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Nitrosative stress contributes to apoptosis in the livers of senescent female rats treated with high fructose

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ABSTRACT

Aims: To evaluate oxidative/nitrosative stress and apoptosis in the livers of senescent female rats treated with high fructose.

Main methods: Young (Y) and senescent (S) female rats received water or fructose (F) for 12 weeks and were allocated to 4 groups: YC, YF, SC or SF. At the end of the experiment, the plasma was collected for analysis of liver function, and liver tissue was analyzed for nitric oxide (NO), superoxide anion, catalase (CAT), superoxide dismutase (SOD), total glutathione (GSH), endothelial nitric oxide synthase (eNOS), 3-nitrotyrosine (3-NT), caspase-3 and qualitative histology.

Key findings: glycemia was reduced in SF vs. YF; food intake was reduced in YF, SC and SF vs. YC and augmented in SF vs. SC; water intake presented an increase in YF and SF vs. YC and SC; body mass increased in all groups vs. YC. Superoxide anion was elevated in SF vs. other groups; total GSH was decreased in SF vs. YF and SC. The eNOS had a significant reduction in SC vs. YC but increased in SF vs. SC. 3-NT increased in the senescent groups vs. YC and YF. Caspase-3 showed an increase in SC vs. YC and in SF vs. YC, YF and SC, with a higher rate of apoptosis and greater evidence of hepatic steatosis in SF.

Significance: Our study suggests that high fructose contributes significantly to S-nitrosylation and with a higher rate of apoptosis in the livers of aged animals, suggesting that diets rich in sugar can cause irreversible damage to the body during aging.

Keywords: Nitrosative stress, Apoptosis, Liver, Aging, Female rats.

1 INTRODUCTION

Aging is characterized as a multifactorial process caused by the progressive decline in physiological functions and environmental factors. Currently, aging is considered the natural decrease in the ability of the organism over time as a result of cumulative unrepaired damage [1]. Several factors, including the sharp peak of births from World War II (called the “baby boom”), increased life

expectancy in the world (from 48 years in the 1950s to an estimated 76 years in 2050) and declining fertility, contributed to the significant increase in the elderly population (aged over 60 years) [2]. More than 300 theories have been proposed to explain the aging process; among the most important are the theory of free radicals (FR) and mitochondrial theory; still others, such as the immune theory and the theory of inflammation, all provide important information for understanding the physiological changes that occur during the aging process [3].

Oxidative stress is defined as the imbalance between the formation and removal of oxidizing agents in the body due to excessive generation of reactive species of oxygen (ROS) and/or reactive species of nitrogen (RNS) and reduced endogenous antioxidants [4]. ROS are highly reactive oxidizing species organically produced and related to aging by FR theory, which, in turn, suggests that the age-dependent deterioration of cell function is associated with the accumulation of oxidative molecular damage [5]. An increase in ROS production, particularly as a result of mitochondrial dysfunction, has been recognized as the major cause of oxidative stress. However, changes associated with aging may also contribute to increased oxidative stress. Free tyrosines bind to proteins attacked by various RNSs, including peroxynitrite, to form nitrotyrosine (3-NT), and its detection in proteins is considered a biomarker for endogenous peroxynitrite activity [6][7].

High oxidative stress levels induce cell death through necrosis and apoptosis mechanisms, leading to cell and tissue damage. The liver is a major organ attacked by ROS; parenchymal cells are the first to be subjected to oxidative stress in liver injury [7].

Excessive ROS in the body is counteracted by antioxidants that act enzymatically, such as glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD), or nonenzymatically, such as glutathione (GSH) [8]. NO is produced by the enzyme nitric oxide synthase (NOS), and there are three isoforms described, an inducible isoform (iNOS) and two constitutive isoforms (cNOS); the constitutive isoform initially is found in the endothelium (eNOS) and under basal conditions produces NO by shear force generated through blood flow [9].

NO can act by directly modifying the cysteine residues of the proteins. Cysteine S-nitrosylation is considered a selective and specific signal controlled by cellular NO and has been associated with protein localization, stability and function. NO is a posttranslational modification in which NO regulates protein function and signaling. S-nitrosylation participates in liver physiology and pathophysiology [10, 11].

The largest component of ROS is the superoxide anion, which has high cytotoxic activity and is transformed quickly into hydrogen peroxide by SOD; it is converted into water by two enzymes, CAT and GSH-Px [12]. The first mechanism is involved in microvascular dysfunction associated with aging and oxidative stress resulting in cellular dysfunction and apoptosis [13].

Apoptosis is a physiological process in which cells undergo a series of genetically programmed events that lead to death; such events occur in response to various external and internal stimuli [14]. Caspases (cysteine aspartate-specific proteases) belong to the family of cysteine proteases and have the ability to recognize and cleave substrates with aspartate residues. Fourteen human caspases are known, among which six (3, 6, 7, 8, 9 and 10) participate in apoptosis and seven are involved in the maintenance of cytokines [15].

In elderly humans, lower secretion of bile acid, increased cholesterol levels and oxidative stress have been demonstrated, which is mainly related to the reduced capacity to eliminate metabolically produced superoxide anions [16]. One of the most important changes related to liver aging in animal models is the significant decrease in the regenerative capacity of this organ [17].

Indeed, there are studies showing that diet can influence hepatic oxidative stress [18-20]. It has been shown that excessive consumption of fructose leads to accumulation of fatty acids that remain stored in the liver in the form of triglycerides and can lead to the formation of fatty liver, highlighting the role of lipogenic fructose [21]. Sugars are very common in the diet, such as sucrose, fructose and glucose, and given the substantial participation of fructose in our daily diet, it is important to know its metabolic effects and consequences [22]. The aim of this study was to evaluate S-nitrosylation and apoptosis in the livers of senescent female rats treated with high fructose.

2 MATERIALS AND METHODS

2.1 ANIMALS

We used 40 female Wistar rats provided by the National Institute of Pharmacological and Molecular Biology – INFAR/ UNIFESP; 20 young (four months old) and 20 senescent (twenty-four months old) rats were acquired, both with an average weight between 200-300 g.

