



Applied nutritional investigation

The benefits of ω -3 supplementation depend on adiponectin basal level and adiponectin increase after the supplementation: A randomized clinical trial



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ABSTRACT

Objective: The aim of this study was to analyze whether ω -3 supplementation improves cardiometabolic profile in individuals with cardiovascular risk factors and to determine the effect of adiponectin levels on these changes.

Methods: In this double-blind, placebo-controlled, 2-mo clinical trial, we randomized 80 individuals of both sexes (mean age 52 y) with at least one cardiovascular risk factor (excess weight, hypertension, dyslipidemia, diabetes, or smoking) into two groups: ω -3 (supplemented with 3 g/d of fish oil containing 37% eicosapentaenoic acid and 23% docosahexaenoic acid) and placebo (3 g/d of sunflower oil containing 65% linoleic acid). At baseline and after the intervention, we evaluated serum adiponectin, leptin, lipid profile, apolipoproteins (apo), electronegative low-density lipoprotein (LDL⁻), and glucose metabolism (glucose and insulin).

Results: After supplementation, the ω -3 group showed an increase in serum adiponectin. After stratifying the ω -3 group by adiponectin concentration at baseline, participants with lower adiponectin concentration showed a higher reduction of total cholesterol, LDL, LDL/high-density lipoprotein ratio, LDL/apo B, and LDL⁻. Individuals with a higher variation of adiponectin concentration after ω -3 supplementation presented with reduced blood glucose. The variation of serum adiponectin induced by ω -3 supplementation was negatively correlated with the Framingham and Adult Treatment Panel IV scores ($r = -0.4$ and $P < 0.05$ for both).

Conclusions: Adiponectin is shown as one of the mechanisms by which ω -3 improves cardiometabolic profile in persons with cardiovascular risk. Moreover, the benefit varies according to the adiponectin basal level and adiponectin variation after supplementation.

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Introduction

Adipose tissue shows important endocrine function. Adipocytes secrete bioactive peptides and proteins (adipocytokines) that modulate food intake, energy homeostasis, regulation of blood pressure, and insulin sensitivity [1]. In general, increased body fat stimulates the production of most adipocytokines and often is associated with increased risk for developing cardiovascular diseases (CVDs). However, one of them, called adiponectin, presents synthesis inversely proportional to the amount of adipose tissue [2].

High serum adiponectin concentration has been associated with several cardioprotective mechanisms, such as increasing

lipid oxidation and insulin sensitivity and inhibiting the vasoconstriction induced by serotonin. These events contribute to prevention and control of many morbidities related to CVDs, such as hypertension, insulin resistance (IR), and diabetes [3]. The mechanisms involved in the reduction of IR are due, at least in part, to the role of adiponectin as activator of adenosine monophosphate (AMP) and cellular AMP-activated protein kinase (AMP-K), stimulating β -oxidation and glucose use as energy source, in addition to reducing gluconeogenesis [4].

Additionally, experimental studies have found an association between ω -3 fatty acid consumption and increased adiponectin [5–7]. However, in humans, the results are still controversial in clinical trials with ω -3 supplementation from 0.186 to 3 g/d for periods from 6 wk to 5 y [8].

Here, we showed that ω -3 supplementation improves cardiometabolic profile in humans with cardiovascular risk factors and that the benefits vary according to adiponectin basal level and adiponectin increase after the supplementation.

Methods

Study design

This double-blind, placebo-controlled, randomized (allocation ratio = 1:1) clinical trial was a 2-mo intervention study, divided into three phases:

1. Screening: Identification of individuals who met the inclusion criteria.
2. Baseline period: Signature of the informed consent form; assessment of previous cardiovascular event by electrocardiogram; measurement of blood pressure (BP), waist circumference, and weight and height (to calculate body mass index [BMI]); performance of bioimpedance to calculate the percentage of body fat; blood collection; application of the questionnaires to collect sociodemographic, clinical, dietary, and physical activity data; and delivery of the capsules.
3. After 2 mo of intervention: Measurement of BP, anthropometric and bioimpedance assessment; blood collection; and reapplication of the questionnaires.

The data were collected between August 2011 and July 2012, when we reached the sample size requirement. The biochemical analyses were conducted from August 2011 to December 2014.

At baseline, participants received capsules containing ω -3 (3 g/d; 37% eicosapentaenoic acid [EPA] and 23% docosahexaenoic acid [DHA]) or placebo (3 g/d; sunflower oil, with 65% linoleic acid [ω -6]) in a quantity enough for 2 mo. The capsules and their containers were identical to minimize risk for information bias.

