Hypocaloric diet associated with the consumption of jam enriched with microencapsulated fish oil decreases insulin resistance

Anna Paula Soares de Oliveira Carvalho¹, Sofia Kimi Uehara², José Firmino Nogueria Netto¹ and Glorimar Rosa¹


Abstract

Background: The metabolic syndrome is related to the increase in cardiovascular diseases. Polyunsaturated fatty acids from fish oil help in reducing cardiovascular risk factors and are natural bindings of PPARγ.

Objective: To evaluate the impact of hypocaloric diet associated with microencapsulated fish oil supplementation in women with metabolic syndrome.

Methods: We conducted a randomized, single-blind and placebo-controlled clinical trial with adult women who presented metabolic syndrome (n = 30) for 90 days. The volunteers were divided into two groups: placebo group (n = 15) and microencapsulated fish oil group (n = 15) (3 g/day of microencapsulated fish oil containing 0.41 g/day of eicosapentaenoic acid and decosahexanoic acid). Anthropometric, body composition, clinical and laboratory parameters were assessed before and after the intervention. Paired t-test was used for comparisons within groups and Student’s t-test for comparison between groups. We considered p < 0.05 as significant values.

Results: The comparison between groups revealed a significant reduction of blood glucose, insulinemia and the homeostasis model assessment in the microencapsulated fish oil group after 90 days, as opposed to the placebo group. We also observed reduction of the systolic arterial pressure in the microencapsulated fish oil group.

Conclusion: A hypocaloric diet associated with the consumption of microencapsulated fish oil was effective in reducing blood glucose, insulinemia and insulin resistance in women with MS.

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Key words: Metabolic syndrome. Fish oil. Insulin resistance. Hypocaloric diet. Microencapsulation.

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Resumen

Antecedentes: El síndrome metabólico se relaciona con un incremento de las enfermedades cardiovasculares. Los ácidos grasos poliinsaturados del aceite de pescado ayudan a reducir los factores de riesgo cardiovascular y son ligandos naturales del PPARγ2.

Objetivo: Evaluar el impacto de la dieta hipocalórica asociada con suplementación de aceite de pescado microencapsulado en mujeres con síndrome metabólico.

Métodos: Realizamos un ensayo clínico de distribución aleatoria, simple ciego y controlado con placebo en mujeres adultas con síndrome metabólico (n = 30) durante 90 días. Se dividió a las voluntarias en dos grupos: el grupo placebo (n = 15) y el grupo con aceite de pescado microencapsulado (n = 15) (3 g/día de aceite de pescado microencapsulado que contiene 0,41 g/día de ácido eicosapentaenoico y de ácido decosahexanoico). Se evaluaron parámetros antropométricos, clínicos y de laboratorio y la composición corporal antes y después de la intervención. Se emplearon la prueba t pareada para las comparaciones dentro de los grupos y la prueba t de Student para la comparación entre grupos. Consideramos valores significativos de p < 0,05.

Resultados: La comparación entre grupos reveló una reducción significativa de la glucosa sanguínea, la insulinemia y la evaluación del modelo homeostático en el grupo de aceite de pescado microencapsulado tras 90 días, en comparación con el grupo placebo. También observamos una reducción de la presión arterial sistólica en el grupo con aceite de pescado microencapsulado.

Conclusión: La dieta hipocalórica asociada con el consumo de aceite de pescado microencapsulado fue eficaz en la reducción de la glucosa sanguínea, la insulinemia y la resistencia a la insulina en mujeres con SM.

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Introduction

Metabolic syndrome (MS) is characterized by a set of cardiovascular risk factors\(^1\) associated with central obesity and insulin resistance (IR) responsible for increased cardiovascular mortality\(^7\). The frequency of MS has been greater in women in Brazil\(^1\) and in countries like China\(^4\) and Spain\(^9\).

