Original/Alimentos funcionales

A coconut extra virgin oil-rich diet increases HDL cholesterol and decreases waist circumference and body mass in coronary artery disease patients

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Abstract

Introduction: saturated fat restriction has been recommended for coronary arterial disease, but the role of coconut oil (Cocos nucifera L.) extra virgin, lauric acid source in the management of lipid profile remains unclear.

Objective: to evaluate the effect of nutritional treatment associated with the consumption of extra virgin coconut oil in anthropometric parameters and lipid profile.

Methods: we conducted a longitudinal study of 116 adults of both sexes presenting CAD. Patients were followed in two stages: the first stage (basal-3 months), intensive nutritional treatment. In the second stage (3-6 months), the subjects were divided into two groups: diet group associated with extra virgin coconut oil consumption (GDOC) and diet group (DG). Held monthly anthropometric measurements: body mass, waist circumference (WC), neck circumference (PP), body mass index (BMI). Gauged to collected blood pressure and blood samples were fasted for 12 hours, for total cholesterol analysis (BMI). Se tomó la presión arterial y muestras de sangre fueron divididos en dos grupos: grupo asociado con el consumo de aceite de coco virgen extra en los parámetros antropométricos y el perfil lipídico.

Results: the mean age of the population was 62.4 ± 7.7 years, 63.2% male, 70% elderly, 77.6% infected, 52.6% with angina, hypertension and dyslipidemia 100%. In the first stage the nutritional treatment reduced body weight, WC, BMI and PP and insulin concentrations, HbA1C, HOMA-IR and QUICK, without changing the other parameters. In the second stage of the study, we observed that the GDOC maintained the reduction of body mass, BMI, WC, with a significant difference between groups for DC (-2.1 ± 2.7 cm; p < 0.01).

EL ACEITE DE COCO VIRGEN EXTRA RICO EN ÁCIDOS GRASOS INCREMENTA EL COLESTEROL HDL Y DISMINUYE LA CIRCUNFERENCIA DE LA CINTURA Y LA MASA CORPORAL EN PACIENTES CON ENFERMEDADES DE LA ARTERIA CORONARIA

Resumen

Introducción: el aceite de coco (Cocos nucifera L.) virgen extra contiene una alta proporción de ácidos grasos de cadena media que parecen contribuir a la reducción del peso y podría ayudar en la prevención secundaria de la enfermedad arterial coronaria (EAC).

Objetivo: evaluar el efecto del tratamiento nutricional asociado con el consumo de aceite de coco virgen extra en los parámetros antropométricos y el perfil lipídico.

Métodos: se realizó un estudio longitudinal de 116 adultos de ambos sexos que presentan CAD. Los pacien-
tes fueron seguidos en dos etapas: en la primera etapa (basal-3 meses), se llevo a cabo un tratamiento nutricio-
nal intensivo. En la segunda etapa (3-6 días), los suje-
tos fueron divididos en dos grupos: grupo asociado con el consumo de aceite extra virgen de coco (GDOC) y el grupo de dieta (GD). Se realizaron mediciones mensuales antropométricas: peso, circunferencia de la cintura (CC), circunferencia del cuello (PP) e índice de masa corporal (IMC). Se tomó la presión arterial y muestras de sangre recogidas en ayunas durante 12 horas para el análisis de colesterol total y lipoproteínas, apoproteínas (Apo A-I y B), glucosa, hemoglobina glucosilada (HbA1c) e insulina (I). Se compararon los promedios al principio y al final del estudio mediante el test t de Student-independiente. Se ajustó la presión arterial diastólica por el IMC mediante ANOVA. Los análisis se realizaron con el paquete estadístico SPSS, siendo significativa p < 0.05.

Resultados: la edad media de la población fue de 62.4 ± 7.7 años, el 63.2% hombres, 70% mayores, el 77.6% con infarto de miocardio, el 52.6% con angina de pecho y el 100% con hipertensión arterial y dislipidemia. En la pri-
mera etapa del tratamiento nutricional se redujeron las concentraciones de insulina, peso, WC, IMC y PP, HbA1c, HOMA-IR y rápido, sin cambiar otros parámetros. En la segunda etapa del estudio se observó que la GDOC mantie-
ne la reducción del peso, BMI, WC, con una diferencia sig-
nificativa entre los grupos para DC (-2.1 ± 2.7 cm; p < 0.01).

