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Anti-inflammatory and antioxidant effect of long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFAs) in diabetic rats

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Abstract

Introduction: Diabetes Mellitus is a common endocrine-metabolic disease in the world and its incidence has increased over the years, mainly due to poor diet and obesity affecting more than 246 million people worldwide.

Objective: The objective of this study was to evaluate the effect of omega-3 on oxidative stress and inflammation in animals with chronic diabetes.

Methods: A total of 44 male Wistar rats were used. Induction of diabetes was performed by applying a single dose of streptozotocin (i.p) 70 mg / kg. The animals were divided in six control groups (CO), control treated with omega-3 for 15 days (CO + Ô15D), control treated with omega-3 for 30 days (CO + Ô30D), diabetics (DM) omega-3 for 15 days (DM + Ô15D), diabetics treated with omega-3 for 30 days (DM + Ô30D). Animals received omega-3 at the dose of 4 g / kg body weight or 0.9% NaCl solution per gavage. Treatment started on day 7 after induction of diabetes and animals were euthanized after 15 or 30 days of initiation of omega-3 treatment. Blood and liver were removed and serum glucose, triglycerides, cholesterol, and blood tests were performed. In the liver, the activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), thiobarbituric acid (TBARS) and cytokines IL (interleukin) -1β, IL- 6, IL-10 and TNF-α (tumor necrosis factor) and the immunohistochemical labeling for IL-1β, IL-6 and COX2 (cyclooxygenase-2).

Results: Treatment with omega-3 was able to reduce plasma triglycerides and the activity of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase. There was also a significant reduction in the immunohistochemical labeling of IL-1β, IL-6 and COX2, as well as a reduction of TNFα levels without significant alteration in IL-10 in the liver of diabetic animals treated with omega 3.

Conclusion: Treatment with ômegsa-3 in diabetic animals reduces triglyceride levels, decreases the activity of antioxidant enzymes and decreases levels of inflammatory cytokines in the liver. These results seem to indicate that omega-3 may be useful as an adjuvant in the treatment of diabetes.

Keywords: liver, inflammation, antioxidant and omega-3

1. Introduction

Diabetes Mellitus (DM) is a multifaceted chronic disorder that affects different people of all ages, race and sex. In the world, 422 million people suffer from this disease, around 8.5% of adults with diabetes in 2014 [1] In 2015, an estimated 1.6 million deaths were directly caused by diabetes and the World Health Organization projects that diabetes will be the seventh leading cause of death in 2030 [1, 2]

There were 11.9 million people with diabetes in Brazil in 2013, accounting for 9% of the population, and it is expected to be 19 million in 2035, equivalent to 11% of the population [3]. The main characteristic of this illness is elevated blood glucose concentrations due to a loss of insulin producing in the pancreatic b-cells (type 1 diabetes), or loss of insulin responsiveness in its target tissues, including adipose and muscle (type 2 diabetes). There are several acute and chronic complications related to this disease [4-6] such as metabolic disorders, vascular disorders [7], hepatopathy [8] and cardiovascular problems [9, 10].

Diabetic complications are associated with oxidative and nitrosative stress, and decreased levels of antioxidants [11, 12, 13] Oxidative stress is induced by elevations in glucose and free fatty acid (FFA) levels, and has a key role in the pathogenesis of both types of diabetes mellitus. Disruption of antioxidant defense in diabetic subjects (types 1 and 2), as well as increased formation of free radicals reported in many studies, lead to oxidative damage of cell components in several tissues, including the kidney, eyes, and nervous system [14]

The hyperglycemia enhances the levels of non-enzymatic glycation of intra and extracellular proteins and lipids. Excessive glycation leads to the quick formation of advanced glycation end products (AGEs), what activates a series of cellular signaling pathways, such as the nuclear factor-κB (NFκB)-regulated inflammatory pathway, with increased

production of reactive oxygen species (ROS) [15, 16, 11]. NFkB activation, in response to hyperglycemia and increased oxidative stress, leads to the activation of pro-inflammatory genes contributing to the onset and maintenance of the disease complications, such as hyperlipidemia and higher cardiovascular risk [17] It has been suggested that a diet rich in antioxidants, such as vitamins C and E, carotenoids and polyphenols, can improve endogenous antioxidant defenses and lower oxidative stress [18, 19, 20].

