2 are available in the Supplementary Files section.

Figures
Figure 1

Effects of carnosine supplementation on hydrogen peroxide production in retinas from animals undergoing a high caloric diet. Values are means +\- standard error of the mean (SEM); groups: SD, standard diet; SD+Car, standard diet + carnosine; HcD, high sugar-fat diet; HcD+Car, high sugar-fat diet + carnosine. Carnosine 250 mg / (Kg body wt /day) or saline IP for 4 wks; DCF, formation of the fluorescent derivative of oxidized DCFH-DA (oxidation of 2‘, 7‘-dichlorodihydrofluorescein diacetate); Two-way ANOVA, followed by the Newman-Keuls test, used to analyze the treatment effects.
Effect of carnosine supplementation on antioxidant system in retinas from animals undergoing a high caloric diet. Values are means ± standard error of the mean (SEM); groups: SD, standard diet; SD+Car, standard diet + carnosine; HcD, high sugar-fat diet; HcD+Car, high sugar-fat diet + carnosine. Carnosine 250 mg / (Kg body wt /day) or saline IP for 4 wks; Total antioxidant capacity (TAC) (a), total GSH activity (b), and glutathione (GSH)/oxidized glutathione (GSSG) ratio (c). Two-way ANOVA, followed by the Newman-Keuls test, used to analyze the treatment effects (p < 0.05).
Figure 3

Effects of carnosine supplementation on carbonyl contents and sulfhydryl levels in retinas from animals undergoing a high caloric diet. Values are means ± standard error of the mean (SEM); groups: SD, standard diet; SD+Car, standard diet + carnosine; HcD, high sugar-fat diet; HcD+Car, high sugar-fat diet + carnosine. Carnosine 250 mg / (Kg body wt /day) or saline IP for 4 wks; Carbonyl contents, (a), sulfhydryl type levels, (b); Two-way ANOVA, followed by the Newman-Keuls test, used to analyze the treatment effects (p < 0.05).

Figure 4
Effects of hypercaloric diet and carnosine supplementation on metabolic parameters and retinal oxidation

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pdf
- Table2.pdf