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Anti Obesity Action of Omega-3 Supplementation in Wistar Rats

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Abstract

Obesity and Its co morbidity has become a world endemic health problem. Among several triggers to this condition, evolution in diet composition with large amounts intake of energetic foods, added to limited physical activities due to the modern people habits, are notables factors. Change in ratio omega-6/omega-3 fatty acids intake is an important obesity cause and can make the co morbidity become worst. A large number of researches evidences the role of the fatty acids omega-6/ômega3 ratio as effective health promoter by, modulating its bio-active derivatives; the eicosanoids and cytokines. The aim of this study is to evidence the anti obesity effect of a diet supplementation with omega-3 fatty acids, and, specific aims are following up rats corporal weight, adipocytes size and blood levels of total cholesterol, high density lipoprotein (HDL) and triglycerides. This was an experimental study with five Wistar male rats groups (n=6), with body weight upper 260g. One group was euthanized at day one; two groups received intake of water by ga-

vage during 45 and 90 days, respectively, and two groups received intake of 0,1mg/kg/weigh/day of high purified omega-3 (EPA 350 mg/ml + DHA 220 mg/ml) for 45 and 90 days. All animals received intake of water and food at *libitum* during the experience.The results showed decreasing in blood total cholesterol, and triglycerides levels ($p<0,05$), increase in HDL blood level ($p<0,05$), decrease in weight and had reduced its adipocytes area ($p< 0,01$) with omega-3 intake. The group which received omega-3 during 45 days compared to the 90 days group choose less parameter reductions. None of the rats presented any sign of side effects during the study. Conclusion: the current study evidenced ante obesity effect, in rats, of omega-3 fatty acid intake. This effect was time-dependent.

Introduction

Obesity represents a growing global health problem because it is a precursor to chronic diseases, such as those resulting from metabolic syndrome (MS) and other diseases such as pulmonary, gastrointestinal tract, skeletal muscles, joints, endocrine-metabolic imbalances and psychosocial disorders (LANCET, 2017). Body mass index (BMI) is the criterion commonly applied for classification of obesity and overweight. It is defined by the World Health Organization (WHO) as: body weight in kg, divided by the square of height in meters [BMI= Weight(kg) / height2(m)], and its normal value ranges from 18 to 24. Currently, the WHO classifies as obesity in adults, men and women, with BMI>30 and overweight, with bmi >25 (WHO, 2016).

Generally speaking, obesity in the world population has almost tripled since 1975, and is now recognized as an epidemic disease of great repercussions, making valuable any effective measures in the prevention and control of the problem (WHO, 2016). There has been a growing interest in the relationship between obesity and changes in the profile of adipocins and inflammation. The science that adipose tissue exerts a greater rule in the regulation of metabolic functions has brought us new perspectives of the processes and homeostatic mechanisms exercised by adipocytes. Inflammation seems to play a key role in the pathophysiology of metabolic syndrome and adipokines released by adipose tissue regulate various functions in the body, including inflammation (JUNG; CHOI, 2014).

Obesity is the main component of Metabolic Syndrome (MS), which is a condition defined as the set of risk factors for cardiovascular diseases (CVD) and type 2 diabetes mellitus (DM-2). MS abnormalities increase with body fat mass (ECKEL; GRUNDY; ZIMMET, 2005). In the last two decades, the relationship between obesity and adipokine profile and inflammation in adipose tissue has been the object of increasing interest (JUNG, CHOI, 2014).

During the initial phase of inflammation, Omega-6 fatty acids (Aeadonic Acid- AA) are converted to their bioactive derivatives of the unfavorable Eicoanoid group, such as prostaglandins

PGE2, thromboxane (TBX), 5-lipooxygenase (LP) and leukotrien (LC) which activate the Nuclear Factor beta-layer (NF-ķb), crucial mediator of inflammation, which regulates more than 200 genes involved in the pro-inflammatory cascade and apoptosis (SIMOPOULOS, 2002). The resolution of inflammation in turn is activated by the trans cellular process that uses the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosaexaenoic acid (DHA) to synthesize special lipid mediators (adipocins) of the group of resolvernas, which return the inflamed tissue to homeotase (BARDEN *et al*, 2015; KIM *et al* 2016).

Fatty acids (AGs) are active lipid compounds formed by long chains of hydrocarbon with a carboxyl radical at the distal end of the molecule. They can be saturated, mono unsaturated or polyunsaturated. The latter are named according to the location of the carbon carrier of the first most distant unsaturation of the hydroxyl radical. Omega-3 (n-3) and omega-6 (n-6) fatty acids have the first unsaturations, respectively, in molecule carbons 3 and 6.. (SIMOPOULOS, 2002). They are structurally and functionally different. Both perform their functions by competing for specific receptors, free fatty acids reeptors (FFAr), present on the surface of cell membranes, mainly adipocytes, pre adipocytes, macrophages and gastrointestinal tract cells. The AG n-6/AG n-3 ratio in plasma is an important biomarker in food control for health promotion (MOBRATEN; KLEIVELAND, 2013; MILLIGAN *et al*., 2017).

