International Journal of Food Science and Nutrition
ISSN: 2455-4898
Impact Factor: RJIF 5.14
www.foodsciencejournal.com
Volume 3; Issue 4; July 2018; Page No. 170-176

Long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs) inhibit 1IL-β, Cox-2 and TNFα proinflammatory cytokines in an experimental liver obesity model of wistar rats

Michely L Nunes[1], Gabriela F Hahn[2], Vanessa T Bortoluzzi[3], Marilene G Porawski[1,2]

[1] Department of Postgraduate Program in Medicina:Hepatology of the Federal University of Health Sciences of Porto Alegre (UFCSPA), Porto Alegre, RS, Brazil.

[2] Department of Postgraduate Program in Biosciences, Federal University of Health Sciences of Porto Alegre (UFCSPA), Porto Alegre, RS, Brazil

[3] Department of Biological Sciences Biochemistry, Laboratory Inborn Errors of Metabolism, and Federal University of Rio Grande do Sul (UFRGS). Porto Alegre, RS, Brazil

Abstract
The obesity is characterized by a state of inflammation of adipose tissue expansion, causing metabolic diseases. Objetive: to evaluate the effects of ômega-3 in the inflammatory process in liver tissue of rats on experimental obesity model induced by high-fat diet for 16 weeks. 40 Wistar rats were used, the animals were dividid in to 4 groups: control (CO), omega-3 control (CO+O), obese (O) and Obese plus omega-3 (O+O). Omega-3 has been administered at the dose of 1g/kg by gavage during the last 30 day of the diet. The omega-3 significantly reduced triglycerides and cholesterol of obese animals. As measured by TBARS, in SOD and CAT activity there was no difference between the groups.The activity of the GPx enzyme was significantly increased in the obese animals compared to the (CO+O) group and there was a reduction in the (Obese+O) group. The immunohistochemical labeling of TNFα, IL-1 and COX-2 was shown to be decreased in the hepatic tissue of animals (O+O) treated with omega-3 compared to obese and (CO) groups.. Conclusion: the results seem to indicate that the active compound omega-3 had na antioxidante and antiinflammatory in this model.

Keywords: live, antiinflammatory, antioxidant, bioactive compound

1. Introduction
The World Health Organization (WHO) points to obesity as one of the biggest public health problems in the world. The projection is that by 2025, about 2.3 billion adults will be overweight, and more than 700 million will be obese. If nothing is done, the number of overweight and obese children in the world could reach 75 million [1].

Overweight and obesity are the fifth leading risk factor for death in the world. Each year, at least 2.8 million adults die as a result of being overweight or obese. In addition, 44% develop diabetes, 23% develop ischemic heart disease, and 7% to 41% develop some type of cancer related to overweight and obesity [1, 2].

Obesity has multifactorial causes. Genetic factors contribute with less than 10% of cases, and other factors such as eating behavior, sedentary lifestyle, and physical activity practice have greater influence on excess body fat [3].

Body Mass Index (BMI) is the main method for measuring obesity. The BMI is obtained by dividing the body mass in kilograms by the square of the body height in meters (kg/m^2). BMI values above 25.0 kg/m^2 are considered excess weight; values from 25.0 kg/m^2 to 29.9 kg/m^2 are considered overweight, and BMI values $\geq$ 30.0 kg/m^2 are considered obesity [4]. This is an international benchmark recognized by the WHO, but it does not directly measure body fat, because it does not consider the proportion of lean mass, fat mass, fluids, and bone structure of the individual [5].

In obesity there is an increase in the production of cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α), which activate the inflammatory response in adipocytes by increasing the release of free fatty acids from adipocytes and generating a pro-inflammatory and lipotoxic environment. As part of the chronic inflammatory process, hepatic inflammation may occur, in which the activation of the inflammatory pathway could be a result of steatosis and increased responses to oxidative stress in hepatocytes [6].

Omega-3 type polyunsaturated fatty acids are essential nutrients derived from marine or vegetable sources. Eicosapentaenoic acid (EPA, 20: 5) and docosahexaenoic acid (DHA, 22: 6) are derived from fish such as salmon, tuna, and sardines. They exert a wide variety of biological effects being studied in a number of distinct clinical conditions, such as coronary disease [7], hypertension, hyperlipidemia, cancer, diabetes [8], kidney diseases, and inflammatory diseases [9, 10].

The anti-inflammatory properties of omega-3 fatty acids are well established, and a possible antioxidant action has been proposed, especially in relation to docosahexaenoic acid (DHA); it may interfere with the production of reactive oxygen species (ROS) and/or fight them, due to its multiple

double bonds [11].

Thus, omega-3 fatty acid acts by decreasing the formation of eicosanoids with inflammatory characteristics, as it competes with omega-6 fatty acids for the same enzymatic pathway, leading to the inhibition of the TNF-α, IL-1, and IL-6 synthesis, and reducing the intercellular adhesion molecule-1 (ICAM-1) expression. [12] It is also a substrate for the synthesis of series 3 and 5 eicosanoids that have less inflammatory characteristics [13].

