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Omega-3 Fatty Acids Attenuate Brain Alterations in High-Fat Diet-Induced Obesity Model

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Abstract

This study evaluated the effects of omega-3 on inflammation, oxidative stress, and energy metabolism parameters in the brain of mice subjected to high-fat diet-induced obesity model. Body weight and visceral fat weight were evaluated as well. Male Swiss mice were divided into control (purified low-fat diet) and obese (purified high-fat diet). After 6 weeks, the groups were divided into control + saline, control + omega-3, obese + saline, and obese + OMEGA-3. Fish oil (400 mg/kg/day) or saline solution was administrated orally, during 4 weeks. When the experiment completed 10 weeks, the animals were euthanized and the brain and visceral fat were removed. The brain structures (hypothalamus, hippocampus, prefrontal cortex, and striatum) were isolated. Treatment with omega-3 had no effect on body weight, but reduced the visceral fat. Obese animals showed increased inflammation, increased oxidative damage, decreased antioxidant enzymes activity and levels, changes in the Krebs cycle enzyme activities, and inhibition of mitochondrial respiratory chain complexes in the brain structures. Omega-3 treatment partially reversed the changes in the inflammatory and in the oxidative damage parameters and attenuated the alterations in the antioxidant defense and in the energy metabolism (Krebs cycle and mitochondrial respiratory chain). Omega-3 had a beneficial effect on the brain of obese animals, as it partially reversed the changes caused by the consumption of a high-fat diet and consequent obesity. Our results support studies that indicate omega-3 may contribute to obesity treatment.

Keywords Obesity · Brain · Omega-3 · Inflammation · Oxidative stress · Energy metabolism

Introduction

The World Health Organization (WHO) defines obesity as an accumulation of abnormal or excessive fat that can be detrimental to health [1]. Obesity is both a disease and a major risk factor for several other diseases, such as cardiovascular disease, diabetes, respiratory diseases, musculoskeletal disorders, and some cancers [1–3]. In addition, obesity is considered one of the major public health problems worldwide [4].

Obesity causes several body alterations, and among them, there are the presence of inflammation [5, 6], oxidative stress [7, 8], and mitochondrial dysfunction [9, 10]. Most studies have focused on alterations in the peripheral systems and organs. However, some studies have revealed that obesity can also cause abnormalities in the brain [11–13], especially in the cerebral structures hypothalamus [14, 15], hippocampus [16, 17], prefrontal cortex, and striatum [20]. The hypothalamus is the regulating center for energy metabolism [15, 21]; the hippocampus is involved with cognition, memory, learning, and emotions [12, 16]; and the prefrontal cortex and striatum are involved with the reward system [18, 19]. Therefore, alterations in these brain structures can cause several damages to the individual.

Due to the established health risks and the substantial increase in prevalence, obesity has become a major challenge

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for global health [22]. Viewing obesity as an inflammatory disease has aroused interest for therapeutic intervention with focus on inflammation [5]. In this context, certain substances may play an important role in the mediation of inflammation and related changes [23]. Among them, the use of omega-3 polyunsaturated fatty acids may be a strategy, since this substance has known anti-inflammatory effects and may help treat obesity and associated metabolic disorders [24–27].

Omega-3 fatty acids comprise a family of polyunsaturated fatty acids, called essential fatty acids, which are very important for the body [28]. With regard to anti-inflammatory properties, omega-3 in the form of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has received higher prominence [29–31]. DHA, in particular, is one of the most abundant components in the brain's structural lipids [32] and a key component of neuronal membranes at signal transduction sites, suggesting that its action is vital for brain functioning [30, 33]. Incorporating EPA and DHA into the diet may, therefore, affect not only the composition and lipid structure of cell membranes, but also the physiological brain functions [34].

However, there are still many gaps in the effects and possible mechanisms by which omega-3 could help treat obesity, e.g., the brain has been poorly explored, since most studies have focused on the peripheral tissues. In order to study the brain, the use of animal models allows us to study the mechanisms in a way that would be impossible in research with humans [11]. In addition, animal models using high-fat diets allow us to evaluate the interventions to treat obesity in an experimental in vivo environment that is pathophysiologically very similar to the disease in humans [35].

Therefore, considering that further clarification of the role of omega-3 in obesity is still needed, the aim of this study was to assess the effects of omega-3 (fish oil, high in EPA and DHA) on inflammatory, oxidative stress, and energy metabolism parameters in the brain (hypothalamus, hippocampus, prefrontal cortex, and striatum) of mice subjected to high-fat diet-induced obesity model. The effects on body weight and visceral fat weight were evaluated as well.

Methods

Animals

One hundred sixty male Swiss mice (*Mus musculus*), 40 days old, weighing about 30–40 g, were obtained from the Federal University of Santa Catarina (UFSC), Florianópolis, Santa Catarina, Brazil. The mice had free access to food and water and were kept at 22 ± 2 °C under a 12-h light-dark cycle. Eight different animals from each of the experimental groups were used for each analysis group ($n = 8$ inflammatory parameters, $n = 8$ oxidative damage, $n = 8$ antioxidant defense, $n = 8$ Krebs cycle, and $n = 8$ mitochondrial respiratory chain).

Experimental Procedures

Obesity was induced in mice (high-fat diet for 10 weeks) and the effects of omega-3 administration for 4 weeks (starting from week 6) were evaluated. On the first day of the experiment, the animals were weighed and paired in two groups ($n = 80$): (1) control diet (control group) and (2) high-fat diet (obese group). The mice were maintained in this regimen for 6 weeks. At week 6, their body weight was measured, and after confirmed the statistically significant difference, the mice were paired again in 4 groups for treatment with omega-3 ($n = 40$): (1) control diet + saline (control + saline); (2) control diet + omega-3 (control + omega-3); (3) high-fat diet + saline (obese + saline); and (4) high-fat diet + omega-3 (obese + omega-3). Figure 1 shows a schematic representation of the experimental protocol.

Treatment with omega-3 (fish oil, containing 120 mg of DHA and 180 mg of EPA per gram) or with saline (isotonic solution of 0.9% sodium chloride) was administered daily, at a dose of 400 mg/kg (orally, with gavage needle for mice) for 4 weeks. The dose was based on a previous study conducted by Abdel-Maksoud and colleagues [36]. After 4 weeks of treatment, when the experiment completed 10 weeks, body weight was measured again, and the animals were killed by decapitation.