Adherence to intervention was evaluated by counting remaining capsules and analysis of plasma fatty acids. Compliance and adverse effects were monitored using a diary provided to all participants. We evaluated socioeconomic and clinical profiles using a structured questionnaire, which addressed the following variables: sex, age, race, smoking, consumption of alcohol and drugs, and presence of diseases. Participant risks for developing a cardiovascular event were classified according to the Framingham Risk Score updated for the American Heart Association [9,10] and according to the Adult Treatment Panel IV (ATP IV) from the National Heart, Lung and Blood Institute [11].

Sample size calculation

To calculate the sample size, we used a previous study as reference [12]. In that study, 30 women (BMI between 25 and 40 kg/m²) were supplemented with 1.2 g of EPA plus DHA for 8 wk, aiming at change in plasma adiponectin and some parameters associated with metabolic risk. The results showed a significant 19.5% increase in adiponectin concentration, from 11.8 to 13.5 μ g/mL after supplementation.

In the study, the magnitude of effect was 1.7, with an SD of the outcome variable of 2.41 and magnitude standardized effect equal to 0.71. Considering the values of bidirectional α equal to 0.05 and β equal to 0.10 (statistical power of 90%) and a *t* test to compare means for continuous variables, the sample size totaled ≥ 36 individuals per group.

Participants

We selected 80 men and women, ages 30 to 74 y, who had some classic risk factor for CVD (overweight, hypertension, dyslipidemia, diabetes, smoking) with

or without treatment, without previous cardiovascular event (monitored by clinical examination, electrocardiogram, and medical record fulfillment research). These participants were randomly assigned to ω -3 and placebo groups.

Individuals who showed any of the following characteristics were excluded from the study: treatment with antiinflammatory drugs; malnutrition; pregnancy or breast-feeding; participation of other intervention protocols; previous cardiovascular event; acute or chronic severe diseases; illicit drug use; alcohol abuse; allergy or intolerance to any component of the intervention; and uncontrolled psychiatric disorders.

This study was approved by the Ethics Committee of the Faculdade de Saúde Pública (no 2264) and the University Hospital of the Universidade de São Paulo (no 1126/11), where all data were collected. All participants signed an informed consent form. This clinical trial is registered on the *Registro Brasileiro de Ensaios Clínicos* (ReBEC) under the number RBR-2 vfhv.

Randomization

The allocation followed a sequential number in order of arrival. Each participant was allocated in one intervention group. The strategy used was based on a consecutive probability of 50% (i.e., for each participant included in the ω -3 group, the next participant was included in the placebo group, independent of sex or age). As the containers in which the capsules were provided were encoded, neither researchers nor participants knew which intervention group (ω -3 or placebo) each participant was being allocated. The creation of the random-allocation sequence, the enrollment of participants, and the assignment of participants to interventions were done by different researchers.

Blinding

All participants, care providers, researchers, and those assessing outcomes were blinded. The capsules containing ω -3 or placebo were concealed by an independent laboratory. The capsules and containers were encoded and identical for both groups. The written register presenting the codes and respective intervention group were open only after all the analyses were completed.

Blood sampling and analysis

Blood samples were drawn after an 8- to 12-h overnight fast. Serum and plasma were stored at -80°C until analysis.

Serum adiponectin and leptin

Serum adiponectin and leptin concentrations were analyzed using enzyme-linked immunosorbent assay (ELISA) sandwich (EMD Millipore and Enzo Life-sciences, respectively).

Lipid profile

Total cholesterol (TC), triacylglycerols (TG), and high-density lipoprotein (HDL) in plasma were analyzed by standard methods (Labtest, Minas Gerais, Brazil). The content of cholesterol associated with low-density lipoprotein (LDL) was determined using a previously described formula [13], as follows:

$$\text{LDL} = \frac{\text{TC} - \text{HDL} - \text{TG}}{5}.$$

Apo A1 and apo B were determined by standard methods using kits Autokit AI PDB and Autokit B PDB (Wako Chemicals USA Inc., Richmond, VA, USA), respectively, by immunoturbidimetric method. From these results, we calculated TG/HDL, TC/HDL, LDL/HDL, apo B/apo A1, HDL/apo A1, and LDL/apo B ratios.

The nonesterified fatty acids were determined by enzymatic method (acyl-CoA synthetase), using a commercial kit (Free Fatty Acids Quantification Kit, BioVision Products Research, Milpitas, CA, USA).

