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The Effects of a Flavonoid-Rich Diet on Oxidative Stress, Inflammation, and Lipid Profile after Elective Percutaneous Coronary Intervention: A Randomized Clinical Trial

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ABSTRACT: Antioxidant-rich foods may decrease oxidative stress and have a direct impact on atherosclerosis by reducing low-density lipoprotein (LDL) oxidation. Our aim was to assess the impact of a flavonoid-rich diet on oxidative stress, inflammatory response, and lipid profile in patients with coronary artery disease submitted to elective percutaneous coronary intervention (PCI). Thirty-three patients submitted to elective PCI were randomly allocated to follow either a flavonoid rich antioxidant (AOX) diet or a control diet based on National Cholesterol Education Program Adult Treatment Panel III recommendations. Patients were followed for 6 months. Dietary intake was recorded at the start and at the end of the follow-up period, as were oxidative stress markers (ferric reducing ability of plasma and protein sulphydryl) and C-reactive protein (CRP). Patients randomized to follow the AOX diet had a reduction in energy, carbohydrate, and lipid intake, as well as increased flavonoid intake. Compared to the control group, there were no changes in oxidative stress markers or CRP in the patients following the AOX diet, but these patients had a significant decrease in LDL cholesterol levels. In conclusion, the findings of this study suggest that a flavonoid-based antioxidant-rich diet is not associated with reductions in oxidative stress or inflammatory markers 6 months after percutaneous coronary intervention. Nonetheless, patients in the intervention group experienced significant reductions in LDL cholesterol, which may indicate cardiovascular benefits of AOX diets despite of inflammation and oxidative stress markers.

Keywords: flavonoids, oxidative stress, LDL cholesterol, cardiovascular risk

INTRODUCTION

Oxidative stress is a physiological process in cellular metabolism and plays an important role in cell signaling, apoptosis, gene expression, and ion transport (1). However, excessive concentrations of reactive oxygen species (ROS) may have a detrimental effect on a wide range of molecules, including proteins, lipids, RNA, and DNA (2, 3). It also has a central role in atherosclerotic plaque formation and progression, by oxidizing low-density lipoprotein (LDL), increasing interactions between monocytes and endothelial cells and stimulating smooth muscle cell proliferation and growth factor synthesis (4,5).

The intake of certain types of foods can potentially protect against chronic disease. These beneficial effects are believed to be due to antioxidant compounds, such as carotenoids and flavonoids, which may protect key biological sites from oxidative damage. The effect of antioxidant vitamin supplementation has been controversial in observational studies (6-9), and small clinical trials using surrogate endpoints have shown favorable results (10). However, large randomized trials have failed to demonstrate any benefit of antioxidant vitamin supplementation for the prevention of cardiovascular disease (11-14).

Antioxidant (AOX) diets based on flavonoid-rich foods (such as the Mediterranean diet) and moderate red wine intake may be associated with potential cardiovascular benefits (15). However, no randomized clinical trials have demonstrated its beneficial effects in cardiovascular secondary prevention. The objective of the present study is to assess the impact of a flavonoid-rich diet on oxidative stress and markers of inflammation in patients with cardiovascular disease submitted to percutaneous coronary intervention (PCI).

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MATERIALS AND METHODS

Study design and population

This was a randomized clinical trial of patients submitted to elective PCI at the Interventional Cardiology Unit of Hospital de Clinicas de Porto Alegre, a tertiary university hospital in southern Brazil. The study protocol was approved by the local Research Ethics Committee (protocol 06-327).

The study population comprised male and female patients between 18 and 75 years submitted to elective PCI. Patients with acute coronary syndromes, chronic kidney failure, severe congestive heart failure (NYHA functional class III/IV), neoplastic disease, inflammatory connective tissue disease, diabetes mellitus, and pregnant women were excluded.

Trial protocol

PCI was indicated by the attending physician and performed in accordance to routine hospital protocols. All patients received clopidogrel+aspirin before, and heparin (100 IU/kg) during the procedure. All patients received simvastatin 40 mg/d, clopidogrel 75 mg/d, and aspirin 100 mg/d as maintenance therapy. After intervention, patients were informed about the study and were invited to participate. Those who agreed signed an informed consent form and were given further information about follow-up. A 10-mL blood sample was collected by venipuncture and sent to biochemical measures of lipid profile, oxidative stress, and inflammation markers before hospital discharge and in follow-up visits. Follow-up was performed at 3 and 6-months.