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In addition, there was an increase in HDL-C concentrations, Apo A, with significant difference in GD, only for HDL-C (3.1 ± 7.4 mg/dL; p = 0.02).

Conclusion: it was observed that the nutritional treatment associated with extra virgin coconut oil consumption reduced the CC and increased HDL-C levels in patients with CAD.

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Key words: Coronary artery disease. Nutritional treatment. Secondary prevention and extra virgin coconut oil.

Methods

Study subjects and design

We conducted a nonrandomized 6-month clinical trial, with 360 patients being initially screened. The study included patients of both genders aged 45-85 years on secondary prevention of CAD (myocardial infarction and/or stable angina), with the use of lipid-lowering drugs for longer than six months, seen at an outpatient department of a specialized cardiology hospital during January-September, 2012. It excluded those who had coronary artery bypass grafting and previous cardiovascular event within less than 6 months, those who had chronic renal failure with creatinine levels greater than 1.2 mg/dL, patients using coconut oil, food supplements, and those who suffered from liver diseases.

From the screened population, 136 patients met the eligibility criteria for the three-month run-in phase in order to homogenize or standardize their food intake. From the third month the allocation was performed for two intervention groups: diet group (DG) (n = 22), who remained only with diet, and another group that besides diet received extra virgin coconut oil (CODG) (n = 92). The study details are better shown in figure 1.

Patients were seen in a monthly basis at the clinical nutrition department of a specialized hospital where they received intensive dietary treatment with periodic phone calls to assess compliance. In addition, all patients were provided with a telephone number to contact to dispel doubts whenever necessary. Socio economic and demographic data, information on past medical history and present illness, previous cardiovascular event within less than 6 months, those who had chronic renal failure with creatinine levels greater than 1.2 mg/dL, patients using coconut oil, food supplements, and those who suffered from liver diseases.

Conclusion: se observó que el tratamiento nutricional asociado con el consumo de aceite de coco virgen extra redujo la CC e incrementó los niveles de HDL-C en pacientes con CAD.

(Palabras clave: Enfermedad coronaria arterial. Tratamiento nutricional. Prevención secundaria y aceite de coco virgen extra.

Introduction

Secondary prevention for patients with coronary artery disease (CAD) aims to avoid new cardiovascular events. The change towards a healthier lifestyle presents a 44% decrease in mortality from CAD. Recent guidelines emphasize the necessity of reducing visceral fat, and controlling blood pressure and dyslipidemia.

The adoption of a dietary pattern based on good sources of mono- and polyunsaturated fat, fiber, fruit, vegetables, whole grains, olive oil and nuts results in the decrease of risk factors for cardiovascular disease. On the other hand, according to the National Health and Nutrition Examination Survey (2007-2011), conducted on 759 individuals with CAD, data showed very low compliance to the nutritional and clinical treatment, with only 20% displaying adequate body weight, and 59% having lipid profile control.

New therapeutic targets are necessary to increase compliance to dietary treatments. Due to this necessity, the effects of functional foods have been studied. Although no consensus exists over the subject, functional foods appear to exert some beneficial action on lipid profile and promote better compliance to dietary treatment.

In this context, extra virgin coconut oil (Cocos nucifera L.), extracted from the fresh coconut pulp, has been acknowledged for its high proportion of medium-chain fatty acids (MCFA), lauric acid (source of vitamin E), and polyphenols with antioxidant activity. The scientific literature has shown benefits of extra virgin coconut oil to the reduction of body fat, and controlling blood pressure and dyslipidemia.

Thus, the aim of this study was to evaluate the effect of a diet rich in coconut oil concerning the improvement of lipid profile and anthropometric measurements.