LDL(–) was detected by sandwich ELISA following a previously described protocol [14]. We sensitized the plates (Costar®, Model 3690, Corning, NY, USA) with anti-LDL(–) monoclonal antibody (–mAb 1 A3) (0.5 μ g/mL, 50 μ L/well) diluted in carbonate-bicarbonate buffer (0.25 M, pH 9.6) and incubated overnight at 4°C . Thereafter, the free sites were blocked with skim milk (Molico, Nestle, São Paulo, Brazil), diluted to 5% in phosphate-buffered saline 0.01 mol/L (PBS; pH 7.4) and incubated at 37°C for 2 h. Then the plates were washed four times with PBS-Tween (0.05%). It was added to 50 μ L/well of plasma diluted (1:1600) in PBS buffer, and the plate incubated for 2 h at room temperature. After, the plate was washed as described previously and was added 50.0 μ L/well of anti-LDL(–) monoclonal antibody biotinylated (mAb 2 C7–) (0.5 μ g/mL, 50 μ L/well). Plates were again incubated at room temperature for 2 h and then washed as previously described. They were added 50 μ L/well of streptavidin-peroxidase (1:80,000) diluted in PBS. The plates were incubated for 1 h at room temperature and again washed as just described. The color reaction was developed by adding the substrate comprises 3,3',5,5'-tetrametilbenzine,

citrate-phosphate buffer (0.1 M, pH 4.2), and H₂O₂ (30%) (250/12/10 µL/mL/µL). The plates were incubated for 15 min at room temperature and protected from light. The reaction was blocked with 50 mL/well of H₂SO₄ (2 M) and the absorbance monitored at 450 nm. The results were expressed as mean absorbance of the samples minus blank and subsequently applied to the standard curve and multiplied by the corresponding dilution, and results were expressed in U/L. Autoantibodies LDL(–) were determined according to a previous study [15].

Glucose and insulin analyses

We determined plasma glucose using the commercial, enzymatic, and colorimetric kit Glucose PAP Liquiform (Labtest, Minas Gerais, Brazil). Plasma insulin was analyzed by immunoassay technique using the Human Insulin Direct ELISA Kit Novex.

Insulin resistance was calculated by the IR index (homeostatic model assessment [HOMA]-IR) in which

$$\text{HOMA} - \text{IR} = \frac{[\text{fasting insulin concentrations (U/mL)} \times \text{fasting glucose (mmol/L)}]}{22.5}$$

The presence of IR was determined in accordance with model 1 proposed in a previous study [16], in which IR is diagnosed if any one of the following criteria are present: BMI >28.9 kg/m², HOMA-IR >4.65, or BMI >27.5 kg/m² and HOMA-IR >3.6.

Fatty acids in plasma and capsules

To evaluate the adherence of participants to the intervention, we determined the profile of fatty acids in their plasma. Lipids were extracted according to a previously described method [17]. Samples were saponified in the presence of NaOH (10 M) at room temperature in the dark for 90 min. Quantification of fatty acids used the fluorescent reagent 9-antrildiazometano (ADAM). The extracted lipids were dried under nitrogen flow, resuspended in 50 µL of ethyl acetate and ADAM derivatized with 50 µL (5 mg/mL) at 10°C for 30 min. The derivatized fatty acids were analyzed by high-performance liquid chromatography (HPLC) using a Kinetix C18 column (50 × 3 mm with 2.6 µm particle size) and precolumn C18. For each plasma sample run in HPLC, it was also analyzed one blank sample only containing the internal standard (heptadecanoic acid, C17), a sample of pure ADAM, and an external standard sample (mix of separated fatty acids: EPA, DHA, arachidonic, palmitoleic, palmitic, linoleic, linolenic, stearic). This analysis determined the retention time of the fatty acid in each run and allowed their integration according to it. For the separation of fatty acids a gradient of acetonitrile (ACN) and water, from a gradient of 79% ACN, reaching 93% at a rate of 1.45 mL/min and 12 min for each analysis, was used. Fluorescence was monitored at 350 nm and its emission was analyzed at 450 nm. After integration of the peaks obtained in each race, it was calculated the area of each peak percentage. The ω-3 capsules were analyzed using the same method.

Statistical analysis

Statistical analyses were carried out using the IBM SPSS Statistics for Windows, version 20 (IBM, Armonk, NY, USA). Chi-square test was used to analyze the categorical variables, presented in absolute value and their respective percentage. Normal distribution of outcome variables was evaluated using the Kolmogorov-Smirnov test ($P > 0.05$). If needed, variables were log-transformed to obtain better approximations of the normal distribution before analysis. Variables with normal distribution were analyzed by paired *t* test (effect of the time) and *t* test for independent samples (effect of the intervention) and were presented as mean and SD. Variables with nonparametric distribution were analyzed using the Wilcoxon test (effect of the time) and Mann-Whitney (effect of the intervention) and were presented as median (p50) and interquartile range (p25, p75). The Pearson correlation was used to test the association between variation of serum adiponectin and the Framingham Risk Score and the ATP IV. The significance level was set at $P < 0.05$.

