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Original

Systemic inflammation and carotid diameter in obese patients: pilot comparative study with flaxseed powder and cassava powder

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Abstract

Background: Botanical omega-3 fatty acid (alpha-linolenic acid/ALA) has been shown to alleviate the prothrombotic and proinflammatory profile of metabolic syndrome , however clinical protocols are still scarce. Aiming to focus an obese population, a pilot study was designed.

Methods: Morbidly obese candidates for bariatric surgery (n = 29, age 46.3 ± 5.2 years), 82.8% females (24/29), BMI 44.9 ± 5.2 kg/m², with C-reactive protein/CRP > 5 mg/L were recruited. Twenty were randomized and after exclusions, 16 were available for analysis. Flaxseed powder (60 g/day, 10 g ALA) and isocaloric roasted cassava powder (60 g/day, fat-free) were administered in a double-blind routine for 12 weeks.

Results: During flaxseed consumption neutrophil count decreased and fibrinogen, complement C4, prothrombin time and carotid diameter remained stable, whereas placebo (cassava powder) was associated with further elevation of those measurements.

Conclusions: Inflammatory and coagulatory markers tended to exhibit a better outlook in the flaxseed group. Also large-artery diameter stabilized whereas further increase was noticed in controls. These findings raise the hypothesis of a less deleterious cardiovascular course in seriously obese subjects receiving a flaxseed supplement.

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INFLAMACIÓN SISTÉMICA Y DIÁMETRO DE LA CARÓTIDA EN PACIENTES OBESOS: ESTUDIO COMPARATIVO PILOTO CON HARINA DE LINAZA Y CASAVA

Resumen

Introducción: Los ácidos grasos botánicos omega-3 (ácido alfa-linolénico/ALA) se mostran convenientes para atenuar las cáracterísticas protrombóticas y proinflamatórias del síndrome metabólico, pero protocolos clínicos todavía son escasos. Con el objetivo de enfocar una población obesa, un estúdio clínico fue dibujado.

Métodos: Candidatos a cirugía bariátrica morbidamente obesos (n = 29, edad 46,3 ± 5,2 años), 82,8% mujeres (24/29), IMC 44,9 ± 5,2 kg/m², con proteína C reactiva/CRP > 5 mg/L fueron recrutados. Veinte fueron randomizados y após exclusiones, 16 se pudieron analisar. Harina de linaza (60 g/día, 10 g ALA) y harina de casava tostada isocalórica (60 g/día, sin grasas) fueron suministradas em uma rutina doble-ciega por 12 semanas.

Resultados: Mientras se recibió linaza los neutrófilos bajaron y fibrinógeno, complemento C4, tiempo de protrombina y diámetro de la carótida permanecieron estables. El uso de casava se asoció con elevación de todas estas variables.

Conclusiones: Los marcadores inflamatórios y coagulatórios tuvieron un curso mas favorable con la linaza. Asimismo el diámetro arterial se quedó estable, en cámbio hubo peora en el segundo grupo. La hipótesis de una evolucíon cardiovascular menos deletérea en obesos graves recibiendo casava se plantea en estas circunstáncias.

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Palabras clave: *Obesidad. Cirugía bariátrica. Bypass gástrico en Y-de-Roux. Inflamación sistémica. Riesgo cardiovascular. Casava. Ácido alfa-linolénico. Arteria carótida. Ácidos grasos omega-3.*

Introduction

Increased BMI is associated with a proinflammatory and prothrombotic phenotype . Adipokine overexpression and increased acute-phase proteins and cells are further correlated with atherosclerosis, endothelial dysfunction, increased blood viscosity, insulin resistance and other stigmata of the metabolic syndrome cluster.[1,2]