The incorporation of EPA and DHA into the diet can influence the lipid structure of cell membranes and the physiological responses that depend on these membranes, such as cell signaling mechanisms. The omega-3 polyunsaturated fatty acid obtained from diet can contribute to the reduction of inflammatory processes and decrease the incidence of inflammation-related diseases [9, 14].

The present study used a hyperlipid diet-induced obesity model in Wistar rats to study the effects of omega-3 utilization on the biochemical and metabolic parameters of the disease.

2 Materials and methods

The present study was conducted at the Laboratory of Behavioral and Metabolic Physiology of the Federal University of Health Sciences of Porto Alegre (UFCSPA). A total of 40 3-month-old male Wistar rats from UFCSPA were used. The body weight of the rats was recorded weekly until the end of the experiment. The animals were kept in plastic boxes (2 animals per box), at an average temperature of 22° C and a cycle of 12 hours light/dark.

They were divided into four groups (n = 10): a) Group CO: fed ad libitum with control diet; b) Group CO+O: fed ad libitum with control diet plus omega-3, c) Obese group: fed ad libitum with hyperlipidic diet, and d) Group Obese + O: fed ad libitum with hyperlipidic diet plus omega-3. The animals received diet for 16 weeks; omega-3 treatment was administered from the 12th week until the end of the experiment. The control and hyperlipidic diets (45% of calories in lipids) were obtained from PRAGSOLUÇÕES. Sample calculation for a 95% confidence level and a 5% sample error determined an n of 10 animals per group. All experimental procedures performed on the animals were in accordance with the recommendations of the Arouca Law (Law No. 11,794, October 8, 2008) and approved by the local Ethics Committee (CEUA/UFCSPA number 117/13). This was a quantitative experimental study. The bioactive compound used to treat the animals was Omega-3 Catarinense®, (Catarinense Pharma), at a dose of 1 g/kg body weight/day, orally. This dose was defined in previous studies by our research group (data submitted for publication). At the end of the 16-week treatment, the animals were euthanized by overdose of anesthetics with xylazine hydrochloride (Rompun) and ketamine hydrochloride (Ketalar) were removed blood, visceral fat; and hepatic tissue where they were stored in the Freezer -80º for further analysis.

2.1 Anthropometric evaluation by the lee index

The rats were submitted to body weight and naso-anal length (NAL) measurements. From these data we obtained the body mass index, and the Lee index, which is the ratio between the cube root of the body weight (in grams) and the NAL (in centimeters) multiplied by 1000. [$\sqrt[3]{}$ Body weight (g) / NAL (cm)]. The Lee index was used as one of the biometric parameters to indicate the nutritional status of the animal; it is a satisfactory index for estimating body mass in animals of the same age and same nutritional history [15, 16].

2.2 Biochemical evaluation

The evaluation of glycemia, cholesterol, and triglycerides was performed through enzymatic colorimetric tests using commercial kits from Labtest.

2.3 Measurement of lipid peroxidation (LP)

LP was determined by thiobarbituric acid reactive substances (TBARS) test, described by BUEGE AND AUST (1978). One of the substances generated in the LP is malondialdehyde (MDA), which when heated in the presence of thiobarbituric acid forms a pink product, measured in the spectrophotometer at 535 nm [17].

2.4 Superoxide dismutase (SOD) antioxidant enzyme activity

The SOD antioxidant enzyme activity assay evaluates the enzyme's ability to inhibit the reaction of superoxide radical with adrenaline. A 96-well plate was used with 50 mM glycine buffer pH 11, an aliquot of homogenate, and adrenaline. The reading was performed at 480 nm. Data were expressed in units of SOD per milligram of protein (SOD /mg prot) [8].

2.5 Catalase (CAT) antioxidant enzyme activity

The activity of the CAT enzyme was evaluated by determining the decomposition rate of the hydrogen peroxide added to the sample, since the hydrogen peroxide decomposition is directly proportional to CAT activity. The reading was performed in a spectrophotometer at 240 nm and data were expressed in picomoles per milligram of protein (pmoles /mg prot) [19].

2.6 Glutathione peroxidase (GPx) activity

The activity of the antioxidant enzyme glutathione peroxidase was evaluated by the oxidation rate of NADPH in the presence of reduced glutathione and glutathione reductase. Sodium azide was added to inhibit catalase activity. GPx activity was measured in a spectrophotometer at 340 nm, and its activity was expressed in mmol/min/mg protein [20].