To identify the influence of basal adiponectin concentration in the response to the supplementation with ω-3, the ω-3 group was stratified according to the 75th percentile of serum adiponectin concentration at baseline, dividing them into two groups: adiponectin <p75, with a minimum concentration of 1.6 µg/mL and a maximum of 15.5 µg/mL, and adiponectin ≥p75 with a minimum concentration of 17.2 µg/mL and maximum of 42.6 µg/mL. Both groups were similar in relation to mean age (52 y), BMI at baseline (30 kg/m²), and body fat percentage at baseline (33%).

The ω-3 group was also stratified according to the 75th percentile of the variation of serum adiponectin concentration: Δ (at 2 mo – at baseline) adiponectin <p75, with minimal change of –12.9 µg/mL and a maximum of 7.8 µg/mL; and Δ (at 2 mo – at baseline) adiponectin ≥p75, with minimum variation of 9 µg/mL and a maximum of 31 µg/mL. The first group had a mean age of 53 (SD = 8) y and the second, 47.6 (SD = 11) y.

Results

We analyzed 40 participants in each intervention group. The adherence levels to intervention were 83% (ω-3 group) and 84% (placebo group) of the capsules. No participant presented adverse effects that justified the intervention suspension. The main self-reported symptoms were flatulence (9%) and constipation (5%).

At baseline, ω-3 and placebo groups were similar regarding sex, age (mean age 52; SD = 9 versus 51.5; SD = 11 y, respectively), race, smoke status, alcohol consumption, diseases, and medication use (Table 1).

Most participants in the ω-3 (85.5%) and placebo (88.6%) groups reported having at least one disease. For both groups, the most frequent diseases were hypertension and dyslipidemia, followed by diabetes mellitus and hypothyroidism. Most participants used some medication, mainly antihypertensives, statins, and hypoglycemics, confirming self-reported diseases (Table 1).

After the intervention, the ω-3 group increased in weight, BMI, and waist circumference (Table 2), and reduced systolic and diastolic BP. However, these differences were not significant compared with the placebo group. The ω-3 group showed significant reduction of body fat after the intervention period compared with the placebo group (Table 2).

Table 3 shows the biochemical profile of individuals from both groups at baseline and after 2 mo of intervention. The ω-3 group reduced plasma levels of TG, TC, LDL, TG/HDL ratio, TC/HDL ratio, LDL/HDL ratio, and LDL(–). Yet, the ω-3 group increased HDL and HDL/apo AI ratio. However, the placebo group showed similar results ($P > 0.05$). The concentration of plasma EPA in the ω-3 group remained the same after intervention; but DHA increased by 72% (baseline = 6.1 and 2 mo = 10.5 percentage points) after the intervention (Table 3). Also, the ω-3

Table 1
Characteristics of participants at baseline, by intervention group

Variable	ω-3		Placebo		P value
	n	%	n	%	
Sex					
Male	18	45	16	40	0.651
Female	22	55	24	60	
Age (y)					
30–44	6	15	11	27.5	0.182
45–59	28	70	20	50	
60–74	6	15	9	22.5	
Race					
White	28	70	22	55	0.366
African descendant	4	10	7	17.5	
Others	8	20	11	27.5	
Smoking					
Yes	10	25	3	7.5	0.270
No	16	40	27	67.5	
Former smoker	14	35	10	25	
Alcohol consumption					
Yes	18	45	15	37.5	0.496
No	22	55	25	62.5	
Diseases					
Hypertension	21	60	26	74.3	0.203
Dyslipidemia	19	54.3	19	54.3	1.000
Diabetes mellitus	6	17.1	8	22.9	0.550
Hypothyroidism	3	8.6	4	11.4	0.690
Medicines					
Antihypertensive	18	66.7	26	86.7	0.720
Estatin	6	22.2	7	23.3	0.920
Hypoglycemic	5	18.5	9	30	0.315
Thyroid	3	11.1	4	13.3	0.799
Fibrate	1	3.7	1	3.3	0.940

Differences between groups were analyzed by χ^2 test. Level of significance set at $P < 0.05$

Table 2
Anthropometric, body composition, and blood pressure data, by period and intervention group

Variable	ω -3		<i>P</i> value	Placebo		<i>P</i> value
	Baseline	2 mo		Baseline	2 mo	
Weight (kg)	81 (18)	82 (18)	0.004	86 (21)	86 (21)	0.223
BMI (kg/m ²)	29.8 (6.3)	30.1 (6.4)	<0.001	31.8 (6.3)	32.1 (6.3)	0.011
WC (cm)	98 (12)	99 (11)	0.454	104 (15)	104 (15)	0.298
Body fat (%)	34 (13)	33 (12)	0.013*	38 (13)	38 (13)	0.325
SBP (mm Hg)	138 (19)	131 (17)	<0.001	136 (21)	130 (15)	0.018
DBP (mm Hg)	85 (12)	79 (10)	<0.001	81 (10)	77 (7)	0.015

BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; WC, waist circumference

Values expressed as mean (SD). Comparison between periods by paired *t* test. Comparison between groups by *t* test for independent samples. Significance level *P* < 0.005. Values with statistical significance between baseline and 2 mo are shown in bold

* *P* < 0.05: When comparing ω -3 vs placebo.

group increased serum adiponectin after 2 mo of supplementation.