Antioxidant defense includes enzymatic and nonenzymatic pathways. There are quite a number of non-enzymatic antioxidants, namely vitamins (A), ascorbic acid, tocopherol, enzyme cofactors (Q10), nitrogen compounds (uric acid), and peptides (glutathione) [21]. Common enzymatic strategies are superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase. Antioxidant enzymes activity is altered in diabetes, which could be a target for therapy [22, 18, 17, 23].

Omega-3 polyunsaturated fatty acids (LC n-3 PUFA), especially fish omega-3 PUFA (eicosapentaenoic acid - EPA, and docosahexaenoic acid - DHA), may have antioxidant properties, as they reduce cardiovascular disease incidence by lowering cardiometabolic risk factors, such as systemic inflammation. [21, 24, 25, 26]

EPA and DHA show triglyceride-lowering properties [27] and have been linked to promising results in a variety of inflammatory human diseases, including diabetes, atherosclerosis, asthma, and arthritis [28, 29].

Mechanisms underlying the anti-inflammatory actions of LC n-3 PUFAs include altered cell membrane phospholipid composition, disruption of lipid rafts, and the inhibition of the pro-inflammatory transcription factor NFkB, thus reducing the expression of inflammatory genes [30]

As the inflammatory route regulated by NFkB is activated in diabetes and omega-3 can inhibit this activation, the aim of this study was to evaluate the effect of the treatment with omega-3 on oxidative stress and inflammation in animals with chronic diabetes.

2. Methods

2.1 Animals and Experimental Protocol

All procedures were performed in conformity with the Guide for Care and Use of Experimental Animals, published by the National Institutes of Health (NIH publication n. 85–23, revised in 1996) and Arouca Law, 2008 [31]; and the study was approved by the Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) Ethics and Research Committee (protocol 140/12). Male Wistar rats, from the Animal Breeding Unit of UFCSPA, were used. The mean weight of the animals was 200 grams in the beginning of the study. Three rats were housed per cage, receiving food and water *ad libitum* in a room under a 12:12h light–dark cycle, at 22 °C. Diabetes Mellitus (DM) was induced by a single injection of streptozotocin i.p. (STZ, Sigma *Chemical Company*, *St. Louis*, MO, EUA) at a dose of 70mg/Kg of bodyweight [32] (Like & Rossini, 1976. STZ was dissolved in a sodium citrate buffer (0.1 M, pH 4.5) and administered in the left abdominal region of the animal about 10 minutes after dissolution in the buffer solution. The animals in the control group received only NaCl 0.9% i.p. at the same volume of the buffer used to dissolve STZ. The omega-3 was administered in 4g/Kg, once a day, during 15 or 30 days. The administration route was gastric gavage with a final solution of 1mL and treatment was initiated from the 7th day of diabetes induction. The animals were randomized in the different groups: control (CO), control with omega-3 (CO+O15D and CO+O30D), diabetic (DM), and diabetic treated with omega-3 during 15 or 30 days (DM+O15D and DM+O30D). After the 30 days of trial, the animals were euthanized by exsanguination, being anesthetized with xylazine and ketamine. Blood from the retro-orbital plexus was sampled and the liver was collected for histological and biochemical analyses.

2.2 Serum Analyses

The blood samples were placed into a testing tube with heparin (Liquemine). Plasma was used to determine glucose, cholesterol and triglycerides (TG) levels with colorimetric enzymatic test (Kit Labtest, Biodiagnostica) and the absorbance was measured in a spectrophotometer (CARY 3E-UV-*Visible Spectrophotometer Varian*). Animals with a glucose concentration above 250 mg/dL were considered as diabetic [33].