Virtually all mono-and poly-unsaturated lipids have been assayed with regard to inflammation and atherosclerosis , nominally marine omega-3 preparations, for which meta-analyses are available,[3] botanical oils containing omega-3 alpha-linolenic acid and rarely stearidonic acid, gamma-linolenic acid (omega-6), and also olive oil (omega-9).[4,5] Even saturated medium-chain triglycerides could be promising as they are preferentially oxidized by the organism and in experimental models promote weight loss.[6]

By the same token vegetarian diets are well regarded in the prevention of cardiocirculatory diseases, and multiple vegetable foods, fiber supplements, drinks or extracts have been administered to this aim. Among the antioxidants the best known are flavonoids, a family of over 4,000 molecules including such components as quercetin, cathechins and anthocyanins.[7] Other antioxidants and soy protein in various guises[8] could be worthwhile as well to inhibit insulin resistance , proinflammatory state and metabolic syndrome. Complex mixtures partially mimicking the Mediterranean diet, reinforced from the point of view of fruit and vegetables, chocolate, almonds, fish, wine and garlic, should also be mentioned here.[9]

Studies with flaxseed powder *(Linus usitatissimum)* are less common than with fish oil but with roughly parallel results.[1,10,11] To the best of our knowledge it has never been tested for prolonged use in severe obesity , despite the fact that it is inexpensive and readily available in many parts of the world.

Noninvasive large-artery measurements are highly predictive of cardiovascular morbidity and mortality, more than serum markers only, but benefit of diets or supplements on such profile has been rarely addressed, and to our knowledge never with flaxseed.[3,5]

Aiming to ascertain the impact of that seed during a 3-month period, after encouraging results with two-week supplementation,[1] a prospective double-blind pilot study was performed.

The hypothesis was that encouraging short-term antithrombotic and anti-inflammatory changes[1] would be confirmed, and eventually expanded by favorable trends in large-artery measurements. Duration of 12 weeks was considered adequate, as it has been successfully employed in previous flaxseed studies.[10,11]

For control participants cassava powder *(Manihot esculenta)* was selected because it is fat-free. Nevertheless, it is worth mentioning that an experimental study[12] with a resistant fraction of cassava carbohydrate, namely retrograde tapioca starch, elicited a hypocholesterolemic response.

Methods

Study population

Patients with morbid obesity applying for prebariatric evaluation were prospectively screened.

Criteria for inclusion encompassed those of elective bariatric surgery, namely active males or females 18-65 years-old with BMI > 40 kg/m^2 , or > 35 kg/m^2 with comorbidities, plus C-reactive protein (CRP) > 5 mg/L.

Criteria for exclusion were alcohol or drug addiction, fever or infectious foci, cancer with or without chemo/radiotherapy, chronic inflammatory illness (arthritis, enteritis or colitis), transplantation or immunologic disease, trauma, surgery or hospitalization in the last 30 days, organ failures, use of steroidal or non-steroidal anti-inflammatory or immune-modulating agents or antibiotics, use of any nutritional supplement, myocardial infarction, stroke, surgery for central or peripheral arteriopathy, intolerance of the dietary supplement, and refusal to participate in the protocol.

Randomization

This was a single-phase, parallel-arms, double-blind placebo-controlled study. Subjects recruited from the Outpatient Obesity Service who agreed to participate, and who fulfilled inclusion and exclusion criteria, were consecutively randomized to Group I (Flaxseed) and Group II (Controls).

Sample size calculation

A sample size of 9 per group was calculated by means of the Altman nomogram,[13] based on an effect size of a 5% reduction in WBC count,[1], with a type I error of .05 and 80% power, and an assumed correlation of 0.80 between measurement points.

Dietetic routine

Patients received either flaxseed powder (Farinha de linhaca dourada Linolive, CISBRA, Brazil, 60 g/day, 232 kcal), containing 10 g of alpha-linolenic acid – ALA, or isocaloric fat-free roasted cassava flour (Farinha de mandioca torrada Yoki, Brazil, 60 g/day, 228 kcal) for 12 weeks. The two products were packaged in similar-coded 15 g bags, and four units were taken daily.