After supplementation, participants with lower basal levels of adiponectin showed greater reduction in TC, LDL, LDL/HDL, LDL/apo B, and LDL(−), whereas those with a higher concentration of basal adiponectin had further reduction of LDL(−) (Table 4). After stratification according to the 75th percentile of the variation of serum adiponectin concentration, individuals with greater variation ($\geq p75$) in serum adiponectin showed a significant reduction in fasting glucose (Table 5).

For the ω -3 group, the variation of serum adiponectin was negatively correlated with the scores obtained in the Framingham ($r = -0.4$; $P < 0.05$) and ATP IV ($r = -0.4$; $P < 0.05$) scores.

Discussion

In the present study, we observed that ω -3 supplementation improves cardiometabolic profile and increases serum

concentration of adiponectin in people with cardiovascular risk factors. Individuals with low basal concentration of adiponectin are most benefited by the intake of these fatty acids on lipid profile, whereas those with higher baseline concentrations of adiponectin see an improvement in oxidative profile. People with a greater increase in the concentration of adiponectin have an improvement in glucose metabolism.

Experimental studies with animals have shown weight loss [18] and reduction in body fat after supplementation with ω -3 [19]. Studies suggest that these fatty acids have the same effect in humans, particularly when complementary to other treatments for weight loss; but these results are still conflicting [20–22]. In the present study, we found that supplementation with ω -3 led to a reduction in body fat. This effect can be mediated by increased expression of genes involved in lipid oxidation and reduced expression of those related to lipogenesis [23–25].

We also observed an increase in adiponectin concentration after intervention with ω -3. This effect is conflicting in the

Table 3
Biochemical profile of the sample, according to period and intervention group

Variable	ω -3		<i>P</i> value	Placebo		<i>P</i> value
	Baseline	2 mo		Baseline	2 mo	
TG (mg/dL)	146 (80)	109 (59)	<0.001	166 (108)	132 (70)	0.003
TC (mg/dL)	204 (31)	179 (35)	<0.001	200.3 (47)	187 (48)	0.015
LDL (mg/dL)	139 (31)	116 (33)	<0.001	134 (42)	119 (39)	0.001
HDL (mg/dL)	36 (8)	41 (10)	<0.001	36 (11)	41 (13)	<0.001
TG/HDL	4 (2; 6)	2 (1; 4)	<0.001	4 (2; 6)	3 (2; 5)	<0.001
TC/HDL	6 (5; 7)	5 (4; 5)	<0.001	6 (4; 8)	5 (4; 6)	<0.001
LDL/HDL	4 (3; 5)	3 (2; 4)	<0.001	4 (3; 5)	3 (2; 4)	<0.001
apo A1 (mg/dL)	124 (18)	126 (18)	0.363	125 (27)	130 (21)	0.030
apo B (mg/dL)	101 (22)	106 (27)	0.172	101 (27)	104 (29)	0.262
HDL/apo A1	0.29 (0.05)	0.32 (0.05)	<0.001	0.29 (0.07)	0.31 (0.06)	0.013
LDL/apo B	1.4 (0.3)	1.1 (0.4)	<0.001	1.3 (0.2)	1.1 (0.2)	<0.001
LDL(−) (U/L)	6 (3; 16)	4 (2; 15)	0.001	5 (2; 17)	3 (1; 12)	0.038
Anti-LDL(−) (μ U/L)	9 (3; 12)	8 (4; 11)	0.619	8 (5; 12)	8 (4; 11)	0.840
NEFA (mEq/dL)	0.6 (0.3)	0.5 (0.3)	0.171	0.6 (0.2)	0.6 (0.3)	0.375
Glucose (mg/dL)	97 (92; 108)	98 (90; 105)	0.441	97 (90; 116)	95 (91; 107)	0.107
Insulin (μ U/mL)	19 (9)	17 (6)	0.299	17 (9)	19 (9)	0.240
HOMA-IR	5 (2)	4 (2)	0.328	5 (3)	5 (3)	0.268
EPA (%)	1.2 (0.0; 4.1)	1.4 (0.8; 3.4)	0.778	0.7 (0; 5.2)	2.5 (1.4; 3.5)	0.886
DHA (%)	6.1 (2.9)	10.5 (3.5)	<0.001*	5.7 (2.5)	5.2 (1.6)	0.209
ω -6 (%)	34 (8)	29 (9)	<0.001	33 (7)	29 (6)	<0.001
Adiponectin (μ g/mL)	14.8 (10)	18.2 (12.1)	0.021*	19.8 (10.1)	18.7 (10.7)	0.370
Leptin (ng/mL)	42.2 (46.1)	51.1 (55.5)	0.014	56.3 (53)	59.2 (53.7)	0.634
A/L ratio	1.2 (1.7)	1.2 (2.1)	0.602	1.5 (2.6)	1.3 (2.5)	0.289