Diets

Mice diets (purified low-fat diet, used as control diet, and purified high-fat diet) were purchased from a company specialized in the production of standardized diets for animal experimentation (PragSoluções Biociências, Jaú, SP, Brazil). The diet composition was based on a previous study conducted by Cintra and colleagues [37]. The high-fat diet provided more calories and a higher percentage of saturated fat than the standard diet. Diet composition, energy content, and percentage of macronutrients are described in Table 1. The calculations were based on the information provided by the diet manufacturer.

Tissues Removal

After the mice were killed, the abdominal cavity was opened, and the adipose tissue from the mesenteric, epididymal, and retroperitoneal regions was removed. The brain of the animals was also removed, and subsequently, the hypothalamus, hippocampus, prefrontal cortex, and striatum were isolated.

Visceral Fat Weight

The adipose tissue of the mesenteric, epididymal, and retroperitoneal regions was weighed using a high-precision scale. The visceral fat was then measured by the sum of the fat

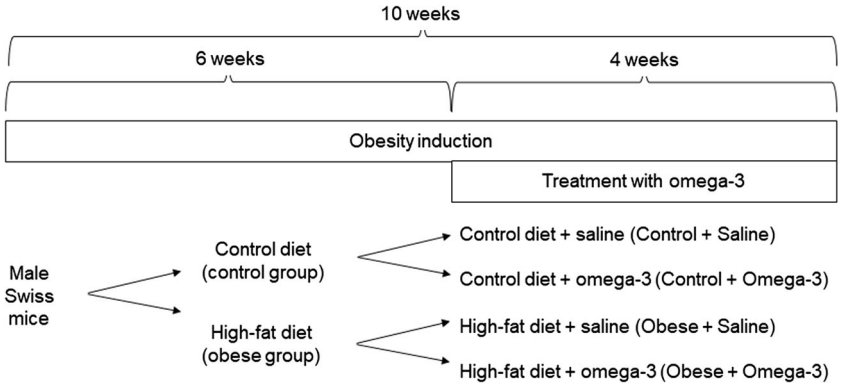

Fig. 1 Schematic representation of the experimental protocol

weight of the mesenteric, epididymal, and retroperitoneal regions, as previously described by Hansen and colleagues [38].

Sample Preparation

All brain structures were homogenized with specific buffer for each technique. Then, the protein content was determined by the method described by Lowry and colleagues [39]. The protein content was used for the normalization of each analysis.

Inflammatory Parameters

Tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-10 (IL-10) levels in the brain structures were determined by enzyme-linked immunosorbent assay (ELISA) on a microplate reader using commercial kits, following the manufacturer's recommendations.

Table 1 Diet composition, energy content, and percentage of macronutrients

Diet composition and energy content per 1000 g (1 kg)				
	Control diet		High-fat diet	
Ingredients	g/kg	kcal/kg	g/kg	kcal/kg
Corn starch	427.5	1710	115.5	462
Casein	200	800	200	800
Sucrose	132	528	132	528
Dextrinized starch	100	400	100	400
Soybean oil	40	360	40	360
Lard	–	–	312	2808
Cellulose	50	–	50	–
Mineral mix	35	–	35	–
Vitamin mix	10	–	10	–
L-cysteine	3	–	3	–
Choline bitartrate	2.5	–	2.5	–
Butyl hydroxytoluene (BHT)	0.028	–	0.028	–
Total	1000.028	3798	1000.028	5358
Macronutrient percentages (%Kcal)				
	Control diet		High-fat diet	
Carbohydrates	69%		26%	
Proteins	21%		15%	
Lipids	10%		59%	

Oxidative Stress Parameters

Oxidative Damage

The formation of malondialdehyde (a lipid peroxidation parameter) was determined by high-performance liquid chromatography, as described by Grotto and colleagues [40]. Oxidative damage to proteins was examined through the quantification of carbonylated proteins by means of the reaction of carbonyl groups in oxidized proteins, based on the reaction with dinitrophenylhydrazine, according to the method described by Levine and colleagues [41]. The absorbance was evaluated at 340 nm.

Antioxidant Defense

Superoxide dismutase (SOD) activity was determined by measuring the inhibition of adrenaline auto-oxidation, as described by Bannister and Calabrese [42]. Adrenaline oxidation leads to the formation of adrenochrome, and the SOD activity was determined by measuring the speed of adrenochrome formation, determined spectrophotometrically at 480 nm. Catalase (CAT) activity was determined by measuring the decay rate of hydrogen peroxide absorbance at 240 nm, according to described by Aebi [43]. Glutathione (GSH) levels were determined as described by Hissin and Hilf [44]. The technique is based on the color development resulting from the reaction between 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and thiols (absorbance was read at 412 nm).

Energy Metabolism Parameters

Krebs Cycle Enzyme Activity

The citrate synthase enzyme activity was evaluated following the method described by Faloone and Srere [45]. The reaction

was initiated by the addition of oxaloacetate and monitored in a spectrophotometer at 412 nm.

The isocitrate dehydrogenase enzyme activity was determined following the method described by Plaut [46]. The oxidized nicotinamide adenine dinucleotide (NAD^+) reduction to reduced nicotinamide adenine dinucleotide (NADH) was monitored in a spectrophotometer at 37 °C at 340 nm.

The α-ketoglutarate dehydrogenase enzyme activity was determined according to the technique described by Tretter and Adam-Vizi [47]. The NAD^+ reduction to NADH was monitored in a spectrophotometer at 37 °C at 340 nm.

The succinate dehydrogenase enzyme activity was determined according to the method described by Fischer and colleagues [48]. The reduction of 2.6-dichloroindophenol (2.6-DCIP) (after the addition of phenazine methosulfate) was determined by using a spectrophotometer at 600 nm.

The malate dehydrogenase enzyme activity was evaluated following the method described by Kitto [49]. The reaction was started by the addition of 0.30 mM oxaloacetate, and NADH consumption was monitored in a spectrophotometer at 37 °C at 340 nm.