The animals were kept in an air-conditioned animal house with a temperature of 21 ± 2 °C, cycle of light and dark of 12/12 h, receiving standard chow and water *ad libitum*. During the first three days, the animals were adapted to the new environment. All experiments were conducted after approval by the Ethics Committee of the Universidade Federal de Sao Paulo under #239114. The animals were allocated into four groups, with 10 rats each:

YC: young control - adult who received water (fructose vehicle)

YF: young fructose - adult who received fructose

SC: senescent control – senescent who received water (fructose vehicle)

SF: senescent fructose – senescent who received fructose

The rats in groups YF and SF received 10% fructose added to the drinking water daily for 12 weeks.

2.2 METABOLIC PROFILE

Blood fasting was measured by a glucometer; food and water intake were obtained weekly by subtraction of the total offered by what was left in the cage of each animal. Weighing was performed once a week from the first day of the protocol.

2.3 EUTHANASIA

The rats were euthanized by decapitation. Briefly, they were immobilized, put with their head in the guillotine opening and decapitated swiftly; blood was collected from the aorta, and plasma was separated and stored at -80 °C for further analysis.

Samples of liver tissue were collected immediately after euthanasia and distributed into two parts. The samples were stored at -80 °C. First, tissue samples were powdered and stored under liquid nitrogen to keep the samples stable and avoid oxidative damage to the proteins and lipids. After the samples were stored at -80 °C, the others were kept in buffered formalin for histological analysis.

2.4 HEPATIC PARAMETERS

The alanine transaminase (ALT) and aspartate transaminase (AST) levels were determined according to the manufacturer's instructions by commercial kits from Lab Test (MG, Brazil); the absorbance was read at 505 nm in a microplate reader (Synergy HT, Biotek, Winooski, USA), and activity was expressed in units/mL (U/mL).

2.5 OXIDANT PROFILE

Tissues were weighed, homogenized in Tris-HCl buffer (pH 7.4) and centrifuged at 3000 rpm for 10 min at 4 °C, and the supernatant was stored at -80 °C for NO and anion superoxide analysis. All these parameters were normalized by protein, which was measured using the Bradford method.

NO was measured in liver tissue samples by chemiluminescence using the Nitric Oxide Analyzer (NOA^{TM} 280, Sievers Instruments Inc, CO, USA), a high-sensitivity detector for measuring NO, expressed in nmol/mg protein [23].

The superoxide anion (OD/mg protein) was detected indirectly according to the adapted nitroblue tetrazolium protocol in liver tissue. The optical density (OD) was read in a microplate reader at 560 nm [24].

2.6 ANTIOXIDANT PROFILE

The antioxidant profile was performed in liver tissue through the commercial kits of Superoxide Dismutase Assay Kit (ESOD-100) and Catalase Assay Kit (ECAT-100), both acquired from Bio Assay Systems, Enzy ChromTM (Hayward, CA, USA) and expressed in U/mg protein, and ApoGSH^{TM}

Glutathione Colorimetric Detection Kit from Biovision (Milpitas, CA) expressed in ng/mg protein. All readings were performed on a microplate reader (Synergy HT, Biotek, Winooski, USA).

2.7 ELECTROPHORESIS AND WESTERN BLOTTING

The liver tissue was homogenized with protease inhibitor cocktail and centrifuged at 2000 rpm for 15 minutes at 4°C for Western blotting. For each sample, we used 80 µg of protein in 8% polyacrylamide gel; subsequently, the nitrocellulose membranes (Bio-Rad Laboratories, Inc., CA, USA) were blocked with 10% skim milk in TBS-T buffer for 1 h and then incubated overnight with antibody against eNOS 1:200 (BD Transduction Laboratories, CA, USA), 3-NT and caspase-3 1:500 (Santa Cruz Biotechnology Inc., CA, USA). The proteins were normalized to actin 1:1000 (Santa Cruz Biotechnology Inc., CA, USA), and the results were quantified using ImageJ software (National Institutes of Health, MD, USA).

2.8 HISTOLOGICAL ANALYSIS

For histological analysis, the liver tissues were fixed in 10% formaldehyde, embedded in paraffin, sectioned to a 4 mm thickness and stained with hematoxylin eosin (HE). The slides were stained at 400× magnification, and the percentage of apoptotic cells was counted in 10 fields of each slide randomly and examined by a pathologist, Dr Valeria P Lanzoni, under blinded conditions.

2.9 STATISTICAL ANALYSIS

The results were expressed as the mean ± standard error (SE) using one-way ANOVA followed by Tukey's post hoc test (parametric data) or Kruskal-Wallis with Dunn's post hoc test (nonparametric data); statistical significance was taken as $p < 0.05$.

3 RESULTS

3.1 METABOLIC PROFILE AND LIVER FUNCTION

Glycemia was altered in the young animals that ingested fructose in relation to the control group (YC); already, the SF group showed a significant decrease in glucose levels compared to the YF group, $p < 0.05$ (**Table 1**).

We noted that food intake was significantly reduced in YF vs. YC; SC showed a significant reduction of approximately 3-fold compared to YC and 2.3-fold compared to YF; SF was significantly reduced vs. YC and YF and significantly increased vs. SC, all with $p < 0.05$, as seen in **Table 1**.

The water intake in YF was significantly increased 2-fold compared to YC; SC presented significant reduction vs. YC and YF; SF was significantly diminished 2-fold vs. YF and with

significant increase vs. SC, all with $p < 0.05$. In relation to body mass, YF, SC and SF were significantly augmented compared to YC, $p < 0.05$ (**Table 1**).

Liver function assessed by plasma levels of ALT and AST were unchanged in the groups, as shown in **Table 1**.

3.2 NO AND SUPEROXIDE ANION

The levels of NO showed no significant change between the groups; the levels of superoxide anion in the SF group were significantly increased when compared with the YC, YF and SC groups, $p < 0.05$ (**Table 1**).