2.7 Immunohistochemistry technique

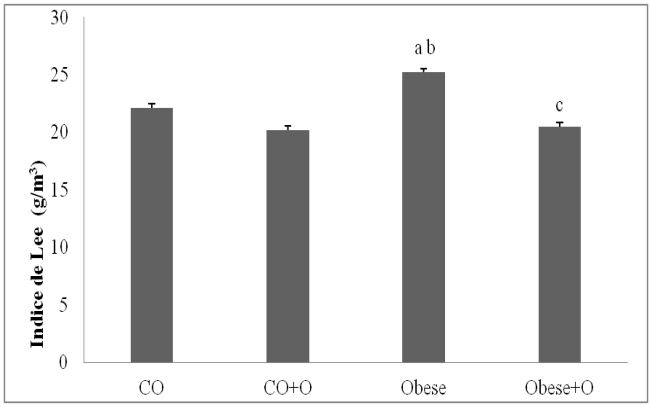
Immunohistochemistry technique was used to localize and quantify interleukin (IL)-1, TNFα, and COX-2 enzyme in the hepatic tissue of obese and omega 3-treated animals. The hepatic tissue was placed in a fixative solution for 48 hours. Afterwards, the pieces were dehydrated, diaphanized, and included in a paraffin block. Three-millimeter thick slices were made using a microtome. Endogenous peroxidase activity was blocked and then incubated with the primary antibodies for 24 hours. In sequence, the sections were washed in PBS-T (phosphate buffered saline buffer with 0.02% Triton X-100) and then, incubated with the secondary antibody at room temperature for 60 minutes. Reactions were labeled with a diaminobenzidine (DAB, Sigma) solution at 60 mg% and counterstained with Harris hematoxylin (Merck) [21].

2.8 Statistical analysis

The results were expressed as mean ± standard error of the mean (Mean ± SEM) for each group. Two-way ANOVA test was used for multiple comparison analysis. To analyze the significance between the groups studied, the Tukey test was used as a post-test. The software Statistical Package for Social Science (SPSS) version 17.0 was used. The level of significance was 5% ($p < 0.05$).

3. Results

The characterization of the obesity status of the animals under study was performed by monitoring the evolution of the body weight of each animal and by calculating the Lee index. This test is very similar to the BMI applied in humans, and the averages of the classification are shown in (Imge 1).

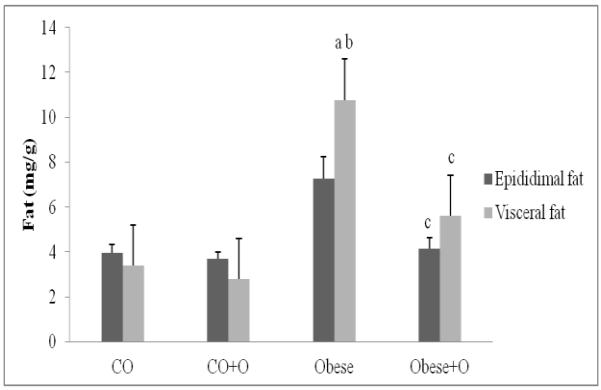


The results are expressed as mean ± SEM ($p < 0.05$)
[a] Significant difference of the control group
[b]Significant difference of control group + omega-3
[c]Significant difference of obese group

Image 1: Lee Index Index Chart

At the end of the experiment, the animals that consumed the high-fat diet had higher body weight and weight gain than those who consumed a standard diet. The Lee Index score shows that there was an increase in the obese group score when compared to the control group. There was a significant reduction in the Lee index score in the obese plus omega-3 group when compared to the obese group.

There was a significant increase of epididymis and visceral fat in the obese group when compared to the other groups. In the obese plus omega-3 group there was a significant reduction in relation to the obese group (Image 2).



The results are expressed as mean ± SEM ($p < 0.05$)
[a] Significant difference of the control group
[b]Significant difference of control group + omega-3
[c]Significant difference of obese group

Image 2: Fat and Epididymal visceral fat

There was no significant difference between groups in the glycemia assessment. In plasma cholesterol levels, there was a significant reduction in the obese group when compared to the control group and obese plus omega-3, and in triglycerides there was a significant reduction in the obese group plus omega-3 compared to the obese group. (Table 1)

Table 1: Plasma levels of glucose, cholesterol and triglycerides

Parameters	Groups			
	Control	CO+O	Obese	Obese+O
Glycemia (mg/dL)	129,37±4,18	135,97±7,18	151,08±5,89	133,32±6,06
Cholesterol (mg/dL)	61,95±4,18	51,06 ±2,74	67,01±5,56[b]	46,22±3,78[c]
Triglycerideos (mg/dL)	70,34±2,33	82,45±5,04	99,74±8,84[a]	70,86±4,26[c]

Results are expressed as mean ± SEM ($p < 0.05$)
[a] Significant difference of the control group
[b] Significant difference of control group + omega-3
[c] Significant difference of obese group

3.1 Determinations of lipid peroxidation (TBARS) and the activity of SOD, CAT and GPx enzymes in hepatic tissue

There was an increase in lipid peroxidation measured by TBARS but not significant among the groups (Table 2). SOD activity presented no significant difference between the groups; the activity of the GPx was significantly increased in the obese animals when compared to the CO+O group, and reduced in the (Obese +O) group. In the enzymatic activity of CAT, there was no significant difference between groups (Table 2)