Randomization

The first outpatient visit was scheduled 14 days after PCI. This visit consisted of anthropometric assessment, patient allocation, and administration of a 24-h dietary recall. After these procedures, patients were randomly assigned to the intervention or control group according to a computer-generated random number.

Patients in the intervention group were instructed to follow an AOX diet meeting their nutritional needs, whereas patients in the control group received dietary guidance with the objective of maintaining their current nutritional status.

Dietary monitoring

Patients allocated to the intervention group received an energy-adequate diet based on National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III recommendations (16) and rich in antioxidant-containing foods (such as cruciferous vegetables, onions, cherry tomatoes, and grapes), for a recommended daily intake of 40 mg/d antioxidant compounds. Patients allocated to the control group received conventional dietary guidance, also for an energy-adequate diet based on NCEP ATP III recommendations.

The diet was tailored individually to the nutritional needs, socioeconomic status, and dietary habits of each patient. It consisted of 60% carbohydrates (no more than 10% simple carbohydrates), 15% protein, and up to 25% fat (excess saturated fat was to be avoided). The maximum daily cholesterol intake was set at 200 mg, the recommended dietary fiber intake at 20~30 g/d, and patients were encouraged to drink water between meals. Patients were instructed to take five or six meals a day (three main meals and two or three light snacks in between), preferably at home. We provided a list of flavonoid-rich foods, which should be consumed preferably four times a day (Supplementary Table). Patients were also advised on food preparation: vegetables should be consumed raw whenever possible, and fruit should only be consumed raw.

Adherence was assessed in follow-up visits. Information on food intake was collected with a 24-hour dietary recall. Furthermore, patients were instructed to keep a 3-day food diary over the course of two weekdays and one Saturday or Sunday. The utensils provided at the beginning of the study were used for measurement of food portions. Food intake was calculated with the Programa de Apoio à Nutrição da Escola Paulista de Medicina 1.6 software package. Calculation of flavonoid intake was based on the U.S. Department of Agriculture Database for the Flavonoid Content of Selected Foods (17).

Blood measurements

The lipid profile consisted of total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL cholesterol, and triglyceride measurements. Blood samples were collected after fasting for at least 12 h. Total cholesterol, HDL cholesterol, and triglycerides were measured with enzymatic colorimetric assays (ADVIA 1800, Siemens Healthineers, Erlangen, Germany), and LDL cholesterol was calculated using the Friedewald equation (18). C-reactive protein (CRP) was determined using an ultrasensitive turbidimetric method (ADVIA 1800, Siemens Healthcare, Tarrytown, NY, USA). Total protein sulphydryl was obtained with Ellman's reagent, and the results were expressed as nmol-SH/mg protein. Ferric reducing ability of plasma (FRAP) was obtained by comparing the absorbance change at 593 nm in the test reaction mixtures with those containing ferrous ions of known concentrations; results were expressed as mmol/L of plasma.

Statistical analysis

We estimated a sample of 15 patients per group (total 30 patients) to detect a 25% difference in FRAP values between groups at the end of the intervention, considering

an α=0.05 and a power of 80%. Continuous variables with parametric distribution were described as mean± standard deviation and analyzed by the model of generalized estimating equations, over time, for both groups. Categorical data were presented as frequencies and their differences were analyzed by chi-square or Fisher exact test. We used sequential Bonferroni correction to detect differences between groups in variables: total calories, carbohydrates, protein, total fat, and fiber. A *P*-value of <0.05 was considered significant. Statistical analysis was performed using SPSS, version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

From seventy-one consecutive eligible patients, twenty-two were excluded due to impossibility of attending follow-up visits. After forty-nine patients were randomized, sixteen of them have dropped out over the course of the study, and thirty-three patients completed the trial. The mean age was 60 years, sixty-one percent of the patients were female, and the mean BMI was 28 kg/m^2. Table 1 shows the clinical and anthropometric profile of the patients enrolled.

Table 2 shows estimated calorie and macronutrient intake as calculated from 24-h dietary recalls. Patients in the control group had a higher total carbohydrate intake than the AOX group (312 vs. 268 g/dL, *P*=0.047), and tended to have a higher calorie intake as well (2,444 vs. 2,097 cal, *P*=0.088). Analysis of dietary intake showed a significant reduction in total calorie intake (*P*=0.002), total protein, and total fat intake in the AOX diet group over the 6-month follow-up period. Compared to baseline, fiber intake increased significantly in the AOX group at the end of the follow-up period (*P*=0.045) (Fig. 1).