The current interest of researchers in the benefits of omega-3 intensified in the 1980s from the observation of low rates of obesity, cardiovascular disease (CVD) and almost no DM-2 (diabetes mellitus) among eskimos, whose basic feeding source is rich in fish oil, compared to the inhabitants of Denmark. Eskimos also exhibited low serum cholesterol and triglyceride levels, high levels of high-density lipoprotein (HDL) and lower AG n-6/n-3 ratio. Since then, many experiments have been carried out, though not all consistent (LANDS; BIBUS; STARK, 2017; BANG; DYERBERG; SINCLAIR, 1980; BANG; DYERBERG; NIELSEN, 1971).

Polyunsaturated, omega-3 and omega-6 Ag are essential elements supplied, almost in their entirety (95%), by food. In the early days of mankind, probably the n-6/n3 ratio was 1. With the evolution of agriculture, Linoleic Acid (AL 18:2n-6,) present in vegetable oils such as soybean, cotton and sunflower and in animal membranes, were highlighted in omega-3 AG, alpha linoleic acid, ALA 18:3-n-3, present in some fruits, such as nuts, nuts, almonds and seeds, eicosapentaenoic acid (EPA) and docosaexaenoic acid (DHA), present mainly in fish oil.

Regarding supplementation with omega-3 GA, considering that changes in dietary patterns have reversed the n-6/n-3 ratio, more recently randomized controlled trials, primarily using more than 1 g of, EPA and DHA in risk groups for cardiovascular diseases and in obese individuals, including those with triglyceride levels > 150 mg/dl or LDL > 130 mg/dl, evidenced benefits that provided additio-

nal information for the clinical use of supplementation in the prevention of CVD, DM-2 and obesity (SPERLING; NELSON, 2016; TAVAZZI, TAVAZZI MAGGIONI; MARCHIOLI, 2008; KUHN; BINART; LOMBES, 2012).

Thus, the aim os this study was evaluate the action on adipose tissue of omega-3 supplementation in rats.

Methods

Experimental Procedures

The study was submitted and approved by the Ethics Committee for the Use of Animals (CEUA) of UNIFOR, Protocol Aprovation No. 0003/2017, reproduced under strict genetic and sanitary control.

Five groups n=6 were randomized: (G1, G2, G3, G4, and G5) of heterogeneous Wistar rats, adults and males, weighing more than 260 g. The groups were separated into cages (with 6 units each), kept in an environment with humidity and temperature control, 12-hour sleep and wake cycles, purified water and standard feeding at libitum. The rats were weighed on the first day and every 7 days to control nutritional status and review the dose of omega TEC (Evidence Soluções Farmacêuticas) to be administered. On day UM of the research, rats in group 3 (G3) were euthanized. We consider this group as a basal control. 5 mL of intracardiac blood was collected for laboratory evaluation of the biochemical parameters of each rat and retroperitoneal adipose tissue was removed for histological analysis. Groups G1 and G2 received 0.5 mL of water per gavage daily. Groups G4 and G5 were filtered with 0.1 mL/kg of fish oil weight. Groups G1 and G4, which received water and fish oil, respectively, were euthanized at 45 days. 5mL of intracardiac blood was collected from each rat and retro peritoneal adipose tissue was removed for histological analysis. Groups G2 and G5 were euthanized at 90 days. We took 5 mL of intracardiac blood and removed retroperitoneal adipose tissue for histological analysis. The animals were first anesthetized with Ketamine (90 to 100 mg/kg) and Xylazine (10 mg/kg). They then received a lethal dose of thiopental (100 mg/kg) and lidocaine (10 mg), all intraperitoneally. The blood collected from each rat, without anticoagulant, was centrifuged for 15 minutes at 6000 rpm and sent to the unifor clinical analysis laboratory for analysis of laboratory parameters: total cholesterol (TC), high density lipoprotein (HDL), triglycerides (TG) and glycemia (GL).

In the preparation of adipose tissue blocks of the animals: Soon after removal, the adipose tissue was dipped in glass containers with 10% formaldehyde for fixation for more than 48 hours; They were then dehydrated in 90% alcohol and treated with xylol; Impregnated in blocks with paraffin; Cut into the microtome; The slides were cordoned by the hematoxylin and eosin method.