A/L ratio, adiponectin/leptin ratio; apo, apolipoprotein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance index; LDL, low-density lipoprotein; LDL(−), electronegative LDL; NEFA, nonesterified fatty acids; TC, total cholesterol; TG, triacylglycerols

Values expressed as mean (SD) for variables with parametric distribution, and as median (p25; p75) for variables with nonparametric distribution. Comparison between periods by paired *t* test or Wilcoxon, according to the distribution. Comparison between groups by *t* test for independent samples or Mann–Whitney, according to the distribution. Values with statistical significance between baseline and 2 mo are shown in bold

* *P* < 0.05 compared ω -3 vs placebo.

† *n* = 38, because 2 participants whose samples fell outside the reference range were excluded.

Table 4Biochemical profile of ω -3 group, according to period and 75th percentile of the serum adiponectin concentration at baseline (São Paulo, 2015)

Variable	Adiponectin (<p75)		P value	Adiponectin (\geq p75)		P value
	Baseline	2 mo		Baseline	2 mo	
TG (mg/dL)	147 (81)	106 (50)	<0.001	147 (86)	121 (86)	<0.001
TC (mg/dL)	209 (28)	178 (34)	<0.001*	189 (38)	183 (40)	0.082
LDL (mg/dL)	144 (28)	116 (32)	<0.001*	124 (35)	117 (38)	0.023
HDL (mg/dL)	36 (8)	41 (9)	0.003	37 (10)	42 (11)	0.002
TG/HDL	4 (2.5; 6)	2 (1.5; 4)	0.001	3 (2; 8.3)	2 (1; 6.3)	0.009
TC/HDL	6 (5; 7)	5 (4; 5)	0.000	5 (4; 7.3)	4 (3; 6.3)	0.010
LDL/HDL	4 (4; 5)	3 (2; 4)	<0.001*	3 (2; 5)	3 (2; 4.3)	0.059
apo A1 (mg/dL)	122 (15)	125 (17)	0.272	131 (25)	131 (22)	0.007
apo B (mg/dL)	104 (22)	111 (26)	0.055	93 (20)	98 (21)	0.063
HDL/apo A1	0.29 (0.05)	0.33 (0.05)	0.001	0.28 (0.06)	0.32 (0.07)	0.001
LDL/apo B	1.43 (0.34)	1.05 (0.21)	<0.001*	1.33 (0.20)	1.18 (0.18)	0.018
LDL(–) (U/L)	6 (3; 15)	4 (2; 13)	0.013*	6 (3; 19)	3 (2; 11)	0.007
Anti-LDL(–) (mU/L)	7 (2.4; 11.5)	8 (2.6; 11.1)	0.754	11 (5; 12)	10 (6.3; 12)	0.878
NEFA (mEq/dL)	0.6 (0.2)	0.5 (0.3)	0.271	0.7 (0.3)	0.6 (0.3)	0.065
Glucose (mg/dL)	100 (92.5; 112)	99 (93; 105.5)	0.387	92 (88.5; 97.5)	91 (80; 101)	0.683
Insulin (μ U/mL)	19.1 (9.2)	17 (5)	0.322	17.8 (9.5)	17.9 (7.6)	<0.001
HOMA-IR	4.7 (2.2)	4.3 (1.4)	0.349	4.2 (2.4)	4.2 (2.0)	0.001
Adiponectin (μ g/mL)	9.8 (3.9)	13.5 (8.2)	0.015	29 (7.7)	31.2 (12.2)	0.553

A/L ratio, adiponectin/leptin ratio; apo, apolipoprotein; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance index; LDL, low-density lipoprotein; LDL(–), electronegative LDL; NEFA, nonesterified fatty acids; TC, total cholesterol; TG, triacylglycerols

Values expressed as mean (SD) for variables with parametric distribution, and as median (p25; p75) for variables with nonparametric distribution. Comparison between periods by paired *t* test or Wilcoxon, according to the distribution. Comparison between groups by *t* test for independent samples or Mann–Whitney, according to the distribution. Values with statistical significance between baseline and 2 mo are shown in bold

* *P* < 0.05 comparing people below versus equal or above the 75th percentile of adiponectin concentrations.

literature [26,27]. A meta-analysis based on 14 randomized clinical trials showed a significant difference of only 0.37 μ g/mL in adiponectin concentration after supplementation with fish oil [28]. A previous study supplemented 50 healthy adults, descendants of diabetic parents, with 2 g of ω -3 for 12 mo and observed a 1.7 μ g/mL (22%) increase in adiponectin levels in these individuals [29]. More recently, a study of patients with stable coronary artery disease observed a 13% increase in adiponectin concentration after supplementation with 1 g of ω -3 for

4 wk [30]. In the present study, the ω -3 group showed an increase of 3.4 μ g/mL after 2 mo of intervention, which corresponds to an increase of 23% compared with baseline concentration.