Regarding the lipid profile, significant reductions in LDL cholesterol were observed in the AOX group, as well as a trend toward lower total cholesterol and higher HDL cholesterol levels (Fig. 2). Fig. 3 shows the impact of the AOX diet on inflammatory and oxidative stress markers. There were no significant differences between the groups in CRP, sulphydryl, or FRAP levels at any point during the follow-up period.

Table 1. Distribution of clinical variables in the control and intervention (AOX) groups

Variable	Control diet (17 patients)	AOX diet (16 patients)	*P*
Female sex	11 (64.7%)	9 (56.3%)	0.619
Age (years)	58.5±9.2	58.9±7.9	0.893
Weight (kg)	77.5±15.8	73.3±14.0	0.422
BMI (kg/m^2)	28.8±6.1	28.0±3.3	0.611
Waist circumference (cm)	103.5±11.2	98.7±10.4	0.215
Hypertension	17 (100%)	16 (100%)	1.000
Smoking, current	6 (35.3%)	2 (12.5%)	0.225
Smoking, past	11 (64.7%)	10 (62.5%)	1.000
Alcohol intake	4 (23.5%)	2 (12.5%)	0.656
Physical activity >1 h/w	3 (17.6%)	5 (31.3%)	0.523
Aspirin use	17 (100%)	16 (100%)	1.000
Clopidogrel use	13 (76.5%)	11 (68.8%)	0.619
Statin use	17 (100%)	16 (100%)	1.000
Beta-blocker use	11 (64.7%)	11 (68.8%)	1.000
Calcium channel blocker use	3 (17.6%)	5 (31.3%)	0.438
ACE inhibitor or ARB use	9 (52.9%)	9 (56.3%)	1.000
Cholesterol, total (mg/dL)	186.5±65.4	175.0±33.0	0.525
Cholesterol, HDL (mg/dL)	41.4±11.2	46.6±15.7	0.285
Cholesterol, LDL (mg/dL)	119.5±55.4	105.0±29.9	0.354
Triglycerides (mg/dL)	156.5±53.5	118.1±44.9	0.033
CRP (mg/L)	4.39±2.09	3.23±2.62	0.167
Sulfhydryl (nmol-SH/mg)	3.54±0.96	3.47±1.10	0.855
FRAP (mmol/L)	943.7±238.6	912.1±243.8	0.736

Results are expressed in n (%) or mean±standard deviation. AOX diet, antioxidant diet; BMI, body mass index; AAS, acetylsalicylic acid; ACE, angiotensin-converting enzyme inhibitors; AT2, receptor antagonist of angiotensin II; HDL, high density lipoprotein; LDL, low density lipoprotein; CRP, C-reactive protein; FRAP, ferric reducing ability of plasma.

DISCUSSION

The findings of this study suggest that a flavonoid-based antioxidant-rich diet is not associated with reductions in

Table 2. Baseline and 6 months daily dietary intake

Baseline			
Nutrient	Control diet	AOX diet	*P*
Calories (total cal)	2,442±149	2,097±112	0.088
Carbohydrates (g/kg)	312±16	268±12	0.047
Protein (g/kg)	119±10	97±8	0.166
Total fat (g/kg)	84±7	74±6	0.325
Saturated fat (g/kg)	9.6±2.8	10.1±3	0.607
Polyunsaturated fat	7.6±1.3	7.6±2.6	0.964
Monounsaturated fat	10.2±1.9	11.1±2.8	0.258
Cholesterol (g/kg)	295±170	234±139	0.270
Flavonoids (g/kg)	27.7±5.3	30.3±9.9	0.354
Dietary fiber (g/kg)	30±2	24±2	0.026
6 Months			
Nutrient	Control diet	AOX diet	*P*
Calories (cal)	2,223±128	1,885±60	0.022
Carbohydrates (g/dL)	296±17	267±8	0.494
Protein (g/dL)	106±7	85±4	0.052
Total fat (g/dL)	71±5	56±4	0.077
Saturated fat (g/dL)	8.4±2.4	6.9±2.5	0.089
Polyunsaturated fat	7.8±1.6	8.4±1.4	0.255
Monounsaturated fat	9.5±1.7	8.8±2.3	0.341
Cholesterol (g/dL)	226±90	168±80	0.060
Flavonoids (g/dL)	27.1±5.3	31.1±8.9	0.140
Dietary fiber (g/dL)	27±2	32±5	0.814

Results are presented as mean±standard deviation. AOX diet, antioxidant diet.