Analysis of results

The analysis of laboratory parameters was performed with the following methods: Enzymatic Colorimetry for

Total Cholesterol and Triglycerides (Labteste commercial kit®). Precipitation for HDL (Labteste commercial kit®). For statistical analysis of laboratory parameters, ibm SPSS Statistics Base (version 21.0) was used, in this phase descriptive analysis was used as means and inferential as variance through dunnett test and f test.

Results and Discusion

Triglyceride Analisys

Comparing, in Table 1, the groups that took water for 45 days and omega for 45 days showed no statistically significant difference in triglyceride levels. However, between the water groups for 90 days, and omega for 90 days there was a statistical difference, that is, the rats that took water had a much higher mean triglycerides ($p<0.005$). Rats that took water for 45 days had lower triglyceride levels than rats that took water for 90 days. One possible conclusion for these data is that omega acts as a controlling factor since rats that underwent water treatment had a progressive increase in triglyceride levels over time and those undergoing omega-3 treatment maintained their basal levels.

There is no statistical difference between the mean group that underwent water treatment for 45 days and control; There is no statistical difference between the mean group that underwent omega-3 treatment for 45 days and control. Again the time factor was important.

We observed that the group that received the water treatment during the 90 days obtained some of the highest values analyzed triglyceride levels.

Table 1 - Triglyceride Levels (mg/dl)

Time	Treatment	Average (mg/dl)	Std Deviation	p
45 days	Water	67,50	38,23	-
	Ômega	71,0	9,20	
90 days	Water	239,75	86,98	**
	Ômega	77,75	18,34	
45 days 90 days	Water	67,50	38,23	**
	Water	239,75	86,98	
45 days 90 days	Ômega	71,00	9,20	**
	Ômega	77,75	18,34	
Water	Control	81,50	50,80	***
	90 days	239,75	86,98	

Mean with standard deviation of triglyceride levels in mg/dl, between rats that ingested water for 45 and 90 days and omega for 45 and 90 days. The rats that ingested omega-3 had lower mean triglyceride scores than the rats that ingested water ***$p<0.05$ **$p<0.02$.

Total cholesterol analysis

The statistical analysis of total cholesterol was performed in two stages. The p-value found in the application of the Shapiro-Wilk normality test for the model with TC was 0.0108, that is, according to the Shapiro-Wilk test at 5% significance, the residues cannot be considered normal. As the Shapiro-Wilk Test rejected normality (H_0), we had to make a transformation in the CT variable so that we could normalize the residues and proceed with the analysis. The transformation used to normalize the residues was the application of the natural logarithmic function in THE. From now on all responses and comparations will be given based on the logarithmus of the choleste-

rol value (lnCT). After the transformation is application, we must check whether the residues generated by the model now follow the normal distribution.

The p-value found in the application of the Shapiro-Wilk normality test for the model with lnCT was 0.6799, that is, according to the Shapiro-Wilk test at 5% significance, the residues can be considered normal and with this we can proceed with the analysis.

According to the F-test, the means of the LnCT are statistically different, i.e., the rats that were treated with water for 90 days had statistically higher mean total cholesterol than the rats that underwent omega treatment for 90 days. According to the analysis of the logarithmic function of total cholesterol, rats that were treated with water for 45 days had mean total cholesterol, lower than rats that treated with water for 90 days (Table 2).

From the results we can conclude that rats over time receiving water treatment increased their total cholesterol level. The group that had water treatment for 90 days had a statistically higher mean total cholesterol than the control group, we note that the two assumptions previously raised that rats that underwent water treatment for 90 days have high values of LnCT and that rats that underwent omega treatment for 90 days have the lowest values of ln(TC), which seems to be attributed to the fact that omega-3 administration contains the elevation of their cholesterol levels.

Table 2 Total Cholesterol Analysis –.(mg/dl)

Time	Treatment	Average (g/dl)	Std Deviation	p
45 days	Water	4,179	0,10	-
	Ômega	4,159	0,12	
90 days	Water	4,903	0,36	**
	Ômega	3,902	0,16	
45 days 90 days	Water	4,179	0,10	**
	Water	4,903	0,36	
45 days 90 days	Ômega	4,156	0,12	*
	Ômega	3,902	0,16	
	Control	4,295	0,23	**
	Group – 90 days	4,903	0,36	

The mean values with standard deviation of the logarithm of total cholesterol values were higher in the group that took water for 90 days and lower in rats that took omega-3 for 90 days *p<0.05 **p<0.002.

HDL analysis

According to the F-test, hdl means are statistically different, i.e., rats that treated with water for 90 days had statistically lower HDL means than rats that underwent omega treatment for 90 days (Table 3). With this result, we can conclude that omega treatment promoted an increase in HDL levels.