2.3 Biochemical Analyses of Oxidative Stress and Antioxidant Assay

The liver samples were homogenized with 9mL of phosphate buffer (KCL 140 mm, phosphate 20 mm, pH7.4) per gram of tissue. Protein concentration in the liver homogenates was determined using a standard solution of bovine albumin, according to Lowry *et al*., 1951 [34]. Lipid Peroxidation was determined by the method of thiobarbituric acid reactive substances (TBA-RS) [35] Superoxide dismutase (SOD) activity in the liver was determined using a technique based on the inhibition of adrenochrome formation in the epinephrine autoxidation [36]. Catalase (CAT) activity was determined as described elsewhere [37] and the determination of selenium dependent glutathione peroxidase was obtained with a technique consisting of the measure of NADPH oxidation by glutathione reductase [38].

2.4 Histological Study

For the histological analysis the samples were embedded twice in paraffin. Using a microtome, the paraffin blocks were cut into 3-μm seriate sections. In the staining phase, the slides were immersed in hematoxylin-eosin. During dehydration phase, the structures went through three containers with absolute alcohol and two containers with xylol. Reading was performed with light microscopy (*Nikon Labophot*) at 100×. The analysis was performed by 2 pathologists, who did not know the study details.

2.5 Immunohistochemical Detection of IL-1, IL-6 and Cox2

Immunohistochemical reactions were performed through the technique of streptavidin-biotin peroxidase complex (Strept ABC, DAKO). The slides were previously coated by a silane solution (APTS, Sigma) diluted in 4% acetone. 3-μm thick sections were obtained using a mechanical microtome. The sections were then deparaffinized and successively immersed in xylol and ethanol, and then submitted to antigenic recovery by irradiation heat in (Eterna, Nigro) using citrate buffer

(10mM, pH 6.0) for 15 minutes. Peroxidase blocking was performed using a hydrogen peroxide solution at 3%, followed by incubation with primary antibody against IL-1, IL-6, and Cox2 (Santa Cruz). The reactions were marked with diaminobenzidine (DAB, Sigma) solution at 60mg% and counterstained with Harris's hematoxylin (Merck). For each reaction a positive control was tested, using a tissue that was known to be positive for the antibody. Two negative controls were also used, the first one by absence of the primary antibody and the second by removing the secondary antibody during the reaction steps.

2.6 Liver sample preparation and determination of tissue TNF-α, IL-1, IL-6 and IL-10 protein levels

For cytokine assays, liver samples were homogenized in phosphate-buffered saline (PBS, ph7.4), containing 0.4 mol/L NaCl, 0.05% Tween-20, 0.5% bovine serum albumin, 10 mmol/L EDTA and 20 Kl/mL aprotinin. The homogenates were centrifuged at 12,000×g for 60 min at 4 °C. The supernatant was removed and TNF-α, IL-1, IL-6 and IL-10 levels were determined by multiplex bead array using Milliplex™ MAP rat cytokine kits (RCYTO-80 K) (Millipore, Billerica, MA, USA). Milliplex™ MAP is based on Luminex® xMAP™ technology. Frozen samples were thawed immediately prior to analysis, and then maintained on ice throughout the assay setup. Briefly, according to procedures recommended by the manufacturers and previously described [38] all tissue samples were diluted 1:5 in sample diluent and then incubated in duplicate overnight with capture beads specific for TNF-α, IL-1, IL-6 and IL-10. The beads were subsequently washed and incubated for 2h with a biotin-conjugated detection antibody, and then for 30 min with streptavidin–phycoerythrin. Bead fluorescence was analysed on a Luminex 100 IS Multiplex Bio-Assay Analyser. The concentrations of the cytokines were determined from standard curves of recombinant rat cytokines in which four-parameter logistic curve fitting analysis was used. All cytokines are reported in picograms per milliliter.

2.7 Statistical Analysis

The data are presented as mean ± standard error (SE) and were analyzed through statistical software SPSS 15.0. The variables were tested for normality through the Kolmogorov-Smirnov test. One way analysis of variance (ANOVA) was used for intergroup differences. Tukey post hoc test was used for parametric variables. The level of significance used was $P < 0.05$.

3 Results

3.1 Blood glucose, TG and cholesterol

Glucose concentration in the plasma of streptozotocin-treated rats was significantly higher ($p<0.01$) than in the normal control group, and was not affected by omega-3 treatment (Table 1). There were no significant differences in plasma cholesterol in the different groups, but triglyceride was significantly increased in the diabetic animals, when compared to control, and the treatment of diabetic animals with omega-3 for 30 days reduced plasma triglycerides ($p<0.02$).