3.3 CAT, SOD AND TOTAL GLUTATHIONE

We found that there was no significant difference between the groups in CAT and SOD levels. However, we observed that total GSH was significantly decreased in the SF vs. YF and SC groups, $p<0.05$, as shown in **Table 1**.

Table 1. Metabolic, oxidative stress and antioxidant profile after treatment.

Parameters	YC	YF	SC	SF	
Glycemia (mg/dL)	105 ± 9	122 ± 5	109 ± 6	99 ± 8[b]	
Food intake (g)	181 ± 6	143 ± 6[a]	63 ± 3[a, b]	87 ± 4[a, b, c]	
Water intake (mL)	275 ± 14	555 ± 22[a]	106 ± 9[a, b]	255 ± 25[b, c]	
Body mass (g)	219 ± 2	231 ± 2[a]	243 ± 4[a]	234 ± 2[a]	
ALT (U/mL)	57 ± 11	49 ± 2	77 ± 8	72 ± 7	
AST (U/mL)	117 ± 9	122 ± 2	135 ± 3	111 ± 11	
NO (nmol/mg protein)	21.4 ± 1.8	23.8 ± 1.5	24.2 ± 2.3	21.5 ± 0.9	
Superoxide anion (OD/mg protein)	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.44 ± 0.16[a, b, c]	
CAT (U/mg protein)	0.0096 ± 0.0009	0.0102 ± 0.0002	0.0066 ± 0.0004	0.0080 ± 0.0008	
SOD (U/mg protein)	0.15 ± 0.01	0.16 ± 0.01	0.17 ± 0.02	0.14 ± 0.01	
Total GSH (ng/mg protein)	1.18 ± 0.03	1.22 ± 0.06	1.20 ± 0.04	0.99 ± 0.04[b, c]	

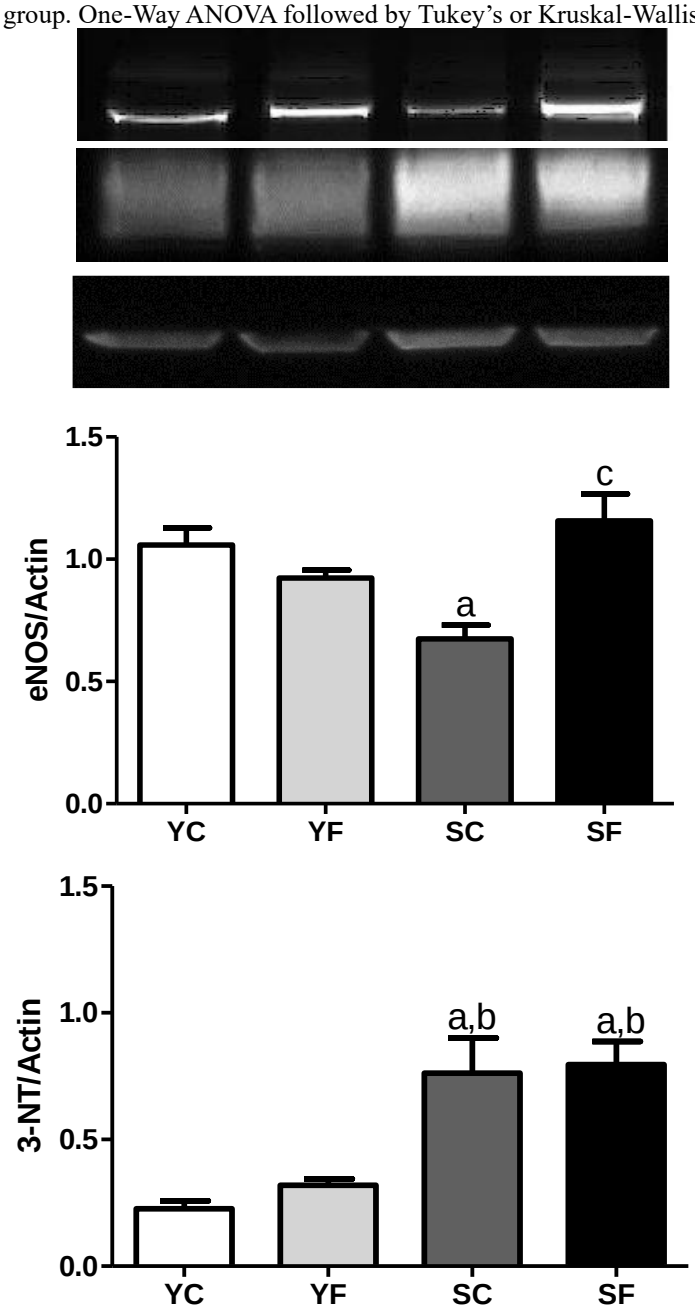
ALT: alanine transaminase; AST: aspartate transaminase; NO: nitric oxide; CAT: catalase; SOD: superoxide dismutase; GSH: glutathione. YC: young who received water; YF: young who received fructose; SC: senescent who received water; SF: senescent who received fructose. Data presented as mean ± SE; n= 5-10 per group. One-Way ANOVA followed by Tukey's or Kruskal-Wallis, $p < 0.05$: [a] *vs.* YC; [b] *vs.* YF; [c] *vs.* SC.

3.4 PROTEIN CONTENT OF ENOS, 3-NT AND CASPASE-3

The eNOS was decreased in SC (0.67 ± 0.06) vs. YC (1.06 ± 0.07) and increased in SF (1.16 ± 0.10) vs. SC, all with $p < 0.05$, as seen in **Fig. 1**.

Regarding 3-NT, there was no significant difference between young rats, YF (0.32 ± 0.02) vs. YC (0.23 ± 0.03); in relation to the older rats, SC (0.76 ± 0.14) and SF (0.79 ± 0.09) were increased vs. YC and YF ($p < 0.05$), as shown in **Fig. 1**.