This result may have contributed to the reduction of body fat. Adiponectin activates peroxisome proliferator-activated receptor (PPAR) α , transcription factors that promote the expression of acyl-CoA oxidase and mitochondrial uncoupling proteins (UCP). The acyl-CoA oxidase participates in the oxidation of fatty acids.

Table 5Biochemical profile of ω -3 group, according to period and 75th percentile of serum adiponectin variation (Δ) (São Paulo, 2015)

Variable	Δ (at 2 mo – at baseline) adiponectin <p75		P value	Δ (at 2 mo – at baseline) adiponectin \geq p75		P value
	Baseline	2 mo		Baseline	2 mo	
TG (mg/dL)	149 (89)	116 (64)	0.003	139 (60)	92 (46)	0.005
TC (mg/dL)	202 (33)	176 (33)	<0.001	211 (30)	193 (38)	0.063
LDL (mg/dL)	137 (33)	113 (31)	<0.001	146 (27)	129 (38)	0.099
HDL (mg/dL)	36 (8)	39 (8)	0.008	38 (10)	46 (12)	0.023
TG/HDL	4 (2; 6.5)	3 (2; 4.5)	0.001	3 (2; 7)	2 (1; 3.5)	0.016
TC/HDL	6 (5; 7)	5 (4; 5.5)	<0.001	5 (4.5; 7.5)	4 (3; 5)	0.010
LDL/HDL	4 (3; 5)	3 (2; 4)	<0.001	4 (3; 5)	3 (2; 4)	0.039
apo A1 (mg/dL)	123 (15)	124 (16)	0.744	129 (28)	138 (22)	0.269
apo B (mg/dL)	103 (22)	109 (26)	0.102	96 (23)	103 (21)	0.247
HDL/apo A1	0.3 (0.1)	0.3 (0.1)	0.002	0.3 (0.04)	0.3 (0.1)	0.003
LDL/apo B	1.3 (0.3)	1 (0.2)	<0.001	1.6 (0.4)	1.2 (0.2)	0.046
LDL(–) (U/L)	6 (3.6; 17)	6 (2; 13)	0.003	4 (2.3; 18)	3 (2; 11)	0.051
Anti-LDL(–) (mU/L)	8 (4.1; 12)	8 (4.7; 11)	0.871	11 (2.4; 12)	10 (2.8; 12)	0.374
NEFA (mEq/dL)	0.6 (0.3)	0.5 (0.3)	0.310	0.7 (0.3)	0.6 (0.3)	0.367
Glucose (mg/dL)	97 (92; 110)	100 (92; 106)	0.789*	94 (86.5; 108)	89 (82; 98)	0.050
Insulin (μ U/mL)	18 (6)	18 (5)	0.982	22 (15)	17 (7)	0.336
HOMA-IR	4.4 (1.7)	4.4 (1.5)	0.847	5.1 (3.6)	3.8 (1.7)	0.266

A/L ratio, adiponectin/leptin ratio; apo, apolipoprotein; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance index; LDL, low-density lipoprotein; LDL(–), electronegative LDL; NEFA, nonesterified fatty acids; TC, total cholesterol; TG, triacylglycerols

Values expressed as mean (SD) for variables with parametric distribution, and as median (p25; p75) for variables with non-parametric distribution. Comparison between periods by paired *t* test or Wilcoxon, according to the distribution. Comparison between groups by *t* test for independent samples or Mann–Whitney, according to the distribution. Values with statistical significance between baseline and 2 mo are shown in bold

* *P* < 0.05 comparing people below versus equal or above the 75th percentile of adiponectin concentrations.

The UCP, in turn, promotes oxidative phosphorylation with no formation of adenosine triphosphate, which leads to an energy dissipation as heat, increasing energy expenditure [31,32].

Several mechanisms may be involved in the increase of serum adiponectin in the ω -3 group. First, adiponectin concentration is inversely proportional to the percentage of body fat [2]. Despite the increase in weight, individuals who consumed ω -3 had a reduction in body fat. Thus, the reduction of body fat could lead to increased concentration of adiponectin, and this, in turn, could further influence the reduction of fat mass.