Table 1: Effect of streptozotocin-induced diabetes and omega-3 on plasma glucose, triglycerides and cholesterol concentration in rats.

Parameters	Groups						
	CO	CO+Ô15D	CO+Ô30D	DM	DM+Ô15D	DM+Ô30D	p
Glucose (mg/dL)	107,06±12,13	63,97±9,22	102,39±12,84	458,28±78,66[a]	475,44±37,45[a]	430,92±272,84[a]	0,01
Cholesterol (mg/dL)	68,12±3,32	55,10±7,48	51,17±4,44	65,52±11,73	65,52±7,42	72,97±4,56	0,28
Triglycerides (mg/dL)	59,02±7,82	57,94±10,35	58,73±10,10	148,54±64,30[a]	210,11±23,07[a]	72,97±58,42[b]	0,02

[a] differ statistically from control, $p< 0,05$

[b] differ statistically from DM+Ô30D group, $p< 0,05$

Results are expressed as mean ± standard error (EP).

3.2 Hepatic markers of oxidative stress

The cytosolic concentration of TBARS was greater in diabetic rats than in controls, whereas concentrations in the diabetic rats that received omega-3 during 15 days were reduced (Table 2). In contrast, control animals that received omega-3 also showed an increase in cytosolic concentration of TBARS (Table 2). SOD activity was higher in diabetic than in control animals, and was not reduced in diabetic rats treated with omega-3. In the other hand, the control group that received omega-3 during 30 days showed increased SOD activity. Catalase activity was significantly enhanced by diabetes and this effect was prevented by omega-3 treatment. Glutathione peroxidase activity was reduced in diabetic rats and omega-3 treatment have further reduced this enzyme activity.

Table 2: Effect of streptozotocin-induced diabetes and omega-3 of lipid peroxidation (TBARS- thiobarbituric acid reactive substances) and hepatic antioxidant enzyme activities in rat liver.

Parameters	Groups					
	CO	CO+Ô15D	CO+Ô30D	DM	DM+Ô15D	DM+Ô30D
TBARS (nmoL/mg protein)	0,20±0,03	0,39±0,05[a]	0,39±0,02[a]	0,41±0,03[a]	0,29±0,02[b]	0,33±0,02
SOD (SOD U/mg protein)	14,29±0,73	16,07±1,38	19,82±1,02[a]	19,94±0,55[a]	19,13±0,82[a]	16,49±0,87
GPx (nmoL/mg protein)	372,58±8,27	267,99±8,68[a]	267,13±6,32[a]	280,45±10,50[a]	271,68±9,04[a]	182,92±33,29[a,b]
CAT (pmoL/mg protein)	0,41±0,07	-	0,73±0,25	4,87±0,50[a]	-	1,79±0,60[b]

Results are expressed as mean ± standard error (EP).

[a] differ statistically from control, $p< 0,05$

[b] differ statistically from DM group, $p< 0,05$

3.3 Immunohistochemical Analysis of IL-1, IL-6 and Cox2

In figure 1 there is the presence of IL-1 identified by brown staining on the liver parenchyma of diabetic animals. In control animals (Figure 1) was not identified by the presence of IL-1 in diabetic animals and treated with omega-3 for 15 and 30 days (in image 1 - D) is observed a reduction in intensity of immunohistochemical staining for IL -1. The figure 1 and figure 2 shows the results of the measurement of intensity of immunohistochemical staining of IL-1, indicating a significant increase of IL-1 in diabetic animals compared to control and significant reduction in diabetic animals treated with omega- 3 for 15 and 30 days compared to diabetic animals.