Table 2: Measurement of lipoperoxidation (TBARS) and activity of enzymes, SOD, CAT and GPx in hepatic tissue

Parametrs	Groups			
	Control	CO+O	Obese	Obese+O
TBARS (nmol/mg)	0,99±0,959	0,88±0,112	1,26±0,218	1,08±0,156
SOD(U/mg)	21,10±3,269	19,02±1,735	17,35±1,872	15,68±1,833
GPx(U/mg)	12,77±2,265	11,90±2,254	47,27±5,457 [a,b]	40,11±6,747 [a, b]

CAT(pmol/mg)	28,81±4,500	21,31±7,937	17,32±3,495	23,46±4,139

Results are expressed as Mean ± SEM ($p < 0.05$)
[a] Significant difference of the control group
[b] Significant difference of control group + omega-3
[c] Significant difference of obese group

3.2 Immunohistochemical labeling of COX-2, IL-1β and TNF-α.

Figure 1 and Image 3 show the labeling intensity of the COX-2 enzyme in the liver; this labeling is identified by brown staining around the hepatocytes. An increase in COX-2 labeling in the liver of obese animals was observed when compared to control animals; in obese animals treated with omega-3, a significant reduction in enzyme labeling was observed when compared to obese animals and control group plus omega-3.

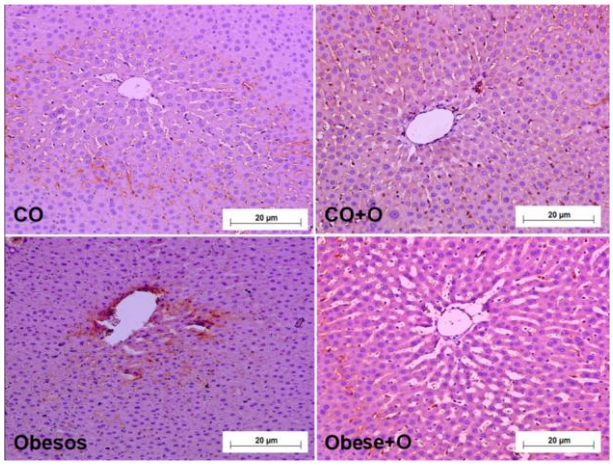
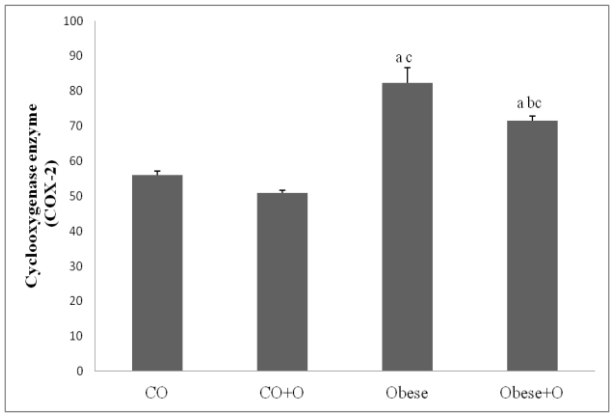


Fig 1: Photomicrography of the hepatic tissue of obese animals with the immunohistochemical labeling of the enzyme (COX-2) increased 200X.



The results are expressed as mean ± SEM ($p < 0.05$)
[a] Significant difference of the control group
[b] Significant difference of control group + omega-3
[c] Significant difference of obese group

Image 3: Quantification of the immunohistochemical labeling of the enzyme cyclooxygenase (COX-2)

Figure 5 and Fig 6 show the labeling intensity of IL-1β Interleukin in the liver; this labeling is identified by brown staining around hepatocytes. A significant increase in IL-1β labeling in the liver of the obese group is observed when compared to the control group; in animals of the obese group plus omega-3 there was a reduction in the labeling of this cytokine when compared to the obese group.

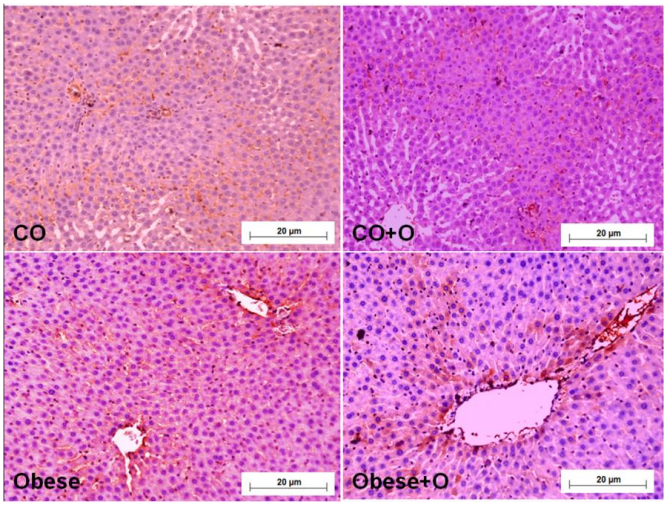
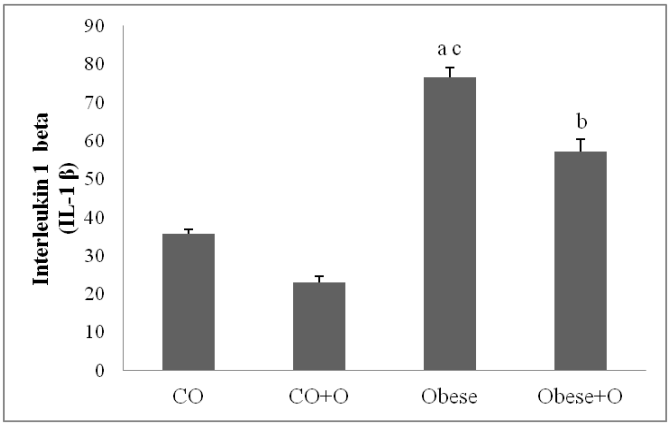