Comparing HDL with respect to water, according to the F test, the means of this factor are statistically different, that is, the rats that were treated with water for 45 days had a higher mean HDL than the rats that underwent water treatment for 90 days. From the results we can conclude that rats over time receiving water treatment significantly reduced their HDL levels. At this point, what we can observe is that the average HDL levels have already been reduced to

45 days of omega.

Table 3 – HDL Analysis (mg/dl)

Time	Treatment	Average (mg/dl)	Std Deviation	p
45days	Water	45,25	4,57	*
	Ômega	41,75	5,91	
90days	Water	5,00	0,82	***
	Ômega	38,75	7,37	
45 days 90 days	Water	45,25	4,57	***
	Water	5,00	0,82	
45 days 90 days	Ômega	41,75	5,91	**
	Ômega	38,75	7,37	
	Control	32,68	15,20	***
	Group – Water 90 Days	5,00	0,82	

The mean HDL values of rats that took Omega-3 for 90 days were higher than hdl values in rats that took water for 45 and 90 days * $p<0.05$ ** $p<0.02$ *** $p<0.01$.

Final Weight

The p-value found in the application of the Shapiro-Wilk normality test for the model with PF was 0.971, that is, according to the Shapiro-Wilk test at 5% significance, the residues can be considered normal and with this we can proceed with the analysis.

According to the F test, the mean final weight is statistically different, i.e., rats that treated with water for 90 days had statistically higher weight averages than rats that underwent omega treatment for 90 days (Table 7).

According to the F test, the mean scores of the final weight are statistically different, that is, the rats that were treated with water for 45 days had a lower mean final weight than the rats that underwent water treatment for 90 days (Table 4).

Table 4 – final weight (g)

Time	Treatment	Average	Std Deviation	p
45 days	Water	329,0	27,78	-
	Ômega	320,4	20,12	
90 days	Water	417,2	20,28	**
	Ômega	354,8	27,34	
45 days 90 days	Water	329,0	27,78	***
	Ômega	417,2	20,28	
45 days 90 days	Ômega	320,4	20,12	**
	Ômega	354,8	27,34	
	Control	347,6	23,78	**
	Group – 90 Days Water	417,2	20,28	

The mean scores of the final weight of rats that took Omega-3 for 90 days were lower than those who took water for 90 days *$p<0.05$ ** $p<0.02$ *** $p<0.01$.

Our results show that, as the days passed, rats that received water as treatment had a gradual increase in body weight, with a statistically significant difference, in relation to rats that received omega-3 for 90 days. However, at 45 days there was no statistical difference between the weights of the rats. That is, omega-3 supplementation contained weight gain in rats, but the time factor was important.

Our results were coincided with the literature data, as in the research conducted by Lombardo, Hein and Chico (2007), which followed three groups of Wistar rats; one control group had sucralose replaced by starch, the second group received a diet rich in sucralose and corn oil, and the third group received sucralose, corn oil and fish oil for 8 months. The group that received the added diet of fish oil had its triglycerides reduced, as well as the size of the adipocytes, besides presenting greater sensitivity to insulin.

Larsen *et al*., (2003), worked with two groups of rats for 4 weeks in which group 1 had their diet enriched with cod liver oil and 2.5g of fish oil, and group 2 received a diet enriched with sunflower oil and palm oil and olive oil. Rats in group 1 had lower triglyceride levels.

Mondragon *et al*., (2013), used 3 sources of fatty acids to evaluate the effect on metabolic syndrome parameters in humans; in their research, three groups of adult individuals (n=15) received for 6 weeks, respectively; 1.8 g of n-3 (1.08 g EPA + 0.72 g DHA); 2g conjugated linoleic acid (50:50) and finally in the third group 40 g of walnuts. As results, the group that received omega-3 (EPA and DHA) obtained a greater reduction in triglyceride indexes (186 ± 35.2 to 149 ± 29 mg/dl). The group that used nuts had a beneficial effect by increasing HDL levels and there were no significant changes in the group that used CLA (conjugated linoleic acid).

Researchers such as Wree *et al*., (2011), Neuhrofer *et al*., (2013), Donahue *et al*., (2011), obtained similar results.

Conclusion

We conclude that supplementation with omega-3 in rats at a dose of 0.1 mg/kg/day, with a concentration of 330 mg/ml of EPA and 220 mg/ml of DHA, proved to be effective with progressive effect in reducing blood levels of total cholesterol and triglycerides, increasing HDL fraction levels, the longer the administration time. Had an effect on obesity since it contained weight gain. The effect was more expressive with a longer time of omega administration.

Thus, we suggest searches for a longer period of time for a defined slimming effect.

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