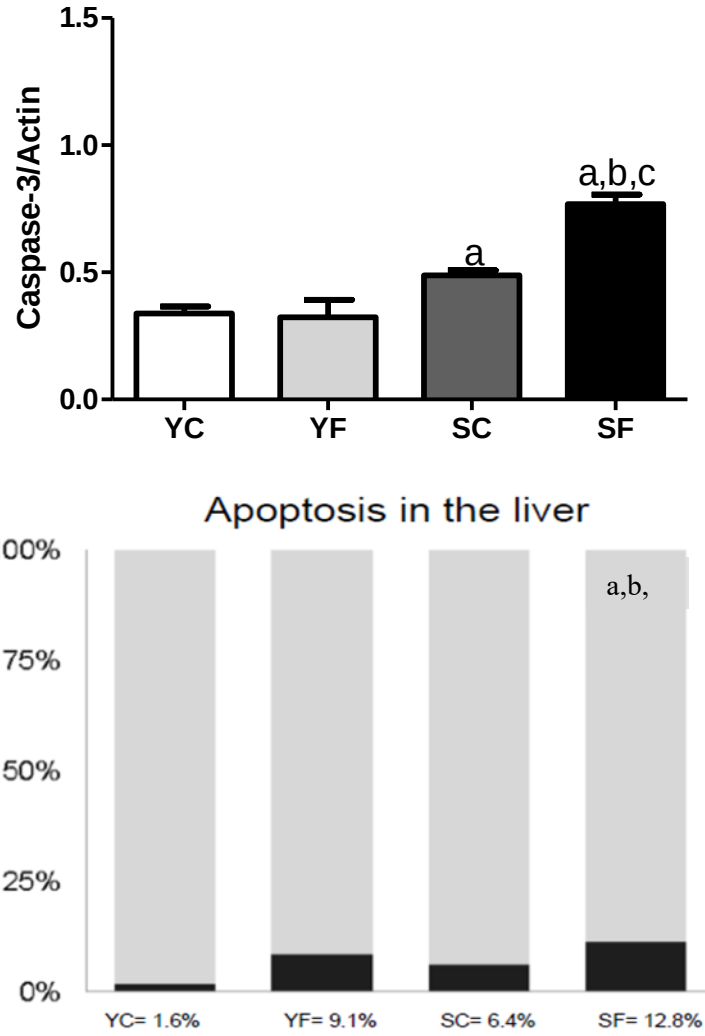
Fig. 1. The eNOS and 3-NT in hepatic tissue after 12 weeks of treatment. YC: young who received water; YF: young who received fructose; SC: senescent who received water; SF: senescent who received fructose. Data presented as mean ± SE; n = 4 per group. One-Way ANOVA followed by Tukey's or Kruskal-Wallis, $p < 0.05$: [a] vs. YC; [b] vs. YF; [c] vs. SC.



Caspase-3 showed an increase in senescent (0.49 ± 0.02) and senescent (0.77 ± 0.04) control rats when compared to their young animals, YC (0.34 ± 0.03) and YF (0.32 ± 0.07), all with $p < 0.05$; in addition, SF had an increase vs. YC, YF and SC groups ($p < 0.05$), as shown in **Fig. 2**.

Still in **Fig. 2,** it was observed the percentage of apoptotic cells, showing that YF and SF, groups treated with fructose, had a higher rate of apoptosis compared to their controls ($p < 0.05$), demonstrating that SF was augmented 2-fold compared to SC.

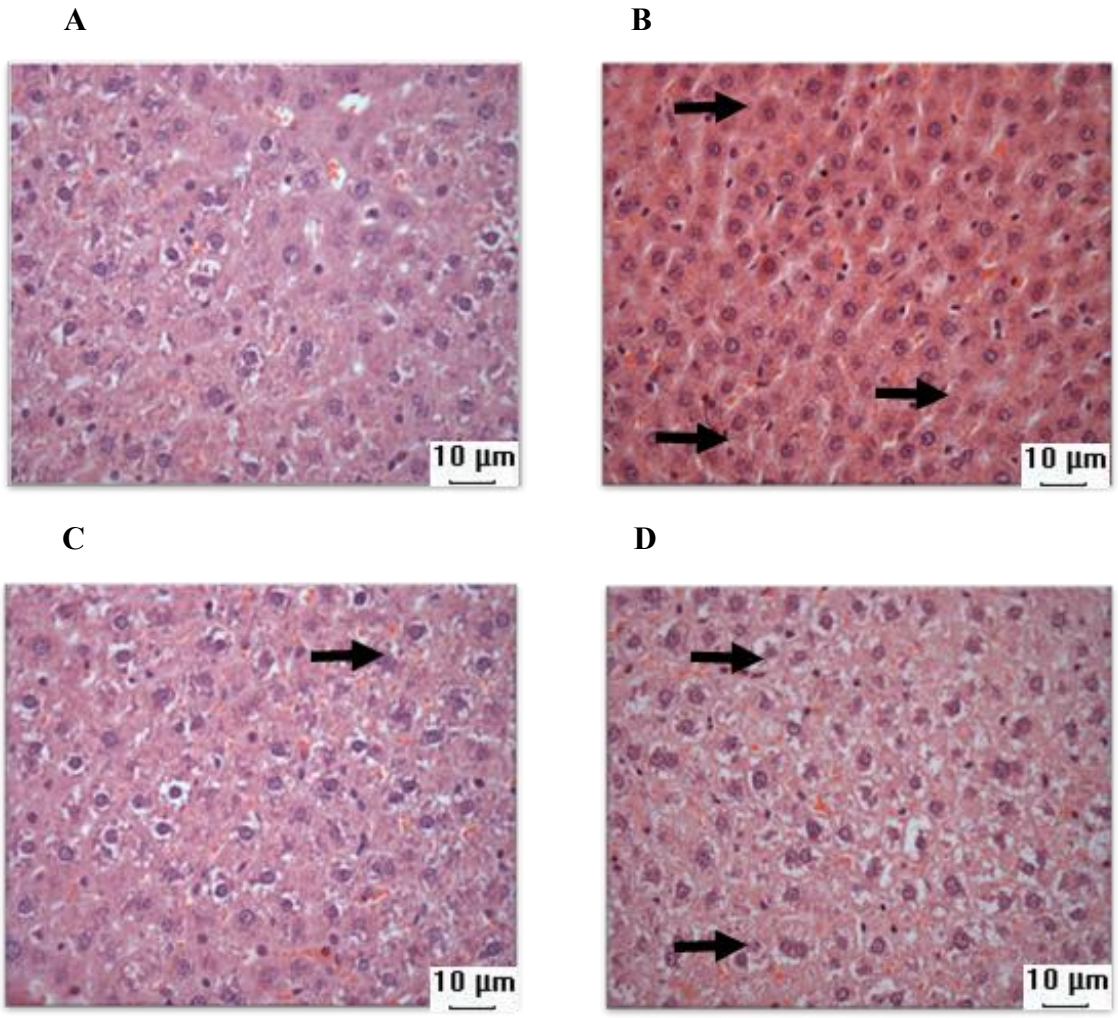
Fig. 2. Caspase-3 protein content and percentage of apoptotic cells on slides of hepatic tissue stained with HE after 12 weeks of treatment. YC: young who received water; YF: young who received fructose; SC: senescent who received water; SF: senescent who received fructose. Data are presented as the mean ± SE; n=4-5 per group. One-way ANOVA followed by Tukey's or Kruskal-Wallis test, $p < 0.05$: [a] vs. YC; [b] vs. YF; [c] vs. SC.