Fig 2: Photomicrography of hepatic tissue of animals with the immunohistochemistry staining of the cytosine IL-1β, increase of 200X.



The results expressed with mean ± SEM ($p < 0.05$)
[a] Significant difference of the control group
[b] Significant difference of control group + omega-3
[c] Significant difference of obese group
[c] Difference in the obese group

Image 5: Quantification of the immunohistochemical labeling of cytokine interleukin 1 beta (1ILβ)

Figure 3 and Image 6 show the labeling intensity of tumor necrosis factor (TNF-α) in the liver; this labeling is identified by brown staining around the hepatocytes. An increase in TNF-α labeling in the liver of obese animals was observed when compared to the control group; in obese animals treated with omega-3 there was a reduction in the labeling of this cytokine when compared to the obese group.

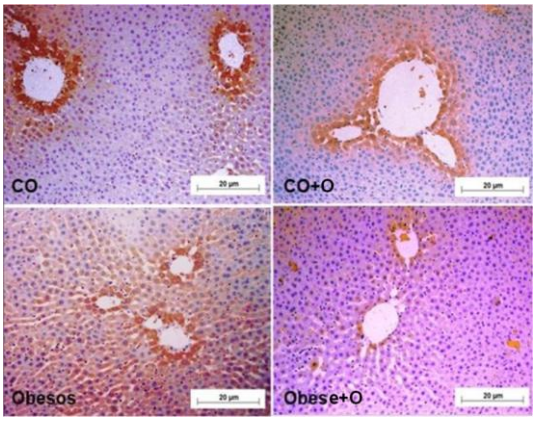
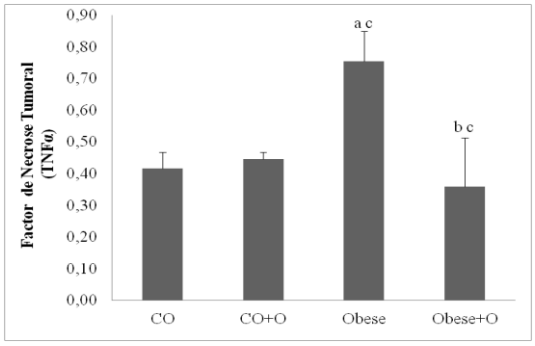


Fig 3: Photomicrography of the hepatic tissue of animals with the immunohistochemical staining of the cytokine TNF-α, increase of 200X.



The results are expressed as (mean ± SEM), ($p < 0.05$)
[a] Significant difference of the control group
[b]Significant difference of control group + omega-3
[c]Significant difference of obese group

Image 6: Quantification of immunohistochemical marking of tumor necrosis factor (TNFα), increase of 200X.

4. Discussion

Omega-3 improves the metabolic profile of obese individuals through action on different mechanisms, that include alterations in the adipose tissue gene expression; changes in adipokine release; reduced appetite; changes in carbohydrate metabolism; increased fat oxidation; increased energy expenditure (possibly through thermogenesis); increased muscle anabolism; and finally, influence on epigenetics [22, 23].

In the present study, obese animals treated with omega-3 lost weight significantly, as evidenced by the reduction in the Lee index and the reduction in the amount of epididymal and visceral fat. The Lee index can be used as an accurate and rapid way to determine obesity in rats submitted to a weight-gain method. Bernardis and Patterson described that determination of obesity in rats proposed by Lee [15] correlates with fat mass [16].

The animals submitted to the hyperlipid diet presented a 50% increase in the epididimal and visceral fat, and the treatment with omega-3 for 4 weeks after the induction of obesity promoted a significant reduction of 50% in the amount of body fat. A 1993 study reported the reduction of body fat deposition in rats receiving omega-3 when compared to animals receiving a high-fat diet, but unlike our study, these animals were not previously obese [24].