N-3 polyunsaturated fatty acids (PUFA) – derived from fish oil (FO), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) – act by increasing insulin sensitivity\(^6\). N-3 PUFA in FO is susceptible to lipid oxidation\(^7\). This characteristic leads to changes in its physical and chemical structure and compromises its sensory characteristics, such as odor and taste\(^6\). Microencapsulation increases the stability of PUFA,\(^7\) in addition to improving its palatability and avoiding the discontinuity of treatments.

Microencapsulation is a procedure that consists in coating of active materials (core) by an encapsulating matrix (water-soluble agents or insoluble in water) with the production of microcapsules whose size can vary from a few nanometers to several micrometers\(^8\). It is a procedure that has been successfully used by cosmetics, pharmaceutical and food industries\(^8\). Microencapsulation can be used in the enrichment of food with FO.

In Brazil, there is no food enriched with microencapsulated fish oil(ME FO). Studies of individuals with MS treated with food fortified with ME FO are nonexistent. Available studies on foods enriched with ME OP are scarce and have been conducted with healthy individuals\(^11\),\(^12\). This fact justifies our study and led to the elaboration of light strawberry jam enriched with ME FO. This study attempted to assess the impact of a hypocaloric diet associated with the consumption of light jam enriched with ME FO on insulin resistance in women with metabolic syndrome.

Patients and Methods

Study group

Adult women (aged 30 to 45 years) diagnosed with MS according to the International Diabetes Federation\(^11\) were recruited through advertisements in newspapers. Exclusion criteria were: allergy to strawberry; pregnancy; lactation; using fatty acid supplements; submission to bariatric surgery; and nutritional or pharmacological treatment for the reduction of body weight during the last three months before the trial.

The volunteers were informed about the research and signed an Informed Consent Form, in accordance with resolution 196/96 of the National Health Council. The study protocol was approved by the Committee of Ethics on Research of the Clementino Fraga Filho University Hospital, Federal University of Rio de Janeiro (181/10 Protocol).

Study design

We conducted a randomized single-blind and placebo-controlled clinical trial. The volunteers (n = 30) were distributed into two groups that received jam enriched with ME FO fortnightly (3 g/day, totaling 0.4 g/day of EPA and DHA) or placebo (jam not enriched) for 90 days. We performed blood collection and assessment of clinical, anthropometric, body composition, biochemical and insulin resistance parameters at the beginning (T0) and the end (T90) of the study.

Nutritional treatment

A hypocaloric diet was prescribed by predicting a body mass reduction of 2 kg/month with an estimated total energy value\(^14,15\). The macronutrient composition was in accordance with the recommendations of the Ministry of Health that suggests a distribution of 50 to 60% of the total energy value (TEV) of carbohydrates, 15% of TEV of proteins or 0.8% - 1 g/weight current/day and 25 to 30% in the form of lipids, in which saturated fatty acids correspond to less than 10% of TEV, polyunsaturated fatty acids up to 10% and monounsaturated fatty acids up to 20%\(^17\). We assessed the adhesion to the eating plan through food records evaluated with the Food Processor software (version 12)\(^12\). During the 90 days of testing, fortnightly consultations, delivery of jams and nutritional follow-ups of the volunteers were conducted.

Microencapsulation, elaboration and enrichment of light strawberry jam with ME FO

We used the complex coacervation method for microencapsulation of FO\(^19\), through which we obtained a paste. Microencapsulation was performed at Mikron Microcapsules Laboratory (São Paulo, Brazil). The percentage of ME FO in the paste was 30%. For the preparation of jam, strawberries purchased on the market, sugar (reduced by 30%), sucralose, pectin and citrus acid were used. The ME FO paste and the jam were mixed (ratio 2:1) manually and packed in food packaging kept under refrigeration until delivery. The placebo was strawberry jam without FO enrichment.

For the calculation of the amount to be provided to volunteers per consultation, we considered 16 days (one extra day as safety margin). The women were guided to consume the jam enriched (30 g/day, totaling 3 g/day of ME FO) at breakfast and keep it stored under refrigeration. The participants were instructed to not washing the pots and returning them fortnightly in order to assess jam leftovers.