Another potential mechanism for the increase of adiponectin involves the reduction of tumor necrosis factor (TNF)- α and interleukin (IL)-6 [33], by inhibition of I κ B- α . This inhibitor protein is linked to the nuclear factor (NF)- κ B, main factor of inflammation signaling [34]. When the α -I κ B is phosphorylated, it suffers an ubiquitin-dependent proteolysis, turning off of NF- κ B. This factor, then, migrates to the cell nucleus, promoting the transcription of genes whose transcription depends on NF- κ B, such as TNF- α and IL-6. These cytokines suppress the promoter of *ADIPOQ*, the adiponectin gene, inhibiting the expression and secretion of adiponectin [1,33]. As ω -3 prevents I κ B- α phosphorylation [35], NF- κ B does not migrate to the nucleus, which suppress TNF- α and IL-6 synthesis and, consequently, favors the adiponectin synthesis by other ways.

Another mechanism involves the activation of PPAR- γ . The ω -3 is a natural ligand of PPAR- γ [36]. By binding to this receptor, ω -3 disconnects the repressor of PPAR- γ and then a coactivator is connected to the PPAR- γ . This receptor then migrates to the nucleus, promoting the synthesis of target genes, such as *ADIPOQ* [31].

The most described role of ω -3 fatty acids involves the reduction of TG and cholesterol. A major mechanism appears to be associated with the inhibition of maturation of the binding protein sterol regulatory element (SREBP), involved in fatty acid synthesis (SREBP-1 c) and cholesterol (SREBP-2) [37–39]. Our results showed that ω -3 lowered TC, TG, LDL, TG/HDL, TC/HDL, LDL/HDL, LDL/apo B, and LDL(–), and increased HDL and the ratio HDL/apo A1; however, the placebo group showed similar results. The participants in this study had a high prevalence of cardiovascular risk factors, confirmed by the use of drugs and family history of chronic diseases. It is possible that the effects of ω -3 has not been robust enough to overcome the effect of drugs, in particular statin and fibrate used for more than 25% of the total sample.

Almost 90% of the plasma adiponectin concentration may be influenced by genetic factors [40]. Although we did not assess genetic variants as potential determinants of response to supplementation, we evaluated the effect of ω -3 on cardiovascular risk, stratified by adiponectin baseline levels. Participants with lower basal adiponectin concentrations presented greater reduction in TC, LDL, and LDL/HDL and LDL/apo B ratios, whereas those with higher basal concentration of adiponectin further reduced LDL(–). In addition to the ω -3 effect on SREBP, another mechanism that may explain these results involves increasing the serum adiponectin, which in turn activates PPAR α . Activation of this receptor increases the uptake of fatty acids, intracellular esterification, and mitochondrial β -oxidation, which decreases free fatty acids [41].

We also observed significant variation in serum adiponectin. This increase is consistent with the reduction of body fat and improving IR. Studies show that ω -3 and adiponectin have agonist actions, which can strengthen their cardioprotective profile. Additionally, ω -3 and adiponectin act as agonists in the activation of AMP-K and PPAR α , increasing fat oxidation and

glucose utilization, which, added to the decrease in gluconeogenesis, would lead to the higher insulin sensitivity [4,42].

In assessing whether the variation (at 2 mo – at baseline) of adiponectin concentration influenced the response to supplementation with ω -3, we found that those participants who showed greater variation had reduced fasting glucose. In fact, the adiponectin activates AMP-K in skeletal muscle, promoting the translocation of glucose transporter type 4 (GLUT4), which makes the glucose enters the cell. Furthermore, adiponectin inhibits the enzymes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, essential for gluconeogenesis. With higher uptake and lower production of glucose, glycemia is then reduced [31,32].

One limitation of the study is that the placebo group showed higher adiponectin concentration at baseline. However, as we compared the variation of adiponectin concentration for each group, the difference found at baseline concentrations did not influence the comparison. Another limitation is that, although we recommended participants not changing their lifestyle and diet, plasma ω -6 decreased in both groups after intervention. We hypothesize that this reduction was caused by a lower use of oils rich in ω -6 to food preparations. As soy, corn, sunflower, and canola oils are the ones most used by Brazilian people in food recipes prepared at home, these sources of ω -6 could easily be reduced by the participants who felt motivated to improve their diet. The reduction was similar for both groups; however, the placebo group received capsules containing ω -6 and an increase in this fatty acid was expected. So the plasma ω -6 reduction in the placebo group was greater, which could lead to the cardiometabolic profile improvements observed in this group. Both limitations, however, may have underestimated the effect of ω -3.

Conclusion

Adiponectin is shown as one of the mechanisms by which ω -3 improves cardiometabolic profile in persons with cardiovascular risk. Moreover, the benefit varies according to the adiponectin basal level and adiponectin variation after supplementation.

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