Mitochondrial Respiratory Chain Complexes' Activity

Complex I activity was measured by the determination of the rate of NADH-dependent ferricyanide reduction, monitored in a spectrophotometer at 420 nm, following the method described by Cassina and Radi [50]. Complex II activity was measured by following the decrease in absorbance due to the reduction of 2.6-DCIP, monitored in a spectrophotometer at 600 nm, according to the method described by Fischer et al. [48]. Complex IV activity was determined by measuring the decrease in absorbance caused by the oxidation of reduced cytochrome c, measured spectrophotometrically at 550 nm, as described by Rustin and colleagues [51].

Statistical Analysis

Statistical analysis was performed using the GraphPad Prism software, version 6 (GraphPad, San Diego, CA, USA). Statistical analysis between two groups was made using Student's *t* test, and statistical differences between four experimental groups were determined by two-way analysis of variance (ANOVA) followed by Tukey's test. The results were presented as mean ± standard error of the mean (S.E.M.). $P < 0.05$ was considered statistically significant.

Results

At 6 weeks, it observed a significant difference in the body weight of the mice that received high-fat diet (obese group) when compared to control group ($p < 0.0001$). After 10 weeks, at the end of the experimental protocol, it also observed a significant effect of high-fat diet [obesity effect, F (1, 49) = 15, 34; $P = 0.0003$]. Treatment with omega-3 did not affect body weight. In addition to the body weight, the obesity led to increased visceral fat weight (the sum of the mesenteric, epididymal, and retroperitoneal regions) [F (1, 67) = 9, 125; $P = 0.003$]. Treatment with omega-3 reversed the increase in visceral fat weight of obese animals [interaction effect, F (1, 67) = 4, 801; $P = 0.03$] (Table 2).

With regard to the inflammatory parameters, the obese animals presented TNF-α increase in the hippocampus [F (1, 13) = 18, 78; $P = 0.0008$], prefrontal cortex [F (1, 12) = 55, 83; $P < 0.0001$], and striatum [F (1, 14) = 176, 8; $P < 0.0001$] (obesity effect). The treatment with omega-3 reversed the increase of TNF-α in the hippocampus [F (1, 13) = 21, 28; $P = 0.0005$] and in the striatum [F (1, 14) = 86, 85; $P < 0.0001$] of the obese animals (interaction effect). Obesity led to significant IL-1β increase in all brain structures analyzed (hypothalamus [F (1, 15) = 10, 52; $P = 0.005$], hippocampus [F (1, 16) = 43, 44; $P < 0.0001$], prefrontal cortex [F (1, 13) = 144, 3; $P < 0.0001$], and striatum [F (1, 14) = 14, 31; $P = 0.002$]). The treatment with omega-3 reversed the increase of IL-1β in the hippocampus [F (1, 16) = 19, 28; $P = 0.0005$] and in the striatum [F (1, 14) = 9, 559; $P = 0.008$], as well as reduced the IL-1β levels in the prefrontal cortex [F (1, 13) = 13, 23; $P = 0.003$] of the obese animals (interaction effect). IL-10 levels increased in the obese animals in the hippocampus [F (1, 13) = 5141; $P = 0$, 04] and in the striatum [F (1, 13) = 8561; $P = 0$, 01]. Relative to the IL-10 in the hypothalamus, it was observed that there is a different result, since there is reduction in the obese group [F (1, 15) = 24, 45; $P = 0.0002$]. Omega-3 treatment reversed the increase of the IL-10 in the hippocampus [F (1, 13) = 12, 75; $P = 0.003$] and in the striatum [F (1, 13) = 10, 68; $P = 0.006$] of obese animals (interaction effect) (Fig. 2).

Regarding the oxidative stress parameters, the obese animals presented increased oxidative damage to lipids (expressed by the augmented malondialdehyde levels) in the hypothalamus [F (1, 20) = 19, 68; $P = 0.0003$] and in the hippocampus [F (1, 18) = 5, 455; $P = 0.03$], as well as increased oxidative damage to proteins (expressed by the increase of protein carbonylation) in the hypothalamus [F (1, 20) = 8, 427; $P = 0.008$], hippocampus [F (1, 44) = 15, 32; $P = 0.0003$], and striatum [F (1, 51) = 46, 62; $P < 0.0001$] (obesity effect). The treatment with omega-3 reversed the increase of malondialdehyde levels in the hypothalamus [F (1, 20) = 8, 601; $P = 0.008$] and in the hippocampus [F (1, 18) = 5, 519; $P = 0.03$], as well as the increase of carbonylated proteins in the hypothalamus [F (1, 20) = 6, 815; $P = 0.01$] and hippocampus [F (1, 44) = 18, 07; $P = 0.0001$] (interaction effect) (Fig. 3). With respect to the antioxidant defense, obese animals had reduction of CAT activity in the hippocampus [F (1, 54) = 45, 83; $P < 0.0001$], as well as reduction of GSH levels

Table 2 Body weight and visceral fat weight

Weights (g)	Groups			
	Control		Obese	
Initial body weight	35.86 ± 0.4675		36.03 ± 0.5945	
Body weight at 6 weeks	53.10 ± 1.132		61.30 ± 1.267* ($p < 0.0001$)	
	Control + saline	Control + omega-3	Obese + saline	Obese + omega-3
Final body weight	55.38 ± 1.403	55.36 ± 1.612	61.36 ± 1.676** ($p = 0.03$)	61.50 ± 1.395** ($p = 0.04$)
Visceral fat	4.370 ± 0.21	4.257 ± 0.24	5.488 ± 0.19** ($p = 0.003$)	4.435 ± 0.20# ($p = 0.006$)

Values are expressed as mean ± SEM ($n = 20$); * vs. control, $p < 0.05$ (Student's t test); ** vs. control + saline; # vs. obese + saline (last day of experiment), $p < 0.05$ (two-way ANOVA followed by Tukey's test)

in the hypothalamus [F (1, 18) = 10, 34; $P = 0.004$], hippocampus [F (1, 54) = 41, 74; $P < 0.0001$], and striatum [F (1, 48) = 53, 64; $P < 0.0001$] (obesity effect). Omega-3 treatment did not reverse the alterations in the antioxidant defense. Although it was observed that there is a tendency of omega-3 treatment increase GSH levels in the hypothalamus and in the hippocampus, the results for interaction were not statistically significant. The activity of the SOD antioxidant enzyme was not altered in any of the groups (Fig. 4).