Clinical, anthropometric and body composition assessments

Blood pressure (BP) was measured by auscultatory method with the use of aneroid sphygmomanometer.
and stethoscope\textsuperscript{39}. Body mass (kg), height (m)\textsuperscript{2} and waist circumference\textsuperscript{22} were measured and the body mass index (BMI) calculated\textsuperscript{14}. Fat and fat-free masses were assessed by the method of electric bioimpedance (BIA 450 bioimpedance analyzer-Biodynamics Corporation, USA). The volunteers were previously guided to the assessment of body composition\textsuperscript{39}.

**Blood collection and biochemical assessment**

After a 12-hour night fasting, the volunteers were subjected to blood collection using tubes without anticoagulant (Vacutainer tubes, Becton, Dickinson & Company, USA). Aliquots of serum were obtained by centrifugation (4,000 rpm for 15 minutes) and stored at -20 °C until the analysis.

Serum concentrations of triglycerides, total cholesterol and HDL cholesterol were determined by enzymatic-colorimetric method (commercial kit, BioSystems), with the reading performed in an A15 Automated Analyzer (BioSystems). Serum concentrations of LDL cholesterol were calculated using Friedwald’s formula - [LDL - cholesterol = Total Cholesterol - (HDL-Cholesterol + triglyceridemia/5)], only valid for individuals with triglyceridemia < 400 mg/dL\textsuperscript{24}.

Insulinemia was assessed by the radioimmunounoassay method (commercial ImmuChem\textsuperscript{TM} Coated Tube kit of MP Biomedicals\textsuperscript{®}, Insulin, CT) using the 2470 WIZARD Automatic Gamma Counter (Perkin-Elmer Inc.). Insulin resistance was estimated by Homeostasis Model Assessment: Insulin Resistance (HOMA-IR)\textsuperscript{25}.

**Statistical analysis**

The results were expressed as mean ± standard deviation and percentage (%). We used the Student’s \textit{t}-test and the paired \textit{t}-test. We also used the Statistical Package for the Social Sciences (SPSS), version 17 and \textit{p} value < 0.05 was considered significant.

**Results**

Women with MS selected for the study showed grade 1 obesity (32.6 ± 1.8 kg/m\textsuperscript{2})\textsuperscript{14} and their general characteristics can be observed in table I. Adhesion to jam consumption was assessed by observation of leftovers contained in the packs returned at fortnightly consultations. There were no reports of gastrointestinal discomforts.

The average consumption of macronutrients in the groups revealed to be in accordance with the prescriptions; however, the average energy intake was below the prescription (table I). There were no significant differences within and between groups (ME FO and placebo).

Both groups showed significant reduction of body mass, BMI and waist circumference (table II). Placebo