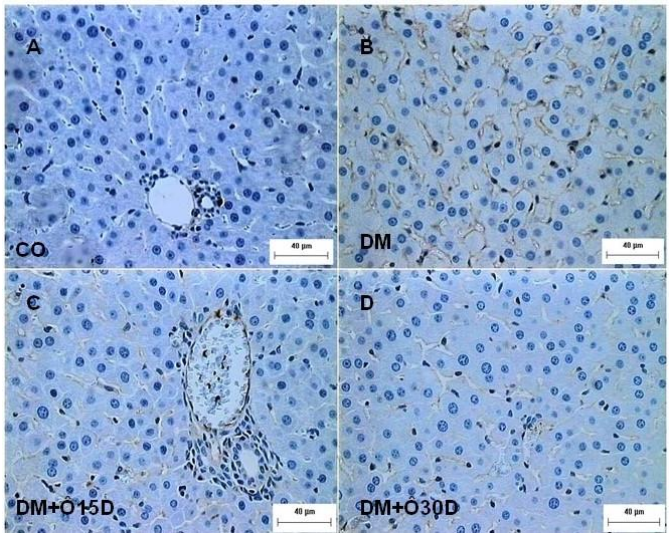
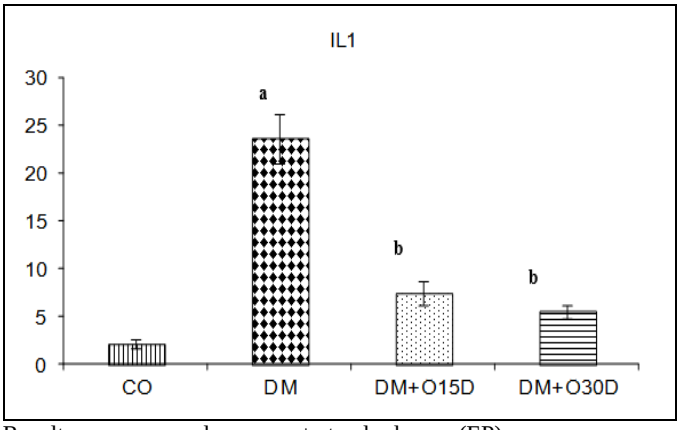


Fig 1: Photomicrograph of liver tissue of animals, immunohistochemical marking of the cytokine IL-1 in hepatocytes seen around the cell cytoplasm (Staining: hematoxylin-eosin, original magnification (400X).



Results are expressed as mean ± standard error (EP).
[a] differ statistically from control, $p < 0,05$
[b] differ statistically from DM group, $p < 0,001$

Fig 2: IL-1

In figure 3 a observed the presence of IL-6 in liver tissue of diabetic animals. In control animals and diabetic patients treated with omega-3 for 15 and 30 days there was a reduction in the markup of immunohistochemistry for IL-6. Figure 3 and figure 4 show the results of the measurement of intensity of immunohistochemical staining of IL-6, indicating a significant increase of IL-6 in the diabetic animals compared to control and significant reduction in diabetic animals treated with omega-3 15 and 30 days.

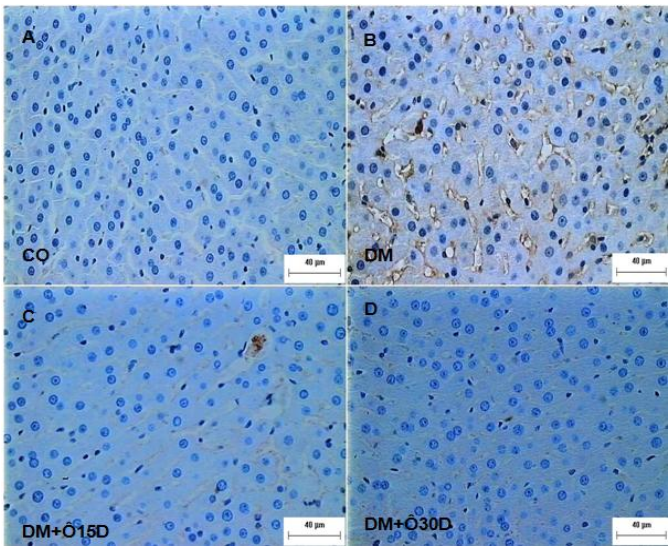
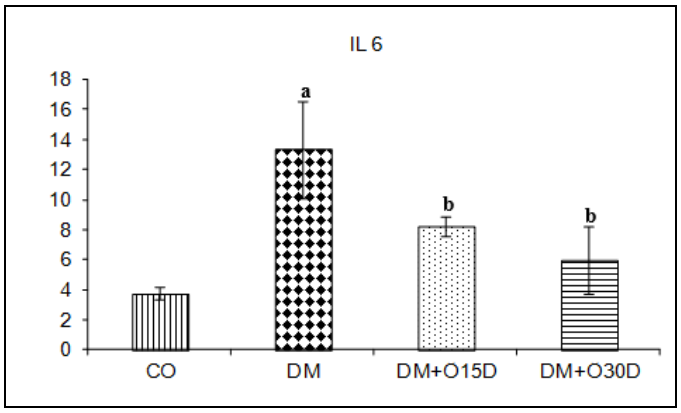