The activity of enzymes of the Krebs cycle and of the mitochondrial respiratory chain was evaluated as parameters of energy metabolism. With regard to Krebs cycle enzymes in brain structures, obesity led to inhibition of the citrate synthase activity in the hippocampus [F (1, 16) = 15, 57; $P = 0.001$], of the isocitrate dehydrogenase activity in the prefrontal cortex [F (1, 14) = 4, 898; $P = 0.04$], and of the α-ketoglutarate dehydrogenase activity in the prefrontal cortex [F (1, 20) = 155, 8; $P < 0.0001$] and striatum [F (1, 19) = 23, 85; $P = 0.0001$]. Furthermore, obesity led to increase of the α-ketoglutarate dehydrogenase activity in the hippocampus [F (1, 19) = 39, 05; $P < 0.0001$], of the succinate dehydrogenase in the prefrontal cortex [F (1, 14) = 7859; $P = 0.01$], and of the malate dehydrogenase activity in the hypothalamus [F (1, 16) = 203.0; $P < 0.0001$]. Omega-3 treatment reversed the inhibition of citrate synthase activity in the hippocampus [F (1, 16) = 10, 09; $P = 0.005$], as well as reversed the activation of succinate dehydrogenase in the prefrontal cortex [F (1, 14) = 13, 25; $P = 0.002$] and of malate dehydrogenase in the hypothalamus [F (1, 16) = 169, 1; $P < 0.0001$] (interaction effect) (Fig. 5). Regarding the mitochondrial respiratory chain, obesity led to

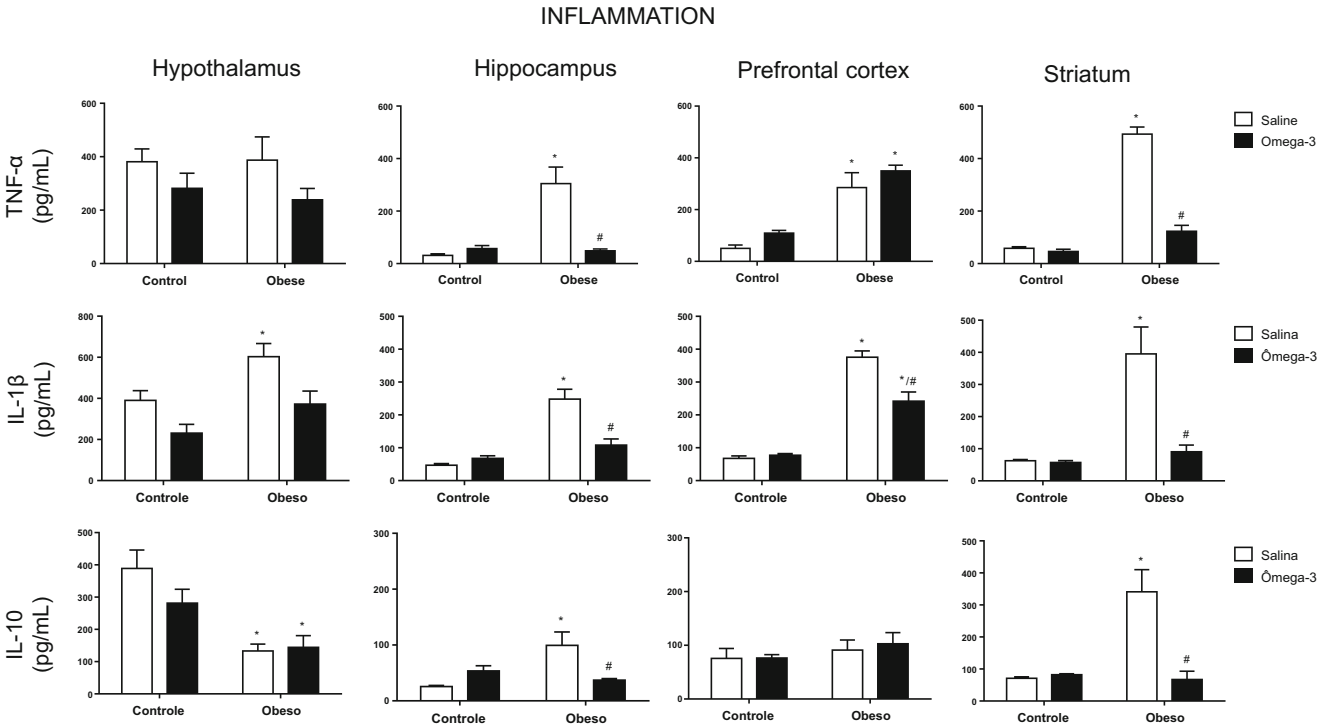

Fig. 2 Effects of omega-3 on inflammatory parameters in the brain of diet-induced obese mice. Values are expressed as mean ± SEM ($n = 8$); * vs. control + saline; # vs. obese + saline; $p < 0.05$ (two-way ANOVA followed by Tukey's test)