3.5 HISTOLOGY OF HEPATIC TISSUE

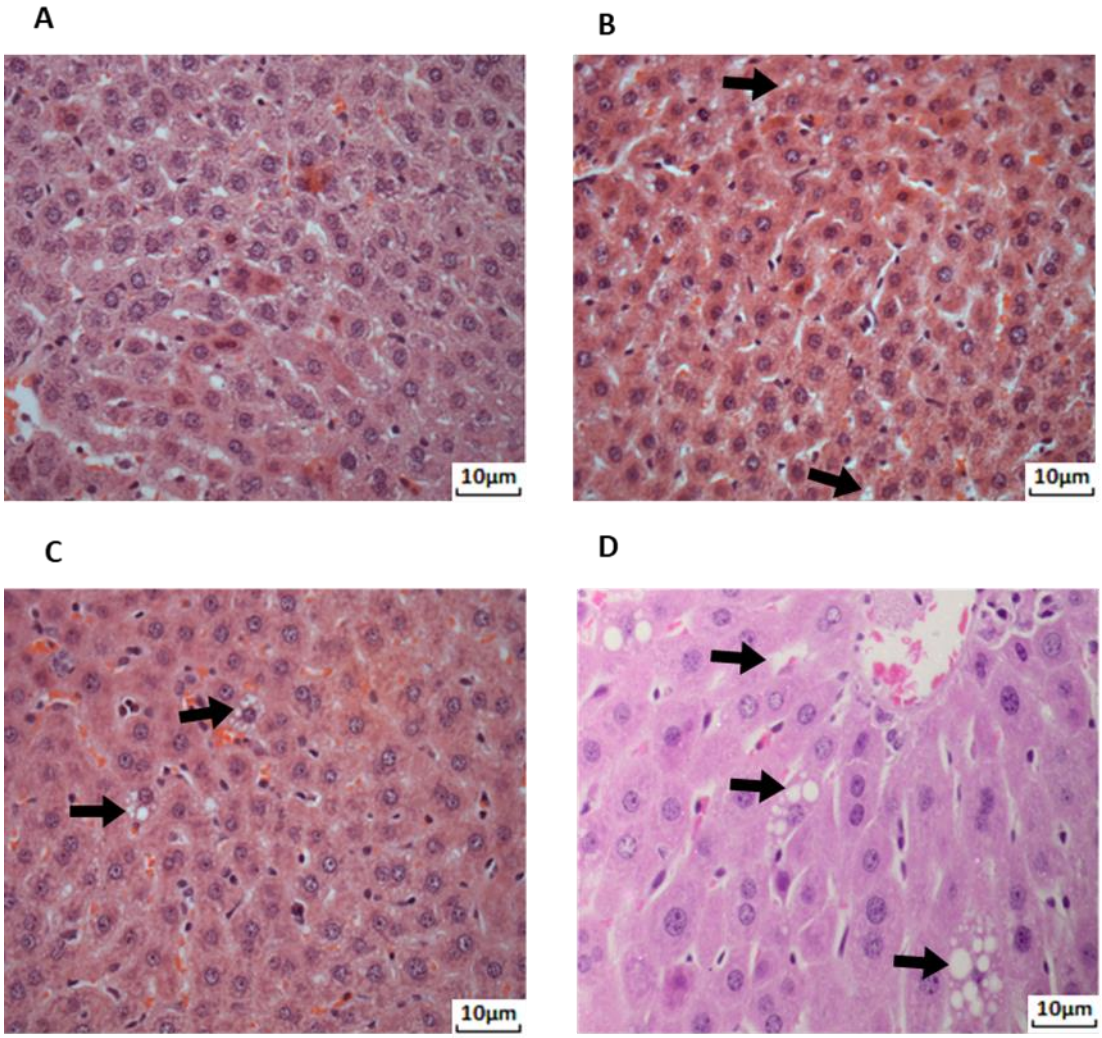
In **Fig. 3**, apoptotic cells are represented by acidophilus corpuscles, and retraction figures (black arrows) in B (YF), SC (C) and SF (D) were more pronounced in the fructose groups.

Fig. 3. Qualitative histological analysis of hepatic tissue after 12 weeks of treatment in animals. HE: hematoxylin-eosin. A) YC: young who received water; B) YF: young who received fructose; C) SC: senescent who received water; D) SF: senescent who received fructose. Black arrows indicate hepatocyte apoptosis, represented by acidophilus corpuscles and retraction. n= 5 slides per group. Magnification of 400 x.