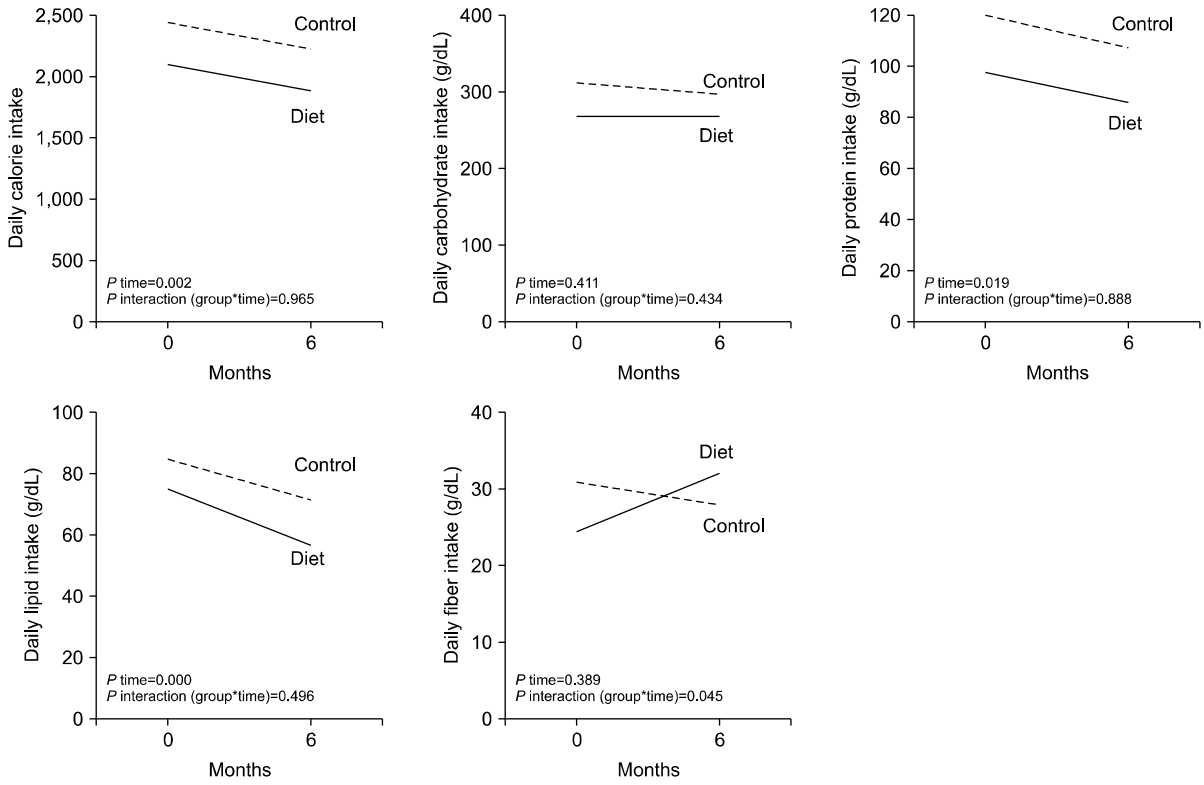

Fig. 1. Average daily intake of calories, carbohydrates, protein, total fat, and dietary fiber between the antioxidant (AOX) and control diets.