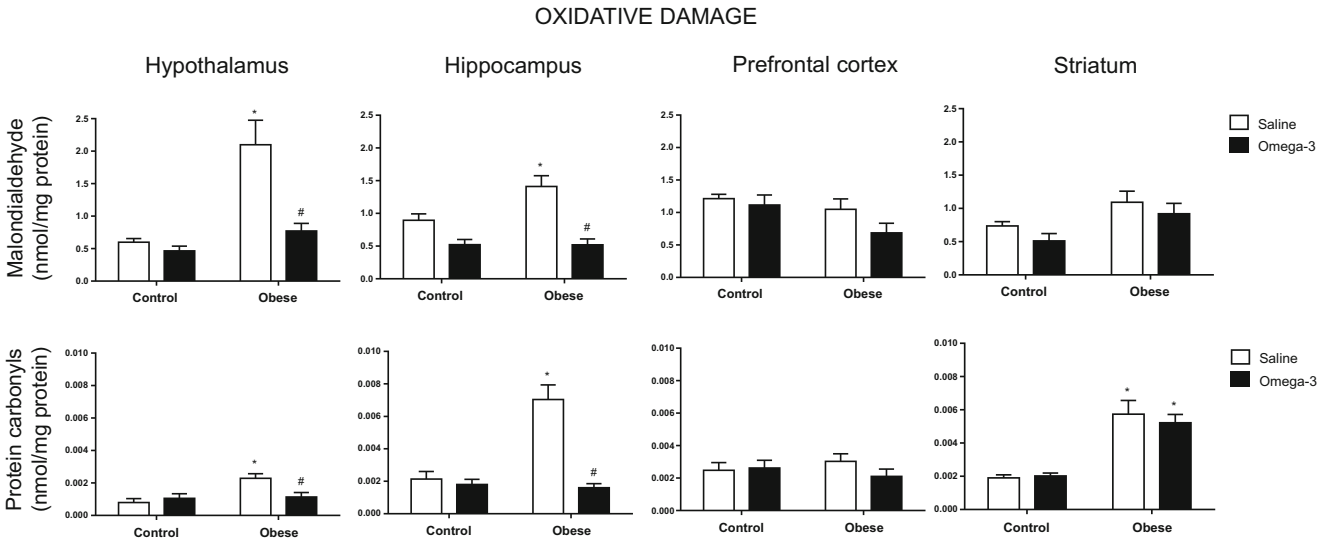

Fig. 3 Effects of omega-3 on parameters of oxidative damage in the brain of diet-induced obese mice. Values are expressed as mean ± SEM ($n = 8$); * vs. control + saline; # vs. obese + saline; $p < 0.05$ (two-way ANOVA followed by Tukey's test)

the inhibition of the complexes I, II, and IV in all brain structures analyzed (hypothalamus: complex I [F (1, 14) = 9346; $P = 0.008$], complex II [F (1, 25) = 4424; $P = 0.04$], and complex IV [F (1, 22) = 27, 89; $P < 0.0001$]; hippocampus: complex I [F (1, 15) = 51, 73; $P < 0.0001$], complex II [F (1, 16) = 8, 031; $P = 0.01$], and complex IV [F (1, 14) = 7, 488; $P = 0.01$]; prefrontal cortex: complex I [F (1, 13) = 11, 07; $P = 0.005$], complex II [F (1, 12) = 9, 364; $P = 0.009$], and complex IV [F (1, 24) = 25, 00; $P < 0.0001$]; striatum: complex I [F (1, 29) = 4816; $P = 0.03$], complex II [F (1, 27) = 4, 705; $P = 0.03$], and complex IV [F (1, 22) = 7, 846; $P = 0.01$]). The treatment with omega-3 reverted the inhibition of complexes I and II in the striatum [complex I: F (1, 29) = 4629; $P = 0.03$; complex II: F (1, 27) = 7348; $P = 0.01$] (interaction effect).

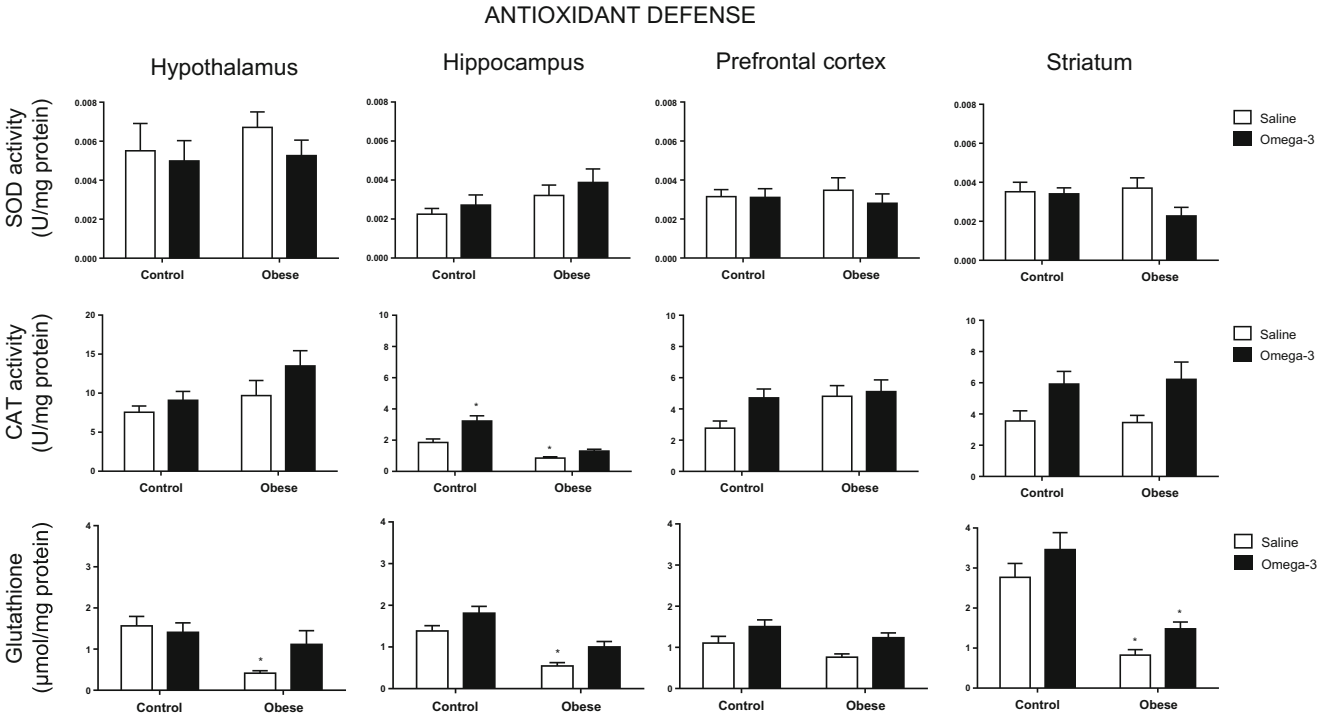

Fig. 4 Effects of omega-3 on antioxidant defense parameters in the brain of diet-induced obese mice. Values are expressed as mean ± SEM ($n = 8$); * vs. control + saline; $p < 0.05$ (two-way ANOVA followed by Tukey's test)