In **Fig. 4**, signs of macro- and microvesicular steatosis (black arrows) were observed in slides stained with HE, noting that these findings were more evident and intense in the D group (SF).

Fig. 4. Histological section of hepatic tissue after 12 weeks of treatment. HE: hematoxylin eosin. A) YC: young who received water; B) YF: young who received fructose; C) SC: senescent who received water; D) SF: senescent who received fructose. Black arrows indicate macro- and microvesicular steatosis; n=5 slides per group. Magnification of 400 x.



4 DISCUSSION

Among the most interesting results of our study, we observed that fructose leads to increased oxidative stress and stimulates higher consumption of water. Wistar rats at 24 months of age show similar blood insulin levels as adult rats; furthermore, they also showed decreased peripheral glucose uptake with aging [25]. In our study, the levels of glucose in blood were significantly increased in the young fructose group but decreased in senescent animals.

The consumption of sugar-sweetened beverages is associated with the development of insulin resistance, fatty liver, type 2 diabetes (T2DM) and cardiovascular disease [26]. Animals feeding a high-fructose diet develop high plasma glucose, insulin, triglycerides, total cholesterol and oxidative stress levels, as well as increased liver mass and lipids [27]. In addition, excessive fructose intake mainly provided by the high amount of corn syrup present in the processed food was considered a major contributor to the increased rate of obesity and metabolic syndrome [26].

Fructose ingestion favors the development of hepatic insulin resistance in human studies and

impairs hepatic insulin action in experimental animal models. Therefore fructose leads to metabolic complications independently of increased weight gain and caloric intake [28]. Although some studies report an increase in body weight associated with fructose consumption [25, 29, 30], these results are conflicting. Suga *et al.* [31] reported no significant impact of fructose consumption on body weight and fat mass. These data corroborate our results since the young or senescent animals did not become obese after receiving fructose.

We did not find changes in the liver function of the animals. This is in agreement with the literature data, since hepatic function is unchanged during aging, which may be explained by the regenerative capacity of the liver [32].

According to Saltiel & Kahn [33], insulin plays a key role in regulating glucose and lipid metabolism in the body. The main pathogenesis of metabolic syndrome is the development of insulin resistance caused by the accumulation of visceral fat, dyslipidemia and deregulation of glucose metabolism; between the insulin target tissues, the liver is the major regulator of glucose and lipid metabolism, controlling hepatic glucose production, glycogen storage and lipogenesis [25-27]. The consumption of fructose-sweetened beverages increases the glucose and insulin concentrations; this, in turn, is metabolized to glucose via fructokinase, which is considered the best way of uptake by the liver, reaching circulation [34].

In the present study, we have shown that hepatic tissue in the senescent group that received fructose presented a significant increase in superoxide anion, demonstrating that fructose contributed positively to increasing oxidative stress in these animals. It is known in the literature that in situations of oxidative stress, there is formation of superoxide anion, which causes deleterious actions on the liver cells producing hydrogen peroxide, as high fructose intake raises the various types of ROS [35]. Once the organism does not eliminate hydrogen peroxide by catalase and peroxidase, hydroxyl radical production can occur; these, in turn, attack tyrosine, which could change and damage protein functions [7]. Furthermore, we showed that in old rats, the antioxidant profile is probably reduced due to increased oxidative stress.

According to Marmol et al. [36], the extent of cell damage and aging are related to a balance between oxidant production and antioxidant system removal. Our study showed a significant decrease in antioxidant activity (glutathione) in older rats that received fructose due to the increase in nitrosative stress in the liver of these rats.

ROS and RNS can react to form peroxynitrite, resulting in nitrotyrosylated proteins, and this mechanism may potentially contribute to redox-mediated dysfunction in this setting [37]. Some proteins can be regulated by this process or S-nitrotyrosylated, as well as caspase in the liver [10], the enzyme methionine adenosyl transferase [38] and flavin-containing monooxygenase [39]. It has also been shown that overall S-nitrosylation levels increase in NO donor-treated hepatic stellate cells [40].

Changes associated with aging have been recently described and contribute to impaired liver function; moreover, aging promotes various structural and functional changes in the microvasculature. Oxidative/nitrosative stress can modify the function of eNOS, which produces superoxide anions instead of NO, and this process is called uncoupling of eNOS. The uncoupling of eNOS is involved in the oxidation of the 4-BH cofactor, L-arginine depletion and accumulation of endogenous methylarginine [41].

In our study, we observed increased eNOS in the older fructose group; in stressful situations, the uncoupling of eNOS favored the production of ROS via NO. However, we did not observe differences in NO production, probably because NO was scavenged by the superoxide anion. On the other hand, eNOS expression was significantly reduced in the senescent group without fructose, which is physiological because aging causes natural endothelial impairment [42].

Santhanam et al [43] demonstrated that NO suppresses activity and increases S-nitrosylation of the matrix cross linking enzyme tissue transglutaminase in cellular models and that S-nitrosylation contributes to vascular stiffening in aging. Another study indicated that altered S-nitrosylation of p53 transcriptional activity during aging could be a contributing factor of sarcopenia condition and of other skeletal muscle diseases associated with oxidative/nitrosative stress [44]. Our data showed that there was S-nitrosylation in the liver of the senescent group, mainly in the animals that received fructose, though there was an increase in the superoxide anion in relation to the controls and young animals treated with fructose, conferring this process on aging.

Apoptosis is described in the literature as a physiologic event that is increased in aging; excessive apoptosis is a feature of organ failure in the liver [45]. There are several factors that may trigger apoptosis, such as low amounts of nutrients, increased levels of ROS or deprivation of growth factors [46]. In our study, we found that caspase-3 was increased in the hepatic tissue in the SF group. In addition, the histological evaluation also showed a higher number of apoptotic cells in the same group. These findings are similar to another study, where the total percentage of cells undergoing apoptosis in older mice was eight times higher than in the liver of young animals [47].