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Prescription</th>
<th>1\textsuperscript{st} Month</th>
<th>2\textsuperscript{nd} Month</th>
<th>3\textsuperscript{rd} Month</th>
<th>1\textsuperscript{st} Month</th>
<th>2\textsuperscript{nd} Month</th>
<th>3\textsuperscript{rd} Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEV (kcal/day)</td>
<td>1,537.5 ± 31.5\textsuperscript{a}</td>
<td>1,533.3 ± 37.4\textsuperscript{b}</td>
<td>1,526.7 ± 30.7</td>
<td>1,529.3 ± 31.3</td>
<td>1,530.3 ± 36.5</td>
<td>1,527.1 ± 42.8</td>
<td>1,524.4 ± 46.6</td>
</tr>
<tr>
<td>Carbohydrates (% TEV)</td>
<td>50-60\textsuperscript{a}</td>
<td>about 50\textsuperscript{b}</td>
<td>53.0 ± 0.5</td>
<td>50.2 ± 0.6</td>
<td>51.2 ± 1.8</td>
<td>51.2 ± 1.9</td>
<td>55.0 ± 1.6</td>
</tr>
<tr>
<td>Proteins (% TEV)</td>
<td>about 15\textsuperscript{a}</td>
<td>22.0 ± 0.8</td>
<td>22.1 ± 0.5</td>
<td>20.3 ± 0.3</td>
<td>21.0 ± 0.6</td>
<td>20.3 ± 0.8</td>
<td>21.5 ± 1.1</td>
</tr>
<tr>
<td>Lipids (% TEV)</td>
<td>25-30\textsuperscript{a}</td>
<td>25.0 ± 0.6</td>
<td>26.2 ± 0.4</td>
<td>28.6 ± 1.7</td>
<td>27.7 ± 1.7</td>
<td>26.9 ± 1.3</td>
<td>26.6 ± 0.7</td>
</tr>
<tr>
<td>SFA (% TEV)</td>
<td>≤ 10\textsuperscript{a}</td>
<td>8.6 ± 0.4</td>
<td>9.8 ± 0.3</td>
<td>10.4 ± 0.0</td>
<td>9.8 ± 1.0</td>
<td>9.0 ± 0.3</td>
<td>9.5 ± 0.5</td>
</tr>
<tr>
<td>MFA (% TEV)</td>
<td>≤ 20\textsuperscript{a}</td>
<td>7.9 ± 0.2</td>
<td>8.0 ± 0.3</td>
<td>8.7 ± 0.6</td>
<td>9.4 ± 0.4</td>
<td>8.2 ± 0.6</td>
<td>8.7 ± 0.2</td>
</tr>
<tr>
<td>PUFA (% TEV)</td>
<td>≤ 10\textsuperscript{a}</td>
<td>4.2 ± 0.3</td>
<td>4.5 ± 0.3</td>
<td>3.8 ± 0.2</td>
<td>4.9 ± 0.2</td>
<td>4.6 ± 0.2</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>TFA (% TEV)</td>
<td>≤ 1\textsuperscript{a}</td>
<td>0.4 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>1.0 ± 0.5</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.05</td>
<td>0.5 ± 0.05</td>
</tr>
<tr>
<td>Cholesterol (mg/day)</td>
<td>&lt; 300\textsuperscript{a}</td>
<td>171.1 ± 17.7</td>
<td>189.0 ± 21.0</td>
<td>207.3 ± 18.9</td>
<td>185.3 ± 24.1</td>
<td>163.4 ± 24.0</td>
<td>194.8 ± 25.6</td>
</tr>
<tr>
<td>Fiber (g/day)</td>
<td>20-30\textsuperscript{a}</td>
<td>16.7 ± 0.8</td>
<td>15.3 ± 0.3</td>
<td>16.5 ± 1.1</td>
<td>17.6 ± 1.6</td>
<td>17.3 ± 2.2</td>
<td>16.4 ± 0.6</td>
</tr>
<tr>
<td>Na (mg/day)</td>
<td>&lt;2400\textsuperscript{a}</td>
<td>1292.7 ± 51.1</td>
<td>1245.6 ± 78.9</td>
<td>1462.1 ± 41.1</td>
<td>1124.4 ± 232.7</td>
<td>1205.3 ± 106.4</td>
<td>1198.8 ± 38.3</td>
</tr>
</tbody>
</table>

TEV: total energetic value; SFA: saturated fatty acids; MFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TFA: trans fatty acids; Na: sodium; \textsuperscript{a}mean ± standard error of TEV prescribed to placebo group and ME FO group, respectively; \textsuperscript{b}1 Brazilian Guideline for Diagnosis and Treatment of Metabolic Syndrome (2005); \textsuperscript{c}World Health Organization (2003).
group showed a significant reduction in body fat. ME FO group found no difference in body composition. The variation of these data did not differ significantly between the groups. ME FO group had a significant reduction in systolic arterial pressure, as opposed to the placebo group (table II).

Blood glucose concentrations, insulinemia and HOMA-IR showed significant reduction in the ME FO group (fig. 1); whereas in the placebo group there was no significant reduction of these parameters (table III).

We did not observe significant differences on the lipid profile between the groups at the end of the study. This shows that the hypocaloric diet was also effective in the improvement of these data (table III).