A lot for six weeks (168 bags, 2,520 g) was prepared for each subject. Patients were requested to bring back the empty packages of used powder, which were carefully checked before redemption of the next batch (mid-study visit) or closure of the supplementation file (last visit). They were further monitored by weekly telephone calls.

Definitions and medications

Metabolic syndrome was established according to NCEP criteria;[14] Insulin resistance (HOMA index) was calculated according to Matthews et al.[15] Diagnosis of comorbidities was based on current treatment. Clinical records and medical prescriptions were carefully checked, and new patients were routinely referred to the appropriate outpatient services for review of diagnosis and management.

General features and laboratory assays

Variables included demographic information, arterial blood pressure, serum albumin, blood glucose, hemoglobin and general hematologic counts, lipid fractions, leptin, insulin, CRP, serum-amyloid A, prothrombin time, fibrinogen, D-dimer, and C3/ C4 complement fractions.

Blood samples were collected in the morning subsequent to overnight fast. Lipid fractions as well as general biochemical variables were measured by a modular analytical system (Roche/Hitachi Diagnostics, Sao Paulo, Brazil). Leptin was estimated by enzyme immunoassay (ELISA) using the Linco kit (Linco Research, St Charles, MI, USA) and insulin concentration was measured with the Immulite kit (DPC, Los Angeles, CA, USA).

Blood cells were documented in a Coulter automatic counter (Beckman Coulter , Hialeah, FL, USA). The intra- and interassay coefficients of variation did not exceed 3%-6% for all laboratory methods.

Large-artery assessment

Common carotid artery intima-media thickness (IMT), diameter (DIAM) and distensibility (DIST), as well as carotid-femoral pulse-wave velocity (PWV) were documented. Carotid-femoral PWV was measured by applanation tonometry to quantify arterial stiffness, by means of accurate positioning of the transducers on the corresponding arteries. The Complior automatic device was employed (Colson, Garges les Gonesses, France).

IMT, carotid diameter and distensibility were registered with a high-definition echo-tracking apparatus (Wall Track System, Medical Systems, Arnhem, The Netherlands), coupled with a conventional, two-dimensional vascular echograph (Sigma 44 Kontrom Instruments, Watford, UK). These measurements were made on the right common carotid artery 1 cm below the bifurcation at the site of the distal wall. Ethical considerations.

Informed consent was given for the study, and this protocol was approved by the Internal Review Board of Hospital das Clinicas (Cappesq).

Statistical analysis

Findings are presented as percentage or mean ± SEM (quantitative results), or as percentage of affected patients (comorbidities and assorted clinical features) . For comparison of qualitative findings (gender, comorbidities) Fisher exact test was selected whereas numerical results were analyzed by ANOVA or Kruskall-Wallis test depending on normal distribution. Analysis of table III and IV was also done employing repeated-measures analysis of variance (ANOVA) to ascertain supplement and/or time interaction. When there was a significant finding, one-way ANOVA was followed by Newman-Keuls multiple-comparison test to identify such a difference.

SPSS for Windows, SPSS Inc., Chicago, Ill., USA, version 14.0 was utilized. P value of less than 0.05 was considered significant.

Results

Among 29 enrolled subjects, 20 agreed to receive a powder supplement during 12 weeks, and were thus randomized to Group I (Flaxseed, n = 11) and Group II (Cassava, n = 9). One patient in each group were excluded because of poor compliance, therefore analyzed subjects were 18 (10 in Group I and 8 in Group II).

Composition of the supplements is shown in table I. Both were isocaloric and included reasonable amounts of fiber, mostly insoluble.

Subjects were well randomized regarding demographics, comorbidities and habits, with no statistical differences, as pointed out in table II.

General profile of the population can be appreciated in table III.

Groups were fairly comparable, with the exception of glucose and insulin, which were numerically higher in Group I, and D-dimer, which tended to be more elevated in Controls. These differences were not statistically significant.