Upon activation of apoptosis, loss of cellular homeostasis can occur, interrupting ATP synthesis and increasing ROS production, causing oxidation of lipids, proteins and nucleic acids, exacerbating the collapse of the inner mitochondrial membrane potential [48]. It is also known that ROS induce the activation of caspases 3 and 9, triggering apoptosis [49]. In our findings, there was increased caspase-3 expression in the senescent groups compared to the young groups, showing that apoptosis was more expressive in the SF group.

The results presented in the study showed that among the modified proteins in liver aging, only the strongly glycated antioxidant enzyme catalase was constitutively present and located in the array. This indicates that glycation is more susceptible to stress; however, we did not observe differences

between the groups.

We can also see in our data an important involvement of ROS in aging and its influence on antioxidant activity, since total GSH activity was significantly reduced in the SF group, and this decrease was inversely associated with increased oxidative/nitrosative stress.

Three antioxidant enzyme systems are known and used as indexes to evaluate the levels of oxidative stress: SOD, CAT and GSH. The SOD enzyme catalyzes the destruction of superoxide anion radicals, converting them into oxygen and hydrogen peroxide; increased levels of SOD allow the removal of superoxide anions, even at low concentrations. There are two forms of SOD in the body: the first contains Cu^{2+} and Zn^{2+} as redox centers and occurs in the cytosol, and its activity is not affected by oxidative stress; the second contains Mn^{2+} as redox centers and occurs in the mitochondria, and their activity increases with oxidative stress. CAT catalyzes hydrogen peroxide into oxygen and water; the third system consists of GSH together with two enzymes, GSH-Px and glutathione reductase GSH-Rd; this system also catalyzes the dismutation of hydrogen peroxide into water and oxygen in the presence of GSH-Px, forming a disulfide bridge, and then is regenerated GSH [7, 8].

A study with healthy people aged 20 - 94 showed an increased incidence of low levels of glutathione in apparently healthy elderly people, 17% lower than the young group, which may be at risk due to the reduced ability to maintain many glutathione-mediated metabolic and detoxification reactions [50].

It was also found in our study that hepatic steatosis, which was represented by the number of fat cells, was present in greater numbers in the SF group. These data are in accordance with the literature, in which animals fed a high sugar diet had a higher hepatic steatosis index. A study by Takahashi et al. [19] revealed micro- and macrovesicular steatosis at histopathology, hypogranuloma and perisinusoidal fibrosis in the groups receiving fructose.

5 CONCLUSION

We observed that fructose contributed significantly to the increase in S-nitrosylation due to the decoupling of eNOS and a higher rate of apoptosis in older animals, demonstrating that diets rich in fructose during aging can cause irreversible damage to the hepatic tissue.

More studies are needed to understand the mechanisms and changes in aging, which could contribute to improving the quality of life in the elderly population.

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DISCLOSURE STATEMENT

The authors declare that there are no conflicts of interest.

REFERENCES

Khan, S.S., B.D. Singer, and D.E. Vaughan, *Molecular and physiological manifestations and measurement of aging in humans.* Aging Cell, 2017. 16(4): p. 624-633.

Bongaarts, J., *Human population growth and the demographic transition.* Philos Trans R Soc Lond B Biol Sci, 2009. 364(1532): p. 2985-90.

Vina, J., C. Borras, and J. Miquel, *Theories of ageing.* IUBMB Life, 2007. 59(4-5): p. 249-54.

Singh, P.P., et al., *Reactive oxygen species, reactive nitrogen species and antioxidants in etiopathogenesis of diabetes mellitus type-2.* Indian J Clin Biochem, 2009. 24(4): p. 324-42.

Gemma, C., et al., *Oxidative Stress and the Aging Brain: From Theory to Prevention*, in *Brain Aging: Models, Methods, and Mechanisms*, D.R. Riddle, Editor. 2007: Boca Raton (FL).

Ahsan, H., *3-Nitrotyrosine: A biomarker of nitrogen free radical species modified proteins in systemic autoimmunogenic conditions.* Hum Immunol, 2013. 74(10): p. 1392-9.

Colantoni, A., et al., *Evidence of oxidative injury during aging of the liver in a mouse model.* J Am Aging Assoc, 2001. 24(2): p. 51-7.

Birben, E., et al., *Oxidative stress and antioxidant defense.* World Allergy Organ J, 2012. 5(1): p. 9-19.

Ahmadinejad, F., et al., *Molecular Mechanisms behind Free Radical Scavengers Function against Oxidative Stress.* Antioxidants (Basel), 2017. 6(3).

Li, J. and T.R. Billiar, *The anti-apoptotic actions of nitric oxide in hepatocytes.* Cell Death Differ, 1999. 6(10): p. 952-5.

Tousoulis, D., et al., *The role of nitric oxide on endothelial function.* Curr Vasc Pharmacol, 2012. 10(1): p. 4-18.

Rahman, K., *Studies on free radicals, antioxidants, and co-factors.* Clin Interv Aging, 2007. 2(2): p. 219-36.

Liguori, I., et al., *Oxidative stress, aging, and diseases.* Clin Interv Aging, 2018. 13: p. 757-772.

Daniel, P.T., *Dissecting the pathways to death.* Leukemia, 2000. 14(12): p. 2035-44.

Shalini, S., et al., *Old, new and emerging functions of caspases.* Cell Death Differ, 2015. 22(4): p. 526-39.

Chiang, J.Y., *Bile acid metabolism and signaling.* Compr Physiol, 2013. 3(3): p. 1191-212.

Biondo-Simoes Mde, L., et al., *Effect of aging on liver regeneration in rats.* Acta Cir Bras, 2006. 21(4): p. 197-202.

Lee, J., et al., *Loss of Hepatic Mitochondrial Long-Chain Fatty Acid Oxidation Confers Resistance to Diet-Induced Obesity and Glucose Intolerance.* Cell Rep, 2017. 20(3): p. 655-667.

Takahashi, Y., et al., *Inhibitory effects of eucalyptus and banaba leaf extracts on nonalcoholic steatohepatitis induced by a high-fructose/high-glucose diet in rats.* Biomed Res Int, 2015. 2015: p. 296207.