Fig 3: Photomicrograph of liver tissue of animals, immunohistochemical marking of the cytokine IL-6 seen in hepatocytes surrounding the cytoplasm of cells (Staining: hematoxylin-eosin, original magnification (400X).



Results are expressed as mean ± standard error (EP).
[a] differ statistically from control, $p < 0,05$
[b] differ statistically from DM group, $p < 0,05$

Fig 4: IL-6

In figure 5 there is an intense immunohistochemical staining for COX-2 in hepatic tissue of diabetic animals. The figure 5 and figure 6. Control animals also showed positive staining for COX-2 and the diabetic animals treated with omega-3 for 15 and 30 days showed a reduction in the intensity of staining was compared to control and diabetic animals. In quantifying the intensity of staining, the diabetic animals showed a significant increase in COX-2 compared to control and

diabetic animals treated for 30 days significantly reduced compared to diabetic animals.

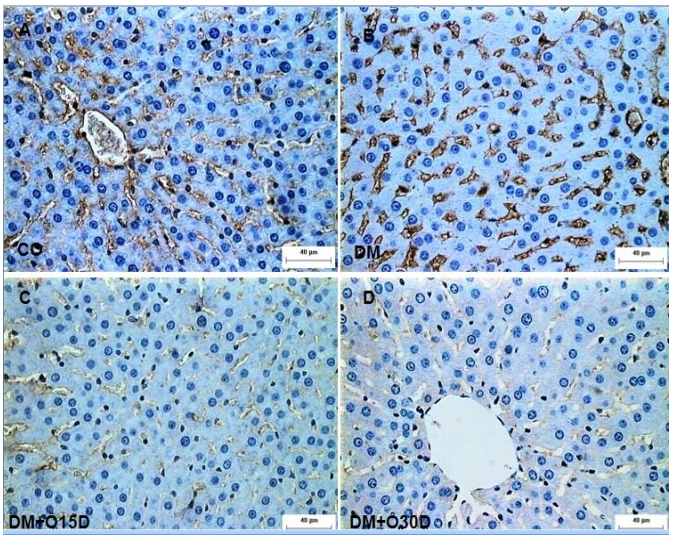
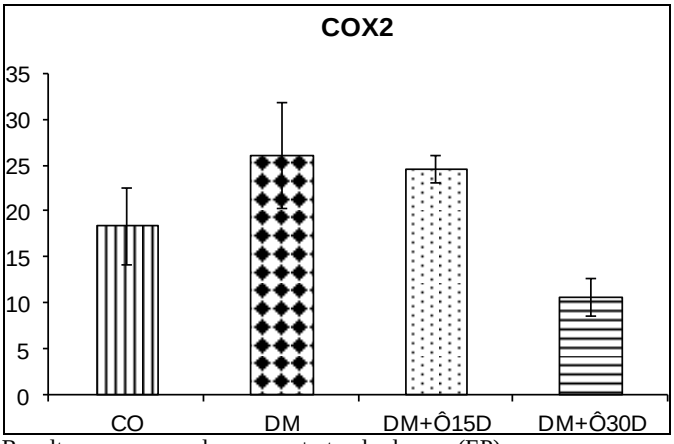


Fig 5: Photomicrograph of liver tissue of animals, immunohistochemical staining of COX-2 seen in hepatocytes surrounding the cytoplasm of cells magnification (400X).