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Anthropometric measurements, physical activity and blood sampling

The anthropometric measurements body mass (kg) and height (m) were taken using a digital platform scale coupled with a stadiometer (Filizola®). BMI was calculated by dividing body mass (kg) by height (m) squared, classified according to the World Health Organization (WHO). WC was measured at the midpoint between the last rib and the iliac crest. NC was measured with the subject standing with the head positioned in the Frankfort horizontal plane, the upper edge of the tape was placed under the cricoid cartilage and applied perpendicularly around the neck. Blood pressure was measured twice in the right arm by the trained investigator, with a mercury sphygmomanometer and stethoscope after subjects had rested for a minimum of 10 minutes.

Physical activity was considered when patients trained at least once a week. Physical exercise was assessed as metabolic equivalent of task (MET) expressed in kcal/day. Patients were considered sedentary when they did not perform physical exercise, or when they exercised with caloric expenditure below 3 METs and a frequency of less than two times per week. Patients were advised to keep the level of habitual physical activity.

Blood samples were drawn after 12 hours of overnight fasting. The samples were taken in blood collection vacuum tubes containing heparin. The collection tubes were then centrifuged for 15 min at 4°C and 3,000 rpm. TG, TC, and HDL-C and LDL-C were analyzed. Serum levels of ApoA-1 and ApoB were measured by
immunoturbidimetric assay\(^1\). Fasting plasma glucose was measured by the spectrophotometric method using the glucose oxidase/peroxidase. The glycated hemoglobin (HgA1c), by by turbidimetric immunoassay. All analyses were performed at the clinical laboratory of INC (Rio de Janeiro, Brazil) through the automated method (ARCHITECT ci8200, Abbott, Abbott Park, IL, USA) using commercial kits (Abbott ARCHITECT ci8200\(^1\), Abbott Park, IL, USA).

**Diet design and supplementation**

The diet was prescribed during the run-in period according to the dietary habits of volunteers and nutritional recommendations for individuals with dyslipidemia\(^2\). The total energy expenditure was calculated considering the recommendations of the Dietary Reference Intake, 2005\(^3\), and of the National Cholesterol Education Program - Adult Treatment Panel III (NCEP ATPIII) (2002)\(^4\) considering the current BM. At each visit, a 24-hour recall was used to assess patient compliance to the offered nutritional treatment. In order to assess changes in the habitual dietary pattern, baseline 24-hour recalls were compared to those three months after intervention. Data were analyzed using the computer program Food Processor Version 7.2 (Esha Research, Salem, USA, 1998).

The CODG received extra virgin coconut oil in sachets containing 13 mL (30 units per month), totaling 90 sachets per patient. Patients were instructed to consume one sachet per day, alone or added to fruit, without subjecting it to heat.

Coconut oil was donated by COPRA Food Industry, Maceió, AL, Brazil.

The composition of fatty acids of coconut oil was obtained by the Analytical Chemistry Organic Laboratory of Centro de Pesquisas e Desenvolvimento Leopoldo Américo Miguez de Mello (CENPES)/Rio de Janeiro, RJ, Brazil. Vitamin E and phytosterols contents were determined by the Instituto de Tecnologia de Alimentos/Centro de Ciências e Qualidade de Alimentos, Campinas, São Paulo, SP, Brazil (Table I).

**Statistical analysis**

The results were expressed as percentage and mean ± standard deviation (SD). The chi-square test (c² test) was performed to compare categorical variables between groups. Kolmogorov-Smirnov adhesion test was performed.

Paired Student’s t-test or Wilcoxon Signed Ranks was used to assess changes in anthropometric and biochemical variables after the intervention period in each group. While the effect of the intervention groups was evaluated by Student t test independent or Mann-Whitney U test according to the distribution of variables.

The difference between DG and CODG was evaluated by Student’s t-test. Through the analysis of variance for repeated measures, DBP was adjusted for BM, and the development of HDL-C and WC in CODG and DG was evaluated. All analyses were performed using SPSS, version 20.0. When \( p < 0.055 \), the finding was considered statistically significant.