Body weight remained stable throughout the protocol. Flaxseed consumption was associated with reduced neutrophil count and stable fibrinogen, complement C4 and prothrombin time, whereas control

Table I
*Nutritional composition of the supplements (60 g)**

Variable	*Flaxseed*	*Cassava*
Energy (kcal/kJ)	232/974	228/957
Carbohydrate (g)	7.2	56
Protein (g)	10.4	1.1
Lipids(g)	18.0	0
Saturated fat (g)	2.0	0
Polyunsaturated fat (g)	12.6**	0
Monounsaturated fat (g)	3.4	0
Fiber (g)***	16.4****	9.6

*Manufacturer's information.
**Alpha-linolenic acid 10 g.
***Includes poorly absorbed carbohydrates.
****Total lignans 32 mg.

Table II
Clinical features of the populations

Variable	*Flaxseed*	*Cassava*
Age (years)	47.8 ± 8.0	50.7 ± 6.4
Diabetes mellitus	20.0% (2/10)	25.0% (2/8)
Dyslipidemia	50.0% (5/10)	50.0% (4/8)
Arterial hypertension	80.0% (8/10)	62.5% (5/8)
Metabolic syndrome	40.0% (4/10)	50.0% (4/8)
HOMA index > 2	10% (1/10)	12.5% (1/8)
Drugs for diabetes/insulin resistance	40% (4/10)	37.5% (3/8)
Previous smokers	30.0% (3/10)	37.5% (3/8)
Occasional angina	10.0% (1/10)	25.0% (2/8)
Intermittent claudication	0% (0/10)	12.5% (1/8)

Obs: None of the differences reached statistical significance.

patients suffered elevation of those prothrombotic and proinflammatory markers. Also D-dimer tended to diminish in the Flaxseed group. As known, fibrinogen, prothrombin time and D-dimer reflect coagulation and fibrinolysis activity, complement C4 is associated with atherosclerosis and cardiovascular risk, and neutrophil count is listed among proinflammatory indices.

In contrast , Total Cholesterol and VLDL diminished in cassava patients only. None of the preparations prevented increase of complement C3, another indicator of the deteriorating cardiovascular environment in this vulnerable population.

Repeated -measures ANOVA detected group interactions for BMI, VLDL, Triacylglycerol (Tri), C4 - complement and Fibrinogen. As shown, all of these except BMI had exhibited differences in the univariate test. No interaction affected differences for Neutrophils and D-dimer.

Likewise, advantage for Prothrombin time activity in Flaxseed Group was conspicuous (P = 0.05), as well as a trend toward greater reduction of Total cholesterol (P = 0.06) in Cassava Group.

Vascular measurements revealed that use of flaxseed was associated with large-artery stability, as in the absence of blood pressure changes, carotid diameter remained unchanged. In the Control group (table IV), which was slightly more hypertensive and with a tendency toward higher initial diameter, additional deterioration was unveiled.