Results are expressed as mean ± standard error (EP).
[a] differ statistically from control, $p < 0,05$

Fig 6

3.4 Liver levels of TNF-α, IL-1, IL-6 and IL-10

The levels of IL-1, IL-6, IL-10 and TNF-α in the liver were higher in diabetic rats than in the control group. Treatment with omega-3 during 30 days reduced significantly TNFα levels (Table 3)

Table 3: Effect of streptozotocin-induced diabetes and omega-3 of inflammatory cytocines in rat liver.

Parameters	Groups				
	CO	CO+O30D	DM	DM+Ô30D	p
IL-1	455,69±46,7	455,18±42,82	766,55±66,86[a,c]	528,74±93,85	0,011
IL-6	949,81±73,43	1152,32±60,17	1985,10±332,86[a,c]	1327,87±155,63	0,008
IL-10	48,28±2,50	54,50±3,10	63,08±1,29[a,]	52,52±3,19	0,013
TNFα	0,464±0,24	0,507±0,76	0,780±0,29[a,c]	0,434±0,84[b]	0,013

Results are expressed as mean ± standard error (EP).
[a] differ statistically from control, $p < 0,05$
[b] differ statistically from DM group, $p < 0,05$
[c] differ statistically from CO+Ô30D group, $p < 0,05$

4. Discussion

This study used an experimental model of diabetes induced by STZ. Glycemia was measured 72 hours after the injection of STZ and during the euthanasia (after 30 days). The diabetic animals had the blood glucose significantly higher, compared to the control group during the experiment, and the daily treatment with omega-3 was not able to reduce blood glucose levels. Other studies using substances with antioxidant potential, such as quercetin [18], aminoguanidine [39] sacaca [40], and resveratrol [41], also did not show a reduction in plasma glucose when the treatment is initiated after the onset of the illness. However, some authors observed a reduction in the blood glucose when the treatment started before the induction of diabetes [42, 12].

Patients with type 2 diabetes using omega-3 supplementation also did not show any change in glycemic control or changes in insulin sensitivity [43, 44]. Studies using omega-3 supplementation reported a significant reduction in plasma triglycerides [45], and this effect was also observed in our study. Elevation in the levels of plasma triglycerides is one of the characteristics of obesity and metabolic syndrome. Sustained high levels of plasma triglycerides are considered a risk factor for diabetes and cardiovascular disease. In type 2 DM, elevated levels of triglycerides are involved in the development of insulin resistance and increased production of reactive oxygen species, what perpetuates and exacerbates the disease.

LC n-3 PUFA promotes hypotriglyceridemic effects by coordinately suppressing hepatic lipogenesis through reducing levels of SREBP-1c, upregulating fatty oxidation in the liver and skeletal muscle through PPAR activation, and enhancing flux of glucose to glycogen through downregulation of HNF-4α [46].

Thus, although treatments with omega-3 do not promote a reduction in glycemia, there may be an improvement in insulin resistance, resulting from the reduction of plasma triglyceride levels.

Increased levels of TBARS were found previously in the liver of STZ induced diabetic rats [47, 48, 18].

Results from the present study also suggest an improvement of oxidative stress by the treatment with omega-3 during 15 days. Prolonged treatment for 30 days does not have the same effect.

Similar results were observed using a treatment with sacaca, a plant with antioxidant properties, for 5 to 20 days in diabetic rats. The short-term treatment has better effects in reducing

oxidative damage than prolonged treatment, probably due to a pro-oxidant effect of long-term administration [40].

In our study, superoxide dismutase (SOD) and catalase (CAT) activities are increased in diabetic animals, as well as the malondialdehyde levels. It is known that hyperglycemia increases the production of superoxide anion radical (O_2^-), and SOD is a major enzyme for its neutralization. Although the activity of SOD is increased, it is not enough to prevent oxidative damage, as measured by TBARS. The increase in CAT activity can also be linked to the neutralization process of ROS, since the hydrogen peroxide formed by the dismutation of superoxide is a substrate for CAT activity.