Wada, T., et al., *Spironolactone improves glucose and lipid metabolism by ameliorating hepatic steatosis and inflammation and suppressing enhanced gluconeogenesis induced by high-fat and high-fructose diet.* Endocrinology, 2010. 151(5): p. 2040-9.

Tappy, L. and K.A. Le, *Metabolic effects of fructose and the worldwide increase in obesity.* Physiol Rev, 2010. 90(1): p. 23-46.

Schaefer, E.J., J.A. Gleason, and M.L. Dansinger, *Dietary fructose and glucose differentially affect lipid and glucose homeostasis.* J Nutr, 2009. 139(6): p. 1257S-1262S.

Hampl, V., C.L. Walters, and S.L. Archer, *Determination of nitric oxide by the chemiluminescence reaction with ozone*, in *Methods in nitric oxide research*, M. Feelisch and J.S. Stamler, Editors. 1996, John Wiley & Sons: Chichester. p. 310-318.

Choi, H.S., et al., *A quantitative nitroblue tetrazolium assay for determining intracellular superoxide anion production in phagocytic cells.* J Immunoassay Immunochem, 2006. 27(1): p. 31-44.

Ghezzi, A.C., et al., *Metabolic syndrome markers in wistar rats of different ages.* Diabetol Metab Syndr, 2012. 4(1): p. 16.

Malik, V.S. and F.B. Hu, *The role of sugar-sweetened beverages in the global epidemics of obesity and chronic diseases.* Nat Rev Endocrinol, 2022. 18(4): p. 205-218.

Park, D.Y., et al., *Dual probiotic strains suppress high fructose-induced metabolic syndrome.* World J Gastroenterol, 2013. 19(2): p. 274-83.

Softic, S., et al., *Fructose and hepatic insulin resistance.* Crit Rev Clin Lab Sci, 2020. 57(5): p. 308-322.

Sertie, R.A., et al., *Metabolic adaptations in the adipose tissue that underlie the body fat mass gain in middle-aged rats.* Age (Dordr), 2015. 37(5): p. 87.

Huynh, M., et al., *Dietary fructose during the suckling period increases body weight and fatty acid uptake into skeletal muscle in adult rats.* Obesity (Silver Spring), 2008. 16(8): p. 1755-62.

Suga, A., et al., *Effects of fructose and glucose on plasma leptin, insulin, and insulin resistance in lean and VMH-lesioned obese rats.* Am J Physiol Endocrinol Metab, 2000. 278(4): p. E677-83.

Schmucker, D.L. and H. Sanchez, *Liver regeneration and aging: a current perspective.* Curr Gerontol Geriatr Res, 2011. 2011: p. 526379.

Saltiel, A.R. and C.R. Kahn, *Insulin signalling and the regulation of glucose and lipid metabolism.* Nature, 2001. 414(6865): p. 799-806.

Coronati, M., et al., *Added Fructose in Non-Alcoholic Fatty Liver Disease and in Metabolic Syndrome: A Narrative Review.* Nutrients, 2022. 14(6).

Zhang, D.M., R.Q. Jiao, and L.D. Kong, *High Dietary Fructose: Direct or Indirect Dangerous Factors Disturbing Tissue and Organ Functions.* Nutrients, 2017. 9(4).

Marmol, F., et al., *Role of oxidative stress and adenosine nucleotides in the liver of aging rats.* Physiol Res, 2010. 59(4): p. 553-60.

Ischiropoulos, H., et al., *Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase.* Arch Biochem Biophys, 1992. 298(2): p. 431-7.

Corrales, F.J., F. Ruiz, and J.M. Mato, *In vivo regulation by glutathione of methionine adenosyltransferase S-nitrosylation in rat liver.* J Hepatol, 1999. 31(5): p. 887-94.

Ryu, S.D., et al., *Flavin-containing monooxygenase activity can be inhibited by nitric oxide-mediated S-nitrosylation.* Life Sci, 2004. 75(21): p. 2559-72.

Perri, R.E., et al., *Defects in cGMP-PKG pathway contribute to impaired NO-dependent responses in hepatic stellate cells upon activation.* Am J Physiol Gastrointest Liver Physiol, 2006. 290(3): p. G535-42.

Scioli, M.G., et al., *Ageing and microvasculature.* Vasc Cell, 2014. 6: p. 19.

Vicencio, J.M., et al., *Senescence, apoptosis or autophagy? When a damaged cell must decide its path--a mini-review.* Gerontology, 2008. 54(2): p. 92-9.

Santhanam, L., et al., *Decreased S-nitrosylation of tissue transglutaminase contributes to age-related increases in vascular stiffness.* Circ Res, 2010. 107(1): p. 117-25.

Baldelli, S. and M.R. Ciriolo, *Altered S-nitrosylation of p53 is responsible for impaired antioxidant response in skeletal muscle during aging.* Aging (Albany NY), 2016. 8(12): p. 3450-3467.

Leist, M., et al., *Tumor necrosis factor-induced hepatocyte apoptosis precedes liver failure in experimental murine shock models.* Am J Pathol, 1995. 146(5): p. 1220-34.

Newgard, C.B. and N.E. Sharpless, *Coming of age: molecular drivers of aging and therapeutic opportunities.* J Clin Invest, 2013. 123(3): p. 946-50.

Pibiri, M., *Liver regeneration in aged mice: new insights.* Aging (Albany NY), 2018. 10(8): p. 1801-1824.

Farrugia, G. and R. Balzan, *Oxidative stress and programmed cell death in yeast.* Front Oncol, 2012. 2: p. 64.

Chandra, J., A. Samali, and S. Orrenius, *Triggering and modulation of apoptosis by oxidative stress.* Free Radic Biol Med, 2000. 29(3-4): p. 323-33.

Lang, C.A., et al., *Low blood glutathione levels in healthy aging adults.* J Lab Clin Med, 1992. 120(5): p. 720-5.