Table III
Nutritional findings and general laboratory assays

Variable	*Flaxseed baseline*	*Flaxseed final*	*Cassava baseline*	*Cassava final*
BMI (kg/m^2)	44.0 ± 3.9	44.8 ± 3.6	45.2 ± 4.2	44.4 ± 4.4
TChol mg/dL	197.2 ± 55.7	179.4 ± 40.9	194.5 ± 17.4	163.3 ± 28.0*
HDL mg/dL	50.9 ± 10.6	47.9 ± 10.6	54.2 ± 16.3	48.7 ± 17.8
LDL mg/dL	122.3 ± 44.4	106.6 ± 28.2	110.0 ± 26.2	94.5 ± 16.3
VLDL mg/dL	25.8 ± 14.5	26.6 ± 14.8	30.3 ± 10.9	20.5 ± 6.2*
Tri mg/dL	124.4 ± 69.4	124.9 ± 74.5	152.5 ± 53.7	102.5 ± 31.7*
WBC /mm^3	7,224 ± 2,499	6,511 ± 2,344	7,317 ± 1,725	7,862 ± 3,353
Neutr /mm^3	4,050 ± 1,456	3,500 ± 1,149	4,150 ± 1,249	4,850 ± 2,988#
Hemogl g/L	130.0 ± 14	132 ± 16	142 ± 19	142 ± 16
Albumin g/L	44 ± 2	44 ± 4	44 ± 3	44 ± 3
CRP mg/L	12.9 ± 7.2	11.1 ± 8.7	10.5 ± 5.5	19.1 ± 15.3
SAA mg/L	14.3 ± 13.6	12.0 ± 10.4	12.4 ± 17.5	21.2 ± 16.9
C3 g/L	0.148 ± 0.024	0.156 ± 0.025*	0.154 ± 0.025	0.160 ± 0.022*
C4 g/L	0.033 ± 0.012	0.032 ± 0.010	0.026 ± 0.008	0.031 ± 0.007*
Gluc mg/dL	98.3 ± 12.9	100.3 ± 15.5	124.2 ± 57.3	118.0 ± 43.4
Insulin uU/ml	19.1 ± 11.3	21.5 ± 17.2	28.5 ± 29.3	24.1 ± 16.5
Leptin ng/ml	44.4 ± 20.2	57.2 ± 32.6	27.6 ± 18.8	31.9 ± 19.2
Proth activ %	105.7 ± 2.4	105.8 ± 4.4	106.0 ± 1.1	110.0 ± 0.0*
Fibrin mg/dL	462.6 ± 97.4	441.5 ± 93.8	401.7 ± 48.6	508.2 ± 114.5*
DD ng/mL	629.4 ± 405.3	461.1 ± 214.4$	431.7 ± 422.1	571.8 ± 456.7

Obs: TChol: Total cholesterol; HDL: HDL-cholesterol; LDL: LDL-cholesterol; VLDL: VLDL-cholesterol; Tri: Triacylglycerol; WBC: White blood cell count; Neutr: Neutrophil count; Hemogl: Hemoglobin; CRP: C-reactive protein; SAA: Serum amyloid A; C3: Complement fraction C3; C4: Complement fraction C4; Proth: Prothrombin time % activity; Fibrin: Fibrinogen; DD: D-dimer, measured as Fibrinogen Equivalent Units.
*Significant difference, final versus baseline; TChol: P = 0.046 ; VLDL: P = 0.035; Trig: P = 0.030; C3 : P = 0.030 (Flaxseed group); P = 0.059 (Cassava group). C4: P = 0.035; Prothr: P = 0.000; Fibrin: P = 0.012.
#Significant difference, final result between groups, with similar initial findings; Neutr: P = 0.045.
$Trend (0.10 > P > 0.05); DD: P = 0.082.

Table IV
Arterial measurements (common right carotid artery)

Variable	*Flaxseed baseline*	*Flaxseed final*	*Cassava baseline*	*Cassava final*
Systolic pressure	126 ± 10	126 ± 10	138 ± 25	140 ± 10
Diastolic pressure	79 ± 3	82 ± 7	92 ± 16	86 ± 10
PWV (m/s)	9.8 ± 1.7	10.1 ± 1.3	10.7 ± 2.4	11.4 ± 2.6
IMT (mm)	0.654 ± 134	0.736 ± 68	0.744 ± 205	0.758 ± 92
Diameter (mm)	696 ± 90	661 ± 49	747 ± 105	771 ± 112*
Dist (1/mmHg 10^2)	5.2 ± 1.5	4.3 ± 1.5	4.8 ± 2.3	4.5 ± 2.8

Obs: Blood pressure in mmHg; PWV: Carotid-femoral pulse wave velocity; IMT: Intima-media thickness; Dist: Distensibility.
*Significant increase, final versus baseline; Diameter: P = 0.022.