Plasma levels of cholesterol and triglycerides were also significantly reduced in obese animals treated with omega-3, and we did not observe changes in glycemia in the different groups. These results demonstrate that omega-3 was able to decrease body fat deposition, and improved the lipid profile of obese animals. Other studies have also demonstrated the beneficial effects of omega-3 on improved lipid profile [25], decreased triglycerides [26], and increased HDL-c in obese rats. [27, 28] Studies using 3T3-L1 adipocyte cell line showed that treatment with EPA markedly reduced the size of lipid droplets and the total lipid accumulation in the cells, probably by suppressing the genes involved in lipid accumulation without altering lipolytic genes [29].

It has also been shown that treatment with EPA induces the expression of genes involved in mitochondrial biogenesis and oxidative metabolism, increasing lipid catabolism [30, 31].

And with this, they decrease the synthesis of the lipid droplets and, consequently, the total accumulation of lipids. These combined effects may be responsible for the reduction of fat mass observed in obese animals treated with omega-3. This effect is relevant because increased visceral fat leads to a number of metabolic changes, such as adipokine production and/or decreased cortisol metabolism, which may result in an increase in blood pressure and peripheral resistance to insulin action [32, 33]. When the large amount of free fatty acids from this fat reaches the liver, it leads to an increase in gluconeogenesis, a decrease in muscle glucose uptake, and a decrease in the hepatic metabolism of insulin [33].

Adipocyte size is an important determinant of the secreted adipokine profile, with large adipocytes predominantly releasing pro-inflammatory factors such as MCP-1 and IL-6, and reducing anti-inflammatory adipokines including leptin and adiponectin [34].

There is evidence that omega-3 improves the insulin signal transduction in adipocytes, which in turn affects insulin-stimulated glucose uptake by regulating GLUT4 expression [35]. In vitro studies on cultured adipocytes demonstrated that treatment with EPA (200 M, 96 h) increased glucose uptake in rat adipocytes. In addition, adipocyte culture from rats supplemented with fish oil for one week showed increased levels of GLUT4 and GLUT1 with consequent insulin-stimulated glucose uptake [36]. According to a study by Rossmeisl and colleagues, the reduction of adipose tissue and the improvement of the inflammatory profile associated with omega-3 can improve insulin sensitivity in obese animals and thus contribute to the maintenance of glycemia. In the present study, this improvement in the glycemic profile was not observed [37].

In the present study, we observed that in the lipid peroxidation measured by TBARS, there was no significant difference between the studied groups. The SOD activity showed no significant difference between the groups. The activity of the enzyme glutathione peroxidase was significantly reduced in obese animals treated with omega-3. Regarding the activity of the CAT enzyme, there was no significant difference between the groups. According to the results, it seems that when there is an increase in lipid peroxidation, a protective effect of the tissues occurs, that is, the bioactive compound omega-3 had antioxidant action in the path of lipogenesis. [38] In the present study we observed a significant increase of the COX-2, IL-1β and TNFα cytokines in the hepatic tissue of the obese animals

and a significant reduction in the obese animals treated with omega-3, indicating an anti-inflammatory action of the omega-3 in this model. In addition to membrane maintenance, polyunsaturated fatty acids (PUFAs) present in phospholipids are also precursors of eicosanoid synthesis. Besides modifying the profile of eicosanoids involved in inflammatory processes, omega-3 PUFA affects the production of many inflammatory proteins including cytokines and adhesion molecules. [39]

It has been accepted for many years that the final step in the synthesis of EPA and DHA fatty acids occurs in the endoplasmic reticulum; predominantly in liver cells Polyunsaturated fatty acids EPA and DHA are precursors of lipid mediators called resolvins and protectins. The route of production of these mediators also involves the cyclooxygenase and lipoxygenase enzymes, which have anti-inflammatory and immunomodulatory characteristics [40].

Resolvins and protectins have been shown to be anti-inflammatory and immunomodulatory. Resolvins E1 and D1, and protectin D1 may inhibit transendothelial migration of neutrophils, preventing the infiltration of these immune cells at the site of inflammation [41, 42].

Resolvin E1 may further inhibit the production of IL-1, and protectin E1 inhibits IL-1 and TNF-α production. Thus, these lipid mediators may help in the resolution phase of inflammation and in limiting tissue damage [40]. According to the results of this model of experimental obesity, omega-3 promotes improvement in lipid profile and acts as an antioxidant action, reducing inflammatory processes. However, it is necessary to carry out more studies to prove the efficiency of this bioactive compound in these metabolic routes.

5. Conclusion

The study concludes that omega-3 supplementation reduces weight gain to adiposity, improves lipid profile and reduces inflammatory markers in the liver of rats with obesity induced by a hyperlipid diet, but in this study there was no beneficial effect on the glycemic profile. However, more studies are needed to understand the mechanism of action of omega-3 in the model of obesity.

5.1 Declaration of conflicts and interests

We forwarded the manuscript titled "The long-chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs) inhibit the proinflammatory cytokines 1IL-β, COX-2 and TNFα in an experimental model of obesity in wistar rats" for possible publication of The International Journal of Food Science and Nutrition the liability statement becomes public liability for its content, that links were omitted or agreements between authors and companies that may be interested in the publication of this article.