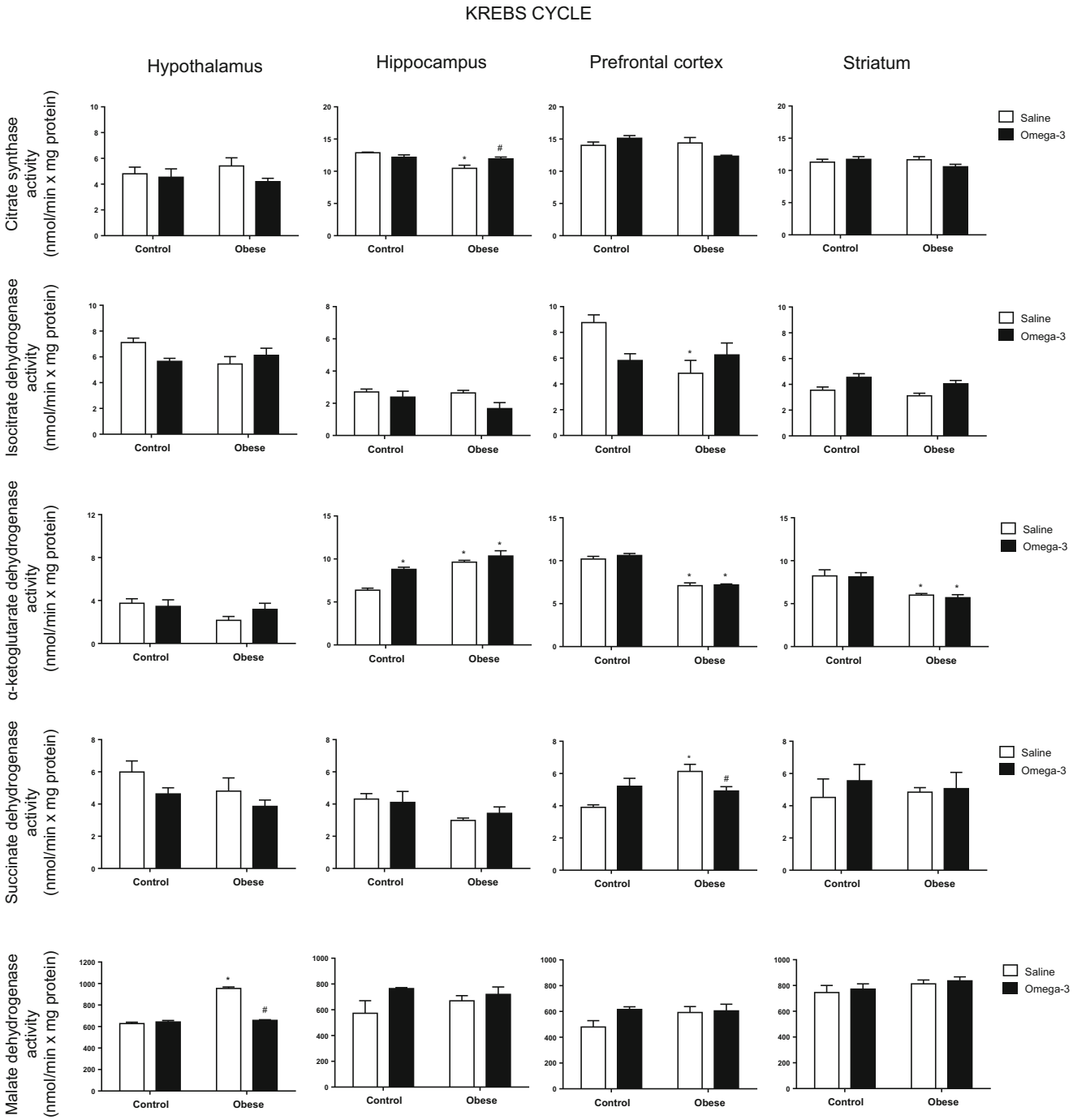

Fig. 5 Effects of omega-3 on the activity of Krebs cycle enzymes in the brain of diet-induced obese mice. Values are expressed as mean ± SEM ($n = 8$); * vs. control + saline; # vs. obese + saline; $p < 0.05$ (two-way ANOVA followed by Tukey's test)

Omega-3 treatment also alleviated complex II alterations in the hypothalamus and hippocampus, but the results for the interaction did not achieve statistical significance (Fig. 6).

Discussion

This study evaluated the effect of fish oil, rich in omega-3 polyunsaturated fatty acids, on parameters of inflammation, of oxidative stress, and of energy metabolism in the brain of high-fat diet-induced obesity mice. Body weight and visceral fat weight were also measured.

Animals fed a high-fat diet gained more body weight than those who received a control diet, which confirms the obesity induction. Treatment with omega-3 did not affect the animals' body weight. Yook and colleagues [52] have also shown that treatment with fish oil did not affect body weight of animals fed a high-fat diet. Cintra and colleagues [37] have shown that