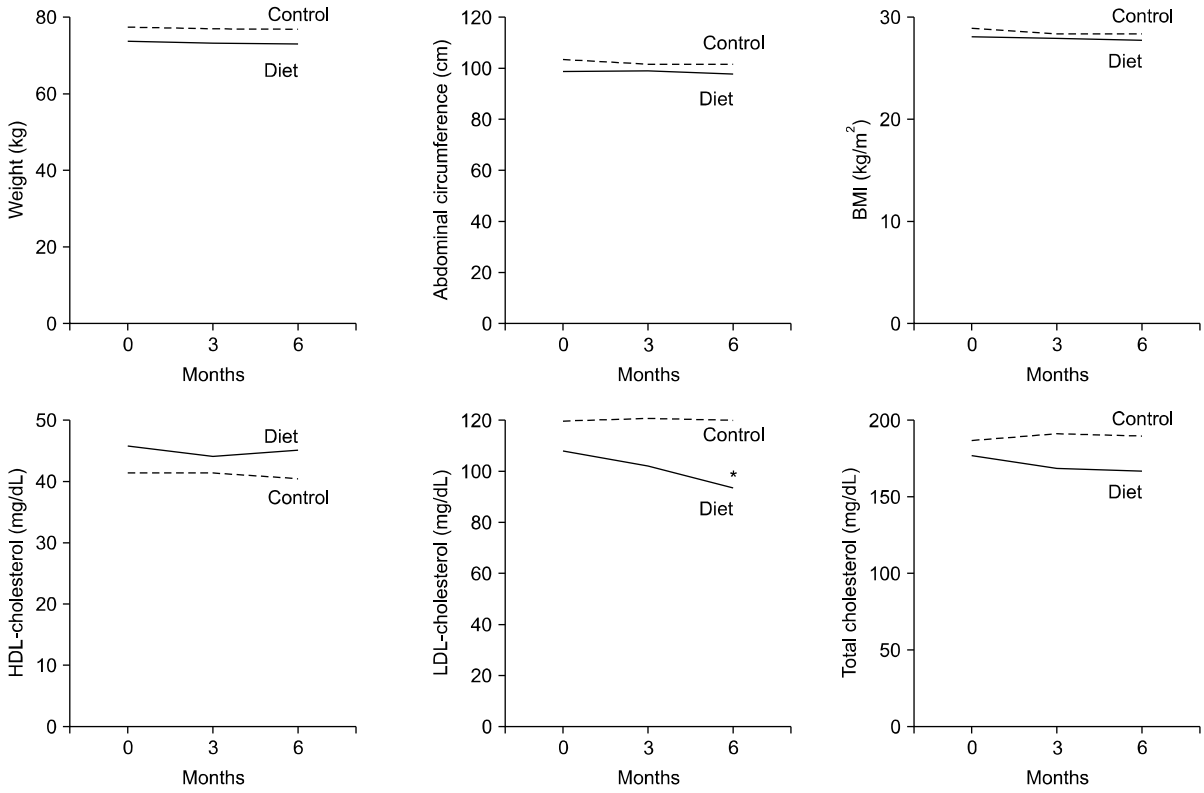

Fig. 2. Effect of diet on anthropometric and metabolic variables during follow-up. A solid line represents patients in the AOX group and the dotted line represents the control group. Group AOX: antioxidant diet. BMI, body mass index (weight/height^2); HDL, high density lipoprotein; LDL, low density lipoprotein. Time P=0.016, P interaction (group×time)=0.046. Other differences P>0.05.

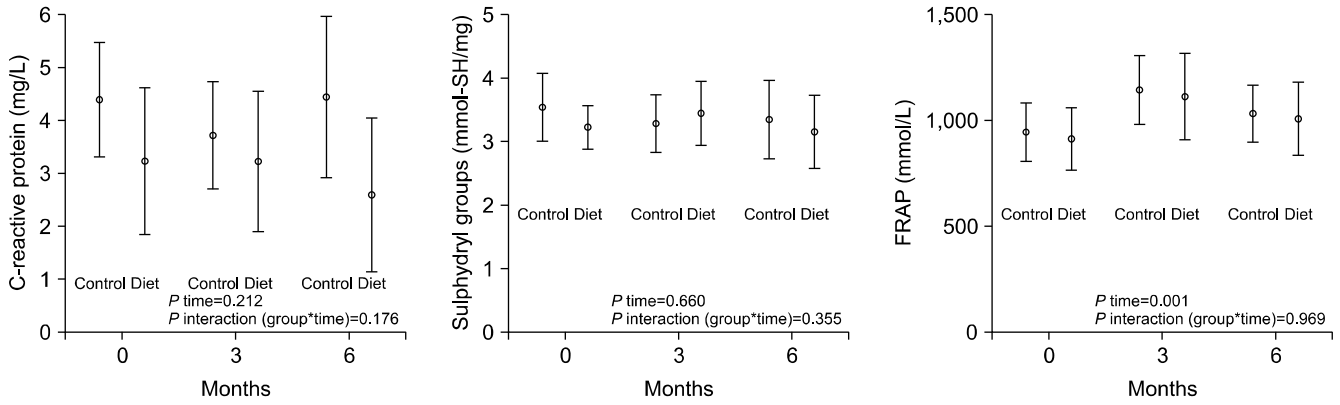

Fig. 3. Effect of diet on levels of C-reactive protein (CRP), sulphydryl, and FRAP at baseline and follow-up in both groups.

oxidative stress or inflammatory markers six months after PCI. However, patients receiving the AOX diet showed improvements in lipid profile, with significant reductions in LDL cholesterol. To the best of our knowledge, this was the first study to investigate the effects of such diet in this type of population.