Increased arterial diameter is accepted as a result of maladaptive arterial remodeling . Group interaction was not demonstrated for Diameter, whereas a trend favoring Flaxseed Group continued to appear (P = 0.022 in univariate analysis, P = 0.095 in Multiple ANOVA).

Discussion

Flax is known for 8,000 years, but its economic importance stemmed from the weaving of linen clothes and the manufacturing of paint and varnish with the extracted oil. Flaxseed meal has traditionally been used for animal feed only, but since the 50's some nutritional preparations for children and adults started to include modest amounts of linseed (flaxseed) oil, which was well tolerated.[16]

As early as 1964 Owren et al recommended alpha-linolenic acid for the prevention of thrombosis and myocardial infarction.[17] Yet, only in the last two decades did flaxseed receive serious attention as an alternative to fish oil. Fish stocks are rapidly diminishing worldwide, especially wild, non-farmed fish, at the same time as obesity and metabolic syndrome are reaching the highest ranks of Public Health concerns. It is appropriate to recall that human genome developed at times during which high quantities of omega-3 fatty acids of plant origin, not marine food, were part of daily diet.

For many years ALA elongation and desaturation was considered erratic and negligible. At least three enzymatic reactions are necessary to generate biologically-active EPA, and conversion rate has been suggested to be as low as 5%,[18] although more substantial proportions usually occur.[1,4,10,11,19]

Various factors tend to interfere with such outcome. Vegetarians achieve notably improved plasma n3-n6 ratios, probably because of liver enzymatic induction, and women also excel in the conversion, in comparison with men. With a diet rich in omega-6 fatty acids synthesis of EPA from ALA is negatively affected, because all long-chain polyunsaturates compete for the same enzymes.[1,4,10,11,18,19]

All these concerns notwithstanding, prescriptions of just 2.4 or 3.6 g flaxseed oil during 12 weeks were sufficient to increase ALA, EPA and DHA in erythrocytes, but without impact on inflammatory markers.[20] The useful range in populations with elevated CRP should probably be around 5 g/day, as employed in a previous investigation,[1] up to 10 g/day as here prescribed. Nevertheless, as much as 30g/day of ALA (60 g of flaxseed oil) during 12 weeks was well tolerated in adults, and indeed permitted highly meaningful changes in plasma fatty acids. Even though results with fish oil at the same dose were more dramatic, EPA was enhanced by 66%.[19]

Carotid diameter in this series increased in controls but not in subjects, even though blood pressure remained stable (P = 0.022 in univariate analysis, P = 0.095 in Multiple ANOVA). This outcome is not unexpected, in the light of experimental benefits of flaxseed for endothelial function and vascular physiology.[21]

Flaxseed lignans could also underlie part of the improvement through their role on inflammation and oxidative stress.[22]

Positive response of total cholesterol occurred in the control group. In human volunteers neither whole-root cassava[23] nor cassava fiber (tapioca starch)[24] influence plasma lipids, at least in a mixed diet . However experimental response with a resistant starch fraction is available.[2]

One weakness of this study is the lack of a placebo group . Such a population was omitted because severe obesity is a well-documented disease, with progressive deterioration of all metabolic and cardiovascular markers in the absence of effective treatment. The authors are experienced with bariatric candidates in the waiting line, which in a public hospital may reach 1-2 years. Incidentally, mortality within such short period may be as high as 3-5%.[25]

Currently there is no dearth of nutritional alternatives to modulate inflammation, coagulation and atherosclerosis in subjects with moderate or high risk for cardiovascular disease. What is still missing is an effective and palatable supplement tailored for daily use by large populations, because wholesale changes in diet and lifestyle by subjects displaying obesity and metabolic syndrome, although imperative, are unrealistic in the short term.

Pharmacologic options are available but entail more substantial costs and adverse effects, especially for long-term use, whereas bariatric surgery is highly effective for appropriate patients but is not a mass-product either. Flaxseed powder and also cassava powder are potential candidates deserving closer scrutiny.

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Disclosure Statement

No competing financial interests exist.

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