On the other hand, glutathione peroxidase (GPx) activity is decreased in diabetic animals what may mean that the redox balance between reduced glutathione (GSH) and oxidized glutathione (GSSH) is not able to respond to the increase in the ROS formed during the illness.

Similar results were found by who used the same model of study, with the administration of treatment after the onset of chronic diabetes. The SOD activity was increased in DM, CAT has not changed and GPx was decreased. This probably reflects an adaptation to chronic illness [15].

When the inflammatory characteristics of the disease are evaluated we observed an increase in IL-1β, IL-6, TNF-α and COX2, and the treatment with omega-3 was able to reduce these inflammatory markers in diabetic animals.

Several studies using omega-3 supplements to healthy human volunteers have reported decreased production of TNF-α, IL-1β and IL-6 by endotoxin stimulated monocytes or mononuclear cells. Some of the researches that failed to show an effect of omega-3 on cytokine production have provided less than 2g of EPA+DHA per day, what may be an insufficient dose. In patients with rheumatoid arthritis, fish oil supplements resulted in decreased IL-1 production by monocytes, decreased plasma IL-1β concentrations, and decreased TNF-α serum concentrations [49, 30].

In our study, the daily administration of 4g/Kg for LC n-3 PUFA for 30 days was able to reduce IL-1β, IL-6 and COX2, as well as the protein expression of TNF-α in the liver of diabetic rats. Furthermore, the protein expression of the anti-inflammatory cytokine IL-10 was not affected by the treatment with omega-3, although some animal studies also report that LC n-3 PUFA increase the concentration of IL-10 [50]. The anti-inflammatory action of omega-3 seems to be related to a lower formation of arachidonic acid metabolites. In general, the structural difference between arachidonic acid (ARA) and EPA-derived eicosanoids, renders the latter less biologically potential. One reason for this reduced biological potency is that eicosanoid receptors typically have a lower affinity for the EPA-derived mediator, than for the ARA-derived one [51]

In addition, the metabolism of EPA and DHA form a group of molecules able to stimulate the resolution of chronic inflammation, such as resolvins and protectins [52]

In the blood of humans consuming increased amounts of LC n-3 PUFA there were increased levels of resolvins [53]. Resolvin D1 (RvD1) inhibited IL-1β production, and protectin D1 (PD1) inhibited TNF-α and IL-1β production [54, 55]. Both EPA and DHA were able to prevent lauric acid-induced activation of NFκB and COX-2 expression in macrophages [30]. It has been demonstrated that the production of specialized pro-resolving mediators (i.e., RvD1 and PD1) is deficient in inflamed obese adipose tissue, and it can be the consequence of the structural deficiency in the tissue content of LC n-3 PUFA (DHA and EPA), as substrates for specialized pro-resolving mediators biosynthesis [56].

Thus, treatment with omega-3 may be beneficial for patients with type 2 diabetes because it reduces plasma triglycerides, decrease proinflammatory cytokines leading to the reduction of the NFkB activation pathway, in addition to increasing resolvins and protectins content. The main issue regarding the use of omega-3 in patients refers to the definition of an effective daily dose, since the studies with diabetic patients have large differences in the treatment amount.

5. Conclusion

In summary, in diabetic rats, daily administration of 4g/Kg of omega-3 for 30 days was able to reduce plasma triglycerides and the activity of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase. There was also a significant reduction in the expression of IL-1β, IL-6, TNF-α and COX2, without significant alteration in IL-10. These results seem to indicate that the treatment with omega-3, in diabetic animals, improves the characteristic inflammatory profile of diabetes. Although it does not promote a reduction in blood glucose, the omega-3 administration may be useful in treating diabetes.

Authors' contribution

Michely Nunes: responsible for the elaboration and integrity of the work as a whole. From the preparation of the research project, work development and article writing. In which, the master Marilene Porawski, was guiding and collaborating with the research. The Elizangela Schemitt collaborated with the help in the processing of the samples in the laboratory. The authors declare that they participated in the conception, analysis of results and contributed effectively to the accomplishment of the article.

Declaration of conflicts and interests

We declare and affirm that we have no conflicts of interest with the topic addressed in the article, nor with the products / items quoted.

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