We declare and affirm that we have no conflicts of interest with the topic addressed in the article, nor with the products / items quoted. We declare that the article quoted above is original and that the paper was not submitted to another scientific journal and will not be, as long it is publication is being reviewed by The International Journal of Food Science and Nutrition electronic format.

6. Acknowledgement

Ph.D. Wannmacher C.M.D. Department of Biological Sciences: Biochemistry - Laboratory: Inborn Errors of Metabolism. Federal University of Rio Grande do Sul - UFRGS - Rio Grande do Sul, Brazil.

PhD. Fernandes M. da Cruz Department of Postgraduate in Pathology: Pathology Research Laboratory: Federal University of Health Sciences of Porto Alegre - UFCSPA - Rio Grande do Sul, Brazil,

Financial Support: Post-Graduate Support Program - PROAP-Federal University of Health Sciences of Porto Alegre-UFCSPA

7. References

1. Organization WH. WHO. Obesity and overweight. Fact sheet 2015; [cited 2017 26 Agosto]. no. 311.
2. Organization WH. Obesity and overweight 2013 [cited 2017 23 Dezembro]. Available from: http://www.who.int/mediacentre/factsheets/fs311/en/#
3. ABESO. Associação Brasileira para Estudo da Obesidade [cited 2018 Janeiro]. Available from: www.abeso.org.br/noticia/dia-nacional-de-prevencao-da-obesidade.
4. Clinical guidelines on the identification, evaluation,and treatment of overweight and obesity in adults. Bethesda, MD: Department of Health and Human Services, National Institutes of Health, National Heart, Lung and Blood Institute [Internet]. [cited 2017].
5. ORGANIZATION WH. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. Geneva: WHO 1995 [cited 2017 24 julho].
6. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, andinsulinresistanceGastroenterology.2007;132:2169-80.
7. Lockette WE, Webb RC, Culp BR, B. P. Vascular reactivity and high dietary eicosapentaenoic acid. Prostaglandins. 1982; 24:631-9.
8. Scott H, Goodnight Jr, Harris WS, William E, Connor, Longworth DR. Decrease of platelet activity after intake of small amounts of eicosapentaenoic acid in diabetics. Thromb Haemost. 1982; 3:344.
9. Lorente-Cebrián S, Costa AGV, Navas-Carretero S, Zabala M, Martínez JA , Moreno-Aliaga M J. Role of omega-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: a review of the evidence. J Biol Chem. 2013; 3:633-51.
10. Borgesa M, Santosa F, Telles R, Correiad M, Lannae C. Ácidos graxos poli-insaturados ômega-3 e lúpus eritematoso sistêmico: o que sabemos?. Revista Brasileira de Reumatologia. 2014; 6:459-66.
11. Goldin PR, McRae K, Ramel W, Gross JJ. The neural bases of emotion regulation: reappraisal and suppression of negative emotion. Biological psychiatry. 2008; 5:77-86.
12. Hugues DA, AC. P. n-3 polynsaturated fatty acids inhibitthe antigen-presenting function of human monocytes. Am J Clin Nutr. 2000; 71:357-60.
13. Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F, *et al*. Relationship of plasma polyunsaturated fatty acids tocirculating inflammatory markers. JCEM. 2006; 91:439-46.
14. Calder PC. Polyunsaturated fatty acids and inflammatory processes: New twists in an old tale. Biochimie. 2009; 79:1-5.