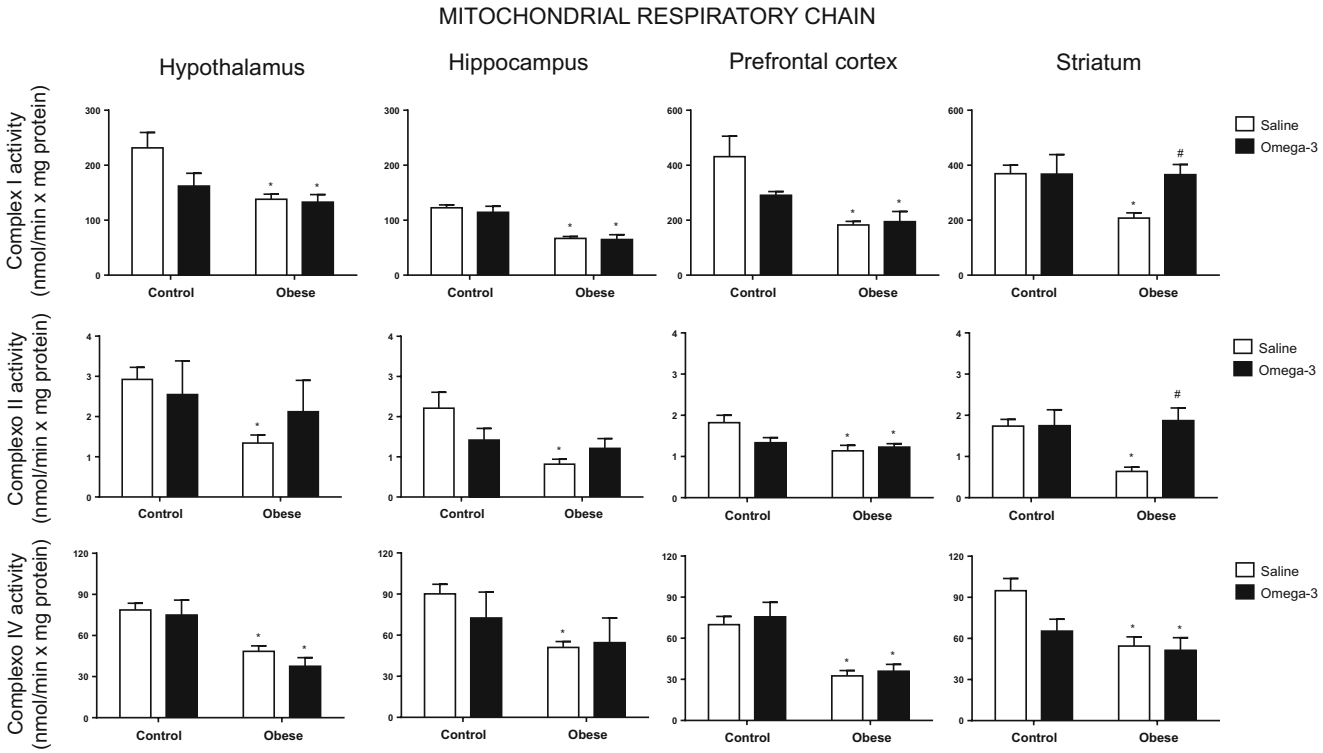

Fig. 6 Effects of omega-3 on the activity of mitochondrial respiratory chain complexes in the brain of diet-induced obese mice. Values are expressed as mean ± SEM ($n = 8$); * vs. control + saline; # vs. obese + saline; $p < 0.05$ (two-way ANOVA followed by Tukey's test)

replacing dietary saturated fatty acids with unsaturated fatty acids, as well as the intracerebroventricular administration of omega-3 and omega-9 led to weight loss in mice and rats. The discrepant results can be explained by methodological differences.

In addition to body weight, the increase in visceral fat can also be used to confirm obesity. Omega-3 treatment reversed the increased visceral fat in obese animals. Likewise, Pimentel and colleagues [53] have shown that rats fed a fish oil-rich diet had a lower accumulation in the mesenteric, epididymal, and retroperitoneal regions compared to those fed a soybean oil-rich diet. Liu and colleagues [54] have shown that dietary supplementation of EPA was effective in suppressing epididymal fat accumulation in mice with obesity induced by a diet high in saturated fat and fructose. Sá and colleagues [55] have also identified lower fat gain in the retroperitoneal region of mice fed a high-fat diet after supplementation with fish oil.

It is now known that alterations related to obesity are not restricted to the peripheral tissues. Peripheral cells of the immune system can be recruited to the central nervous system (CNS) [56] and provide an inflammatory brain response [57, 58]. Moreover, dietary components, such as saturated fatty acids, by itself may cause direct damage to the CNS. Saturated fatty acids can activate innate immune pathways through the binding and activation of toll-like receptors (TLR), stimulating the production of proinflammatory cytokines [57, 59].

In that regard, studies have shown that obesity and excessive consumption of saturated fats are involved with brain alterations [11–13], especially in the hypothalamus [14, 15, 21, 59], hippocampus [12, 16, 60], prefrontal cortex, and in the striatum [18, 19, 61]. Furthermore, it is known that the hypothalamus communicates with other brain areas, such as those that make up the memory and emotion (hippocampus) and reward system (prefrontal cortex and striatum), interacting with them to control the consumption and energy expenditure [20]. Therefore, this study examined the effect of omega-3 on parameters of inflammation, oxidative stress, and energy metabolism in the brain (hypothalamus, hippocampus, prefrontal cortex, and striatum) of mice with obesity induced by high-fat diet.

With regard to inflammatory parameters, the increase of proinflammatory cytokines (TNF-α and IL-1β), as well as alterations identified in anti-inflammatory cytokine (IL-10), indicated the presence of an inflammatory process in the brain structures of obese animals. Omega-3 treatment partially reversed the alterations in the inflammatory parameters in the brain structures. The effects of omega-3 on the CNS on animal models of obesity were poorly studied so far. Moreover, previous studies have only focused on the hypothalamus, probably because of its more direct relationship with food consumption and obesity. Cintra and colleagues [37] have shown that replacing dietary saturated fatty acids with unsaturated fatty acids, as well the intracerebroventricular administration of omega-3 and omega-9, was able to reduce the TNF-α and

interleukin-6 (IL-6) levels in the rodent hypothalamus. Pimentel and colleagues [62] have compared the intake of soybean oil- or fish oil-rich diets (without previous obesity induction) and showed that omega-3-rich diet was able to reduce the levels of inflammatory markers (TNF-α and IL-6) in the rat hypothalamus, which indicates a protection against obesity. Viggiano and collaborators [63] have also found beneficial effects of an omega-3-rich diet in the modulation of hypothalamic inflammation in rats (reduction of the TNF-α levels; without previous obesity induction).

The anti-inflammatory effect of omega-3 can be explained by its ability to interact with inflammatory signaling pathways, having a suppressive effect on the production of pro-inflammatory cytokines [64, 65]. Given the fact that obesity is an inflammatory disease, the anti-inflammatory activity of omega-3 may play an important role in obesity treatment. This effect, without any diet changes, as shown in this study, reinforces the beneficial effects of omega-3 even when eating too much saturated fat, which is quite common for obese patients. Moreover, to the best of our knowledge, this is the first study to extend the assessment to other brain structures beyond the hypothalamus.