**Results**

One hundred and thirty-six patients were included in the study. Among them, one hundred and fourteen (85.3%) managed to complete the run-in period. The majority of the participants who abandoned the study

<table>
<thead>
<tr>
<th>Table I</th>
<th>Nutrient composition of a serving of coconut oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coconut oil composition(^1)</td>
<td>Composition of fatty acids (%/100g)(^2)</td>
</tr>
<tr>
<td>Energy, kcal/kJ</td>
<td>127/533</td>
</tr>
<tr>
<td>Carbs, g</td>
<td>0</td>
</tr>
<tr>
<td>Protein, g</td>
<td>0</td>
</tr>
<tr>
<td>Total Fat, g</td>
<td>14</td>
</tr>
<tr>
<td>Saturated fat, g</td>
<td>13</td>
</tr>
<tr>
<td>Trans fat, g</td>
<td>0</td>
</tr>
<tr>
<td>Monounsaturated fats, g</td>
<td>0.8</td>
</tr>
<tr>
<td>Polysaturated fats, g</td>
<td>0.2</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>0</td>
</tr>
<tr>
<td>Fiber, mg</td>
<td>0</td>
</tr>
<tr>
<td>Sodium, mg</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^1\)Composition held in 15ml of extra virgin coconut oil = 1 tablespoon. 
\(^2\)Source: Laboratory of Analytical Chemistry Organic Cenpes/Rio de Janeiro, RJ, Brazil. 
\(^3\)Source: Institute of Food Technology, Science Center and Food Quality, Campinas, São Paulo, SP, Brazil Laboratory. 
\(^4\)ND: not detected value; \(^a\): Average and estimated standard deviation; mg/dL: milligram per deciliter; IU: international unit.

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did not return after the baseline visit due to scheduling difficulties (Fig. 1).

The main characteristics of the population are shown in table II; there was no significant difference between the studied groups since the beginning of intervention.

The mean age of the studied population was 62.4 ± 7.7 years, with 70% of elderly individuals, and 63.2% of males. There were 100% hypertensive and 94.5% dyslipidemic patients on regular medication to control these diseases.

During the run-in period, there was significant decrease in body mass (BM), body mass index (BMI), neck circumference (NC), waist circumference (WC) and glycemic profile (data not shown).

Table III shows the effect of an extra virgin coconut oil-rich diet on anthropometric data and on the blood pressure (BP) after three months of intervention.

Data showed that the CODG significantly decreased their BM, BMI, NC, WC, with a statistical difference between the groups for WC (-2.1 ± 2.7; p < 0.01) (Fig. 2A). We also observed the reduction of diastolic blood pressure (DBP) in the CODG; however, after adjustment for BMI, no significant difference was found (data not shown). There was no significant difference in physical activity between both groups at the beginning and during the phases of the study (data not shown).

CODG presented an increase on serum concentrations of HDL-C (CODG: 3.1 ± 7.4 mg/dL; p < 0.01 vs. DG: -1.2 ± 8.5 mg/dL; p = 0.52) and apoprotein A (apoA) (CODG: 4.7 ± 12.7; p = 0.01 vs. DG: -3.9 ± 2.7; p = 0.27).

We notice the effect of a coconut-rich diet on the levels of HDL-C in figure 2B. We may also observe a small increase on the concentrations of apoprotein B (apoB) in the CODG; however with no difference on seric concentrations of low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC).

The dietary assessment, undertaken through the 24-hour recalls, showed decrease in the total energy expenditure (-748.9 ± 1110.6 kcal; p < 0.01), lipids (-4.1 ± 11.4; p < 0.01), saturated fat (-2 ± 5.1%; p < 0.01), cholesterol (-70.9 ± 199.1 mg/dL; p < 0.01) and sodium (-814.5 ± 1583.2 mg/d; p < 0.01), after the run-in period (data not shown). After the run-in period, the CODG presented increased intake of lipids and saturated fatty acids, with reduced carbohydrate intake. There was no modification in the DG group. The statistical analyses showed no difference between the groups.