Increasing the intake of fruit, vegetables and nuts, while concomitantly limiting the intake of sodium, dairy and animal protein, is associated with reductions in cardiovascular events, as shown in studies comparing different types of diets such as Dietary Approaches to Stop Hypertension-DASH (19) and Mediterranean (15). However, these studies did not assess the role of oxidative stress in cardiovascular risk reduction in patients following theses diets. Our findings suggest that the potential cardiovascular benefits of these diets may not be the result of a reduction in oxidative stress.

In the present study, most patients were unable to reach the recommended flavonoid intake levels, which may have been responsible for the lack of elevation in plasma antioxidant levels. However, the patients who successfully adhered to our recommendations exhibited higher antioxidant profiles, far above the AOX group average. It is widely known that implementation of dietary recommendations and lifestyle changes in daily practice is extremely challenging. Even without the ideal adherence, patients in the AOX diet group experienced reductions in LDL cholesterol levels, which demonstrates the potential benefit of this diet to mitigate other risk factors.

Inflammation plays a central role in atherosclerosis, and increased levels of CRP are an independent risk factor for cardiovascular disease and a marker of chronic inflammation (20). CRP metabolism can be modulated by antioxidants, and whole dietary antioxidants probably play a greater role than any single antioxidant compound (such as vitamins C or E). Alizadeh et al. (21) conducted a randomized trial and compared an antioxidant-rich diet and L-arginine+selenium supplementation in premenopausal women with central obesity. After a 6-week follow-up, only patients on a vegetable-enriched diet exhibited reductions in CRP levels. Szeto et al. (22) found that vegetarian patients had lower CRP levels compared to non-vegetarian subjects, suggesting that long-term adherence to an antioxidant-rich vegetarian diet may have anti-inflammatory effects.

The effect of different types of diet in cardiovascular mortality is somehow unpredictable, due to highly variable foods and nutrient types and habits across different global regions. While a recent metanalysis of 15 randomized clinical trials suggested a small but potentially important reduction in cardiovascular risk on reduction of saturated fat intake (23), a large prospective cohort study from 18 countries in five continents (24) showed that fats including saturated and unsaturated fatty acids were associated with lower risk of total mortality and stroke. The authors did not find any detrimental effect of fat intakes on cardiovascular disease events.

There is a direct relationship between high LDL levels and recurrent coronary events in patients with established CAD, and reducing LDL have been proven to reduce cardiovascular outcomes (25,26). Reducing LDL levels may be achieved with both pharmacological and dietary strategies; the latter include reduction of lipid-rich food intake and increased intake of fruit, vegetables, and dietary fiber (27). Our study showed a significant reduction in LDL cholesterol levels among patients in the AOX diet group compared to the control, which corroborates the findings of other studies regarding the beneficial effects of this diet in cardiovascular protection.

Our study has some limitations. First, the limited sample size and a considerable amount of losses after randomization may have biased our results. However, we have reached the estimated sample size in this single center randomized clinical trial, which demands great patient availability. Second, the low adherence to the AOX diet may have led to the negative results regarding oxidative stress and inflammation markers. However, our trial protocol with dietary guidance was provided within the framework of regular clinical follow-ups, showing a real-life situation rather than in a research setting. On

the other hand, the fact that the control group also experienced decreased intakes of calories, protein and lipids during the study period shows that every intervention (i.e. outpatient visit, phone calls) leads to a behavioral change, which was a conservative bias in our trial. Another limitation was the relatively short follow-up (6 months), as it is widely known that changing dietary habits is challenging and patients may need more time to adapt. Therefore, a longer follow-up period might have shown different results.

In conclusion, our results suggest that adherence to a diet rich in flavonoid-based antioxidants is not associated with a reduction in inflammation and oxidative stress levels in patients submitted to PCI. Nonetheless, patients in the intervention group experienced significant reductions in LDL cholesterol, which may indicate that the cardiovascular benefits of antioxidant-rich diets may not be related to inflammation and oxidative stress. Further studies with larger a sample size and longer follow-up periods are encouraged to assess the real impact of vegetable antioxidant intake on cardiovascular outcomes.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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