15. Bernardis LL. Prediction of carcass fat, water and lean body mass from Lee's "nutritive ratio" in rats with hypothalamic obesity. Experientia. 1970; 7:89-90.
16. Bernardis LL, Patterson BD. Correlation between 'Lee index' and carcass fat content in weanling and adult female rats with hypothalamic lesions. The Journal of Endocrinology. 1968; 52:7-8.
17. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods in enzymology. 1978; 30:2-10.
18. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. The Journal of biological chemistry. 1972; 317:0-5.
19. Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. The Biochemical journal. 1973; 7:07-16.
20. Wendel A. Glutathione peroxidase. Methods in enzymology. 1981; 32:5-33.
21. Riera J, Astengo-Osuna C, Carlos M, Longmate JN, Jeffrey A, Battifora H. The immunohistochemical diagnostic panel for epithelial mesothelioma: a reevaluation after heat-induced epitope retrieval. The American journal of surgical pathology. 1997; 14:09-19.
22. Dea V. Ácidos graxos das séries ômega-3 e ômega-6 e sua utilização no tratamento de doenças cardiovasculares. RSC Online [Internet]. 2016; 5:65-83.
23. Albracht-Schulte K, Kembra AS, Kalupahana NS, RamalingamLS, Shaikh Rahman M, Comb J R, Moustaid-Moussa N. Omega-3 fatty acids in obesity and metabolic syndrome: a mechanistic update. Journal of Nutritional Biochemistry. 2018; 58:1-16.
24. Rustan AC, Hustvedt BE, Drevon CA. RAHBD. Dietary supplementation of very long-chain n-3 fatty acids decreases whole body lipid utilization in the rat. Journal of lipid research. 1993; 34:99-309.
25. Molena-Fernandes CA, Schimidt G, Neto-oliveira ER, Bersani-amado CA, Cuman rkn. Avaliação dos efeitos da suplementação com farinha de linhaça (Linum usitatissimum L.) marrom e dourada sobre o perfil lipídico e a evolução ponderal em ratos Wistar. Brasileira Plantas Médica. 2010 Apl/Jun.
26. Batetta B, Griinari M CG, Murru E, Ligresti A, Cordeddu L . Endocannabinoids may mediate the ability of (n-3) fatty acids to reduce ectopic fat and inflammatory mediators in obese Zucker Rats. Journal Nutrition. 2009; 139:1495-1501.
27. Cicero AFG , Derosa G, Gregori VD, Bove M, Gaddi VA, Borghi C. Lipid Research Omega 3 polyunsaturated fatty acids supplementation and blood pressure levels in hypertriglyceridemic patients with untreated normal-high blood pressure and with or without metabolic syndrome: a retrospective study. Clinical Experimental Hypertension. 2010; 32:137-144.
28. Ebrahimi M, Ghayour-Mobarhan M, Rezaiean S, Hoseini M, Parizade SM, Farhoudi F, *et al*. Omega-3 fatty acid supplements improve the cardiovascular risk profile of subjects with metabolic syndrome, including markers of inflammation and auto-immunity. Acta cardiologica. 2009; 64:1-7.
29. Manickam E, Sinclair AJ, Cameron-Smith D. Suppressive actions of eicosapentaenoic acid on lipid droplet formation in 3T3-L1 adipocytes. Lipids in health and disease. 2010; 9:50-57.
30. Flachs P, Horakova O, Brauner P, Rossmeisl M, Pecina P, Franssen-van Hal N, Ruzickova J. *et al*. Polyunsaturated fatty acids of marine origin upregulate mitochondrial biogenesis and induce beta-oxidation in white fat. Diabetologia. 2005; 23:65-75.
31. Flachs P, Rossmeisl, M Bryhn, M Kopecky, J. Cellular and molecular effects of n-3 polyunsaturated fatty acids on adipose tissue biology and metabolism. Clin Sci. 2009; 116:1-16.
32. Leite LD RE, Brandão Neto J. Obesidade: uma doença inflamatória. Revista Ciência & Saúde. 2009; 2:85-95.
33. Lima LM, Carvalho MG , Vale AAL, Fonseca Neto CP, Garcia JCF, Saad JA, Souza MO. Proteína C-reativa ultra-sensível em pacientes com diagnóstico de doença arterial coronariana estabelecido por angiografia. J Bras Patol Med Lab. 2007; 43:83-6.
34. T Skurk, C Alberti-Huber, C Herder. Relationship between Adipocyte Size and Adipokine Expression and Secretion. J Clin Endocrinol Metab. 2007; 92:23-33.
35. Gonzalez-Periz A. Horrillo R. Ferre N. KGronert B. Dong E. Moran-Salvador and *et al*. Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: a role for resolvins and protectins. FASEB J Off Publ Fed Am Soc Exp Biol. 2009; 23:46-57.
36. Rossmeisl M, Medrikova D, van Schothorst EM, Pavlisova J, Kuda O, Hensler M, *et al*. Omega-3 phospholipids from fish suppress hepatic steatosis by integrated inhibition of biosynthetic pathways indietary obese mice. Biochim Biophys Acta. 2014; 2:267-78.
37. Martínez-Fernández L, Laiglesia LM, Huertaab AE, Martíne JA, Moreno-Aliaga MJ. Omega-3 fatty acids and adipose tissue function in obesity and metabolic syndrome. Prostaglandins & Other Lipid Mediators. 2015; 121:24-41.
38. Rodrigues GN, F.C; Silveira, K.C.S; Porawski, M.G; Marroni, N.P; Nunes, M.L. Radicais Livres no processo da saúde-doença: da Bancada á Clínica. CRV, editor. Curitiba, 2012.
39. Calder, PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochimica et biophysica acta. 2015; 1851:69-84.
40. Calder, PC. Omega-3 fatty acids and inflammatory processes. Nutrients. 2010; 2:355-374.
41. Serhan CN, Chiang N, Dyke TEV. Resolving inflammation:dual anti-inflammatory and pro-resolution lipid mediators. Nature Rev Immunol. 2008; 8:349-361.
42. Serhan CN, Hong S GK, Colgan SP, Devchand PR, Mirick GMR. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirintreatment that counter proinflammatory signals. J Exp Med. 2002; 196:1021-25.
43. Hong SG, Devchand K, Moussignac P, Serhan RL. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood and glialcells: autocoids in anti-inflammation. J Biol Chem. 2003; 278:7-8.