**Discussion**

The results of this study show that the inclusion of 13 mL of extra virgin coconut oil in a diet increases significantly the HDL-C levels and decreases the WC. Previous studies involving the intake of coconut oil have linked it to the reduction of abdominal fat, as the

<table>
<thead>
<tr>
<th>Table II</th>
<th>Baseline characteristics of the groups CODG and DG1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>CODG (n = 2)</td>
</tr>
<tr>
<td>Age, years</td>
<td>62.5±8.02</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>79.7±15.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.9±5.8</td>
</tr>
<tr>
<td>Inactivity, % (n)</td>
<td>71.1 (66)</td>
</tr>
<tr>
<td>Diabetes mellitus type 2, % (n)</td>
<td>50 (46)</td>
</tr>
<tr>
<td>Hypoglycemic, % (n)</td>
<td>48.9 (45)</td>
</tr>
<tr>
<td>Angina, % (n)</td>
<td>46.7 (43)</td>
</tr>
<tr>
<td>Acute myocardial infarction, % (n)</td>
<td>77.2 (71)</td>
</tr>
<tr>
<td>C-Total, mg/dL</td>
<td>177.5±51.8</td>
</tr>
<tr>
<td>LDL-col, mg/dL</td>
<td>108.3±45.1</td>
</tr>
<tr>
<td>HDL-col, mg/dL</td>
<td>37.5±9.2</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>153.7±71.2</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>129.0±19.0</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>77.8±11.5</td>
</tr>
</tbody>
</table>

Abbreviations: SBP: SBP; DBP: diastolic blood pressure; C-total: total cholesterol; HDL-C: high density lipoproteins cholesterol; LDL-C: low density lipoprotein cholesterol; TG: triglycerides.

1Results are expressed as mean ± SD or percentage.
2T-Student test between CODG and DG.
3Chi-square χ² test between the groups, statistically significant for p < 0.05.
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Another result that draws attention is the reduction of DBP in the group that consumed extra virgin coconut oil. Animal experiments have found the protective effect of coconut oil on blood pressure\textsuperscript{33,34,35}; authors attribute this effect to the presence of polyphenols in the oil.

The current study showed the beneficial effects of an extra virgin coconut oil-rich diet on the significant increase of serum levels of HDL-C (5%; \textit{p} = 0.01) with no change in the levels of TC, LDL-C and triglycerides (TG). Feranil \textit{et al.}\textsuperscript{36} also found positive association between coconut oil intake and the increase of serum levels of HDL-C. Nevertheless, Assunção \textit{et al.},

### Table III

<table>
<thead>
<tr>
<th>Variables</th>
<th>CODG (n = 92)</th>
<th>DG (n = 22)</th>
<th>p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>78.1±5.2</td>
<td>78.5±13.9</td>
<td>-0.6±1.8</td>
<td>-0.4±2.2</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>29.3±5.5</td>
<td>29.3±5.1</td>
<td>-0.2±0.7</td>
<td>-0.1±0.8</td>
</tr>
<tr>
<td>WC, cm</td>
<td>100.1±11.8</td>
<td>100.2±10.7</td>
<td>-2.1±2.7</td>
<td>-0.2±2.6</td>
</tr>
<tr>
<td>NC, cm</td>
<td>38.0±3.8</td>
<td>38.4±3.8</td>
<td>-0.4±0.9</td>
<td>-0.2±0.8</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>129.0±19</td>
<td>128.1±15.9</td>
<td>-3.3±18.2</td>
<td>0.9±13.7</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>77.8±11.5</td>
<td>81.3±9.9</td>
<td>-3.5±13.8</td>
<td>-4.3±10</td>
</tr>
</tbody>
</table>

Abbreviations: BMI: body mass index; NC: neck perimeter; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure.