Discussion

This study found that consumption of jam enriched with 3 g/day of ME FO, containing 0.41 g/day of EPA and DHA, resulted in the reduction of blood glucose, insulinemia and HOMA-IR after a 90-day intervention, as opposed to the hypocaloric diet.

Giacco et al.26 supplemented 3.6 g/day of FO (2.1 g of EPA and 1.5 g of DHA) for 90 days and did not observe any effect of FO on reducing blood glucose. The same result was observed by Brandy et al.27 who used 4 g of FO (2.5 g of EPA and DHA). On the other hand, 3 g/day of FO (180 mg of EPA and 120 mg of DHA) were used in a study carried out by Simon et al.28 and they observed increased blood glucose after 90 days.

These different results in blood glucose can occur due to the profile of subjects studied and the amount of EPA and DHA supplemented. Giacco et al.26 assessed overweight individuals, whereas the volunteers in our study suffered from grade 1 obesity.

The effect of FO in blood glucose can be attributed to the activation of PPARγ2 by EPA and DHA ligands29. PPARγ2 operates in the metabolism of glucose by increasing the expression of genes related to glucose transporter 4 (GLUT4) in the skeletal muscle and has high affinity for insulin, facilitating the absorption of glucose by the cells, thus aiding in reduction in blood glucose30.

Similar to our research, a study carried out by Ramelet al.31 with grade 1 obese patients undergoing a hypocaloric diet associated with 3 g of FO (1.3 g of EPA and DHA) supplementation also found insulinemia reduction after eight weeks. The same result was observed in a study carried out by Krebs et al.32 who supplemented 1 g of FO (1.3 g of EPA and 2.9 g of DHA). On the other hand, Waite et al.33 did not observe change in insulinemia after 60 days.

A study by Simon et al.28 resulted in an increase in HOMA-IR after 90 days of treatment with 3g/day of FO (180 mg of EPA and 120 mg of DHA). Such results are contrary to those found in the literature and our study, probably due to the amount of EPA and DHA supplemented. We can observe that the higher the dose (> 3 g dose) of EPA and DHA supplemented the more deleterious effect on glycemicmetabolism34.

N-3 PUFA can improve IR by several mechanisms, such as reducing the production of pro-inflammatory eicosanoids by inhibiting the phospholipase A2 enzymes35 and increasing the expression of the gene that encodes the adiponectin36, which promotes the oxidation of fatty acids and increases insulin sensitivity in muscles and liver37. This pathway is mediated by receptors of adiponectin leading to activation of protein kinase activated by adenosine monophosphate (AMPK), which is an important modulator of lipid metabolism and glucose. It increases the oxidation of lipids, reducing the concentration of free fatty acids in the plasma and thus improves the absorption of glucose in muscles by increasing their sensitivity to insulin38. In addition, PPARγ directly modulates the signaling pathway of insulin transduction in adipose tissue by increasing the expression of intracellular proteins that stimulate the transport of glucose39.
that could explain the reduction of IR by using FO40.

This is another mechanism related to the IR. This is another mechanism related to the IR. 

The consumption of jan enriched with ME OF seems to promote greater SAP and DAP reduction than a hypocaloric diet. These results are in accordance with those observed in the literature41. It was demonstrated that adiponectin helps in reducing arterial pressure due to its direct action on the vascular endothelium, promoting endothelium-dependent vasodilation41.

We can observe that the activation of PPARγ by n-3 PUFA present in the FO has direct effects on the regulation of the vascular structure and protective effect on the endothelium by inhibiting the production of endothelin 1 (ET-1)42. ET-1 is an important vasoconstrictor peptide secreted by endothelial cells in response to insulin. This way, high concentrations of ET-1 are observed in individuals with IR43.

**Conclusion**

A hypocaloric diet associated with the consumption of jam enriched with ME FO reduced blood glucose, insulinemia and HOMA-IR in women with MS.

**Acknowledgment**

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