The inflammatory process associated with obesity may increase the production of reactive oxygen species (ROS) and lead to oxidative stress [66]. In this study, the animals of the obese group showed increased oxidative damage to lipids and proteins, reduced activity of the CAT antioxidant enzyme, and reduced GSH levels in brain structures. Such changes indicate the presence of oxidative stress in the brain of obese mice, which supports the findings from previous studies [13, 67]. Omega-3 treatment almost completely reversed the oxidative damage in the brain structures but did not reverse the alterations related to the antioxidant defense. In the hypothalamus and in the hippocampus, it was observed a tendency of omega-3 treatment increase GSH levels in the obese animals, attenuating the reduction induced by high-fat diet. However, the results for interaction did not achieve statistical significance. No studies reporting the effect of omega-3 on brain oxidative stress after obesity induction were found. Viggiano and colleagues [63] showed that a diet rich in fish oil, unlike a diet rich in lard (comparison between diets, without previous obesity induction), did not alter malondialdehyde levels, GSH levels, nor the reduced glutathione/oxidized glutathione ratio (GSH/GSSG) in rat hypothalamus.

Oxidative stress may lead to mitochondrial changes [68, 69]. In mitochondria, the Krebs cycle and the respiratory chain are responsible for most of the adenosine triphosphate (ATP) production [70]. Alterations in processes related to ATP production may lead to mitochondrial dysfunction [71]. Previous studies have shown that obesity is related to mitochondrial dysfunction [9, 10]. In this context, the brain tissue is particularly vulnerable, since it has a high energy demand and strongly depends on an efficient mitochondrial function [72].

In this study, we showed that animals fed a high-fat diet (obese) had alterations in Krebs cycle enzyme activity. Omega-3 treatment attenuated some of these changes. Both activation and inhibition of Krebs cycle enzyme activity may cause intracellular changes. However, the inhibition tends to be more prejudicial, since it may cause fewer electrons to be available for complexes I and II of the mitochondrial respiratory chain, thus inhibiting these complexes, which may lead to lower ATP production. Inhibition of mitochondrial respiratory chain complexes may also lead to greater electron escape and increase ROS formation. It is known that oxidative stress may activate transcription factors and stimulate the increase of pro-inflammatory cytokines [7, 66]. However, in general, only slight changes in the Krebs cycle were observed in the brain after obesity induction. The treatment with omega-3, despite have attenuated only a few alterations, did not worsen Krebs cycle enzymes activity.

The analyses of the mitochondrial respiratory chain revealed that the obese animals had inhibition of the complexes I, II, and IV in all studied cerebral structures, which was attenuated by omega-3 treatment. Inhibition of respiratory chain complexes may lead to a decline in ATP production and to greater electron escape, which may increase ROS formation by the respiratory chain. Fewer amounts of ATP and increased ROS generation may impair cell function. Considering the important functions of the brain structures hypothalamus, hippocampus, prefrontal cortex, and striatum, the inhibition of complexes I, II, and IV of the mitochondrial respiratory chain may be related to the other alterations already identified in these brain structures in obesity. Because of the involvement of the mitochondrial respiratory chain in ROS production, the respiratory chain inhibition may lead to oxidative stress and aggravate inflammation. When this occurs in brain structures related to eating behavior, it may lead to uncontrolled eating, contribute to the higher consumption of rewarding foods, generally high in fat and sugar, and cause an accumulation of fat in the adipose tissue, which characterizes obesity.

Omega-3 treatment reversed only the inhibition of complexes I and II in the striatum, but this reversal is very beneficial given the important role of this brain structure in the reward system. Omega-3 also attenuated the inhibition of complex II in the hypothalamus and hippocampus. It is noteworthy that omega-3 did not show any detrimental effects on the respiratory chain in the brain structures. To our knowledge, this is the first study to report the effect of omega-3 on the activity of Krebs cycle enzymes and of the mitochondrial respiratory chain in the brain of high-fat diet-induced obese mice.

In this study, omega-3 treatment showed numerous beneficial effects in obese mice, which highlights its potential to contribute to obesity treatment. Although treatment with omega-3 did not affect body weight, it promoted visceral fat

reduction. In the brain structures, treatment with omega-3 reduced inflammation and oxidative damage, and attenuated the alterations in the antioxidant defense, in the Krebs cycle, and in the mitochondrial respiratory chain.

A common question regarding animal studies is how to translate the dose of omega-3 used in animals to the dose indicated for humans, which can contribute to interpreting and translating the results to the clinics. Reagan-Shaw et al. [73] provided interesting information regarding a method that considers the body surface area for the translation of doses from animal to human studies. Accordingly, a fish oil dose of 400 mg/kg/day used in this study corresponds to a dose of 32 mg/kg/day in humans or 2 g/day for an individual weighing 70 kg. Similar fish oil dosages have been used in human studies [74–80]. Consequently, the dosage used in this study can be considered equivalent to human studies.

Conclusion

In conclusion, in this study, omega-3 had beneficial effects on the brain of obese animals, as it partially reversed the increase in the inflammatory markers and in the oxidative stress markers, as well as attenuated alterations related to energy metabolism (Krebs cycle and mitochondrial respiratory chain) caused by the consumption of a high-fat diet and consequent obesity. These findings support the data indicating that the use of omega-3 may help treat obesity and associated metabolic abnormalities. It should be also emphasized that when the omega-3 did not reverse or improve the alterations caused by high-fat diet, it also did not affect them negatively.

Author's Contributions AHM and GTR conceived and designed the experiments. AHM and RBS performed the animal experiments. AHM, MPSG, MLG, MLG, GBS, and RPZ made the biochemical analyses. PFS, JB, PCLS, FP, and GTR contributed with reagents, materials, and analysis tools. AHM and GTR analyzed the data. AHM wrote the manuscript. GTR revised the manuscript.

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Compliance with Ethical Standards

Ethics Statement This research was approved by the Ethics Committee on the Use of Animals of the University of Southern Santa Catarina (register n° 15.021.4.01.IV and 16.023.4.01.IV). The use of animals followed the Brazilian Directive for the Care and Use of Animals in Research established by the National Council for the Control of Animal Experimentation (known by the Portuguese acronym "CONCEA").

Conflict of Interest The authors declare that they have no conflict of interest.

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