**Statistically comparing the mean DG and CODG groups (\textit{p} < 0.05).

### Table IV

<table>
<thead>
<tr>
<th>Variables</th>
<th>CODG (n = 92)</th>
<th>DG (n = 22)</th>
<th>p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC, mg/dL</td>
<td>177.4±51.8</td>
<td>176.9±68.6</td>
<td>5.9±35.4</td>
<td>11.2±31.6</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>37.5±9.2</td>
<td>37.6±9.3</td>
<td>3.1±7.4</td>
<td>-1.2±8.5</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>108.3±45.2</td>
<td>114.5±55.6</td>
<td>4.0±31.2</td>
<td>2.6±32.7</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>153.8±71.2</td>
<td>153.0±69.0</td>
<td>-2.0±70.5</td>
<td>23.3±72.4</td>
</tr>
<tr>
<td>ApoA, mg/dL</td>
<td>137.2±18.9</td>
<td>141.8±18.9</td>
<td>4.7±12.7</td>
<td>-3.9±27</td>
</tr>
<tr>
<td>ApoB, mg/dL</td>
<td>94.9±23.4</td>
<td>95.0±21.1</td>
<td>6.4±17.6</td>
<td>7.4±18.1</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>118.0±34.1</td>
<td>116.0±42.7</td>
<td>1.4±23.6</td>
<td>-4.2±25.2</td>
</tr>
<tr>
<td>HgA1, mg/dL</td>
<td>6.2±1.1</td>
<td>6.1±0.7</td>
<td>0.1±0.6</td>
<td>0.1±1.0</td>
</tr>
<tr>
<td>UA, mg/dL</td>
<td>5.8±1.5</td>
<td>6.2±1.5</td>
<td>0.3±1.3</td>
<td>-0.0±1.0</td>
</tr>
</tbody>
</table>

Abbreviations: TC: total cholesterol; HDL-C: high density lipoproteins cholesterol; LDL-C: low density lipoprotein cholesterol; TG: triglycerides; ApoA: apoprotein A; ApoB: Apoprotein B; HbaA1C: glycated hemoglobin; UA: uric acid.

**Statistically comparing the mean DG and CODG groups (\textit{p} < 0.05).

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by comparing refined coconut oil and refined soy oil, did not find any benefit in the lipid profile, and Liau et al. 2011 did not find any effect either. Two other studies with isolated MCT also found no important change in the lipid profile. Experimental studies in which animals were fed diets supplemented with virgin coconut oil showed increased levels of HDL-C and decreased levels of LDL-C, TG and TC. Authors credited the results to the action of polyphenols and vitamin E, present in the virgin coconut oil. Besides, saturated fat is known to have a role in the improvement of HDL-C levels by increasing the activity of lecithin cholesterol acetyltransferase (LCAT). The elevation of HDL-C levels, with no change in LDL-C levels, in our population of chronic CAD patients was highly significant, for evidence point that normal concentrations of HDL-C are associated with minor risk of non-lethal infarct and low concentrations of HDL-C are strong predictors of infarct. And further, that the lower the LDL-col lower the cardiovascular morbidity and mortality.

Dietary interventions that contribute to the increase of HDL concentrations are rare; therefore our findings
were highly significant and unprecedented in this group of patients with chronic coronary disease. The intake of this kind of fat meets strong opposition from people in general, although studies have not proved the association between the intake of saturated fat and cardiovascular disease or CAD. Also, considering a specific population that regularly used this coconut oil, there was no positive association with the onset of cardiovascular disease.

Our study presents some limitations: small sample size in the diet group, absence of randomization when allocating patients to nutritional intervention. However, it is noteworthy that CODG and DG were comparable in relation to anthropometric and biochemical data.

**Conclusion**

Nonpharmacological interventions are essential for risk factor control in secondary prevention among patients with coronary disease. Our study showed that a diet rich in extra virgin coconut oil seems to favor the reduction of WC and the increase of HDL-C concentrations, aiding with secondary prevention for CAD patients.

**Acknowledgment**

This study was funded by Institute of National Cardiology. The authors declare no conflicts of interest.

**Authors’ contributions**

DAC, GMMO, ABM, RRL and GR were responsible for the study conception and design, and the drafting of the manuscript; DAC, GMMO, ABM, RRL and GR participated in the analysis and interpretation of data; GMMO, ABM and GR critically revised the article for intellectual content; RRL developed the statistics; all authors are accountable for the final approval of the manuscript. None of the authors had a conflict of interest.

**References**