Clinica Chimica Acta 461 (2016) 110–113

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Reduced levels of potential circulating biomarkers of cardiovascular diseases in apparently healthy vegetarian men

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ARTICLE INFO

Article history:
Received 7 June 2016
Received in revised form 28 July 2016
Accepted 1 August 2016
Available online 2 August 2016

Keywords:
Vegetarians
MPO
MMP-9
MMP-2
TIMP
Cardiovascular biomarkers
Omnivorous

ABSTRACT

Background: Several evidences report that a vegetarian diet is protector against cardiovascular diseases. Few studies have demonstrated the circulating profile of cardiovascular biomarkers in vegetarians. Therefore, the aims of the current study were compared the plasma concentrations of myeloperoxidase (MPO), metalloproteinase (MMP)-9, MMP-2, tissue inhibitor of MMP (TIMP)-1 and TIMP-2 between healthy vegetarian (Veg) and healthy omnivorous (Omn).

Methods: Using ELISA and multiplexed bead immunoassay, we measured in plasma from 43 Veg and 41 Omn the cardiovascular biomarkers concentrations cited above.

Results: We found significant lower concentrations of MPO, MMP-9, MMP-2 and MMP-9/TIMP-1 ratio in Veg compared to Omn (all $P < 0.05$). Moreover, MMP-9 concentrations were correlated positively with leukocytes and neutrophils count in both groups (all $P < 0.05$).

Conclusion: A vegetarian diet is associated with a healthier profile of cardiovascular biomarkers compared to omnivorous.

1. Introduction

Evidences that consumption of vegetarian diet is protective against cardiovascular diseases (CVDs) are growing. As reviewed by Ashen, vegetarians have lower concentrations of systolic and diastolic blood pressure (SBP and DBP), better insulin sensitivity and blood lipids profile in addition to present lower incidence of ischemic heart disease when compared to omnivorous [1]. Some circulating biomarkers such as C-reactive protein (CRP) and serum lipids are very important to assess and predict the development of many CVDs in healthy and non-healthy population. However, there are only few studies which have evaluated concentrations of circulating CVDs biomarkers in vegetarians [2–4]. Many evidences support the idea that there is an increasing linkage between the high incidence of neutrophils-derived inflammatory markers after their infiltration into the tissues and the concentrations and activity of matrix metalloproteinases (MMPs).

MMPs are a family of structurally related, zinc-dependent proteases that degrade many extracellular matrix components. Among all MMPs, the gelatinases MMP-2 and −9 are the main contributors to the cardiovascular maladaptation associated to aneurysm and hypertension. Increased concentrations of circulating MMPs were also associated with an increase risk to develop atherosclerotic lesions in subjects without any apparent disease [5,6] and associated with coronary artery atherosclerotic disease [7]. It indicates that increased circulating MMPs may be a predictor to develop cardiovascular disorders in healthy subjects. Also, increased MMP-2 activity contributes to hypertension-induced cardiovascular remodeling and dysfunction, and treatment with MMP inhibitors prevented these alterations [8]. Therefore, as chronic cardiovascular remodeling results in fatal events as heart failure and stroke, MMP-2 is essential to be determined as a potential biomarker. Both MMP-2 and MMP-9 are regulated by gene transcription, posttranslational modifications and by the interaction with endogenous tissue inhibitors of MMPs (TIMPs) [9,10], in which TIMP-1 inhibits more MMP-9 while TIMP-2 more MMP-2.

Another important regulator of gelatinases activity that contributes to increase its activity in cardiovascular diseases is oxidative stress. The myeloperoxidase (MPO) is a pro-oxidant enzyme which generates reactive oxygen species (ROS) to defend against infections. Increased concentrations of ROS cause endothelial dysfunction and arterial remodeling and these occurs as anion superoxide reacts with nitric oxide, thus diminishing its bioavailability [11,12]. Increaseed MPO concentrations are found in plasma and plaques of patients with cardiovascular disease [13,14] and are related to atherosclerosis progression in

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http://dx.doi.org/10.1016/j.cca.2016.08.002
0009-8981/

diabetic patients and as a future predictor of coronary artery disease in healthy individuals [14,15].

Despite of epidemiology data showing that vegetarian present lower risk of CVDs, the MMP and TIMP concentrations and also MPO were never studied in this community. As MMPs are essential to contribute to arterial remodeling which precede the development of many cardiovascular diseases, our hypothesis is that vegetarians would have lower concentrations of MMP-2, MMP-9 and MPO when compared to an omnivorous diet.

2. Material and methods

2.1. Subjects and biomarkers assessment

In this observational cross-sectional CARVOS (Carotid atherosclerosis and arterial stiffness in vegetarians and omnivorous subjects) Study, at first 745 adults were recruited in Sao Paulo, Brazil through social activities and internet. From these, 416 volunteers were women, which were not included here. The 329 volunteer men filled out questionnaires regarding past medical history, dietary preferences, family history, physical activity, educational concentration and personal data. Exclusion criteria and respective subjects excluded were: 1) history of diabetes ($n = 9$); 2) history of dyslipidemia ($n = 30$); 3) history of cardiovascular or cerebrovascular diseases ($n = 4$); 4) history of hypertension or intake of antihypertensive medication ($n = 19$); 5) smoking ($n = 21$) and 6) age < 35 y ($n = 36$).

Participants were subgrouped into 2 groups according to the dietary patterns: vegetarians and omnivorous. It also excluded vegetarian <3 years ($n = 21$) and omnivorous who eat meat <4 times per week ($n = 34$). Vegetarian was defined as having exclusive consumption of a vegetarian diet void of meat, fish, and poultry for at least 4 years and are lacto-ovo-vegetarians, lacto-vegetarians or vegans. Matched omnivorous men were considered as consuming any type of meat at ≥5 servings per week. During June 2013 to January 2014, after applying inclusion and exclusion criterions, 155 apparently healthy men were recruited to the study, although 67 declined to participate in this phase and 4 were excluded because the lack of plasma samples to perform assays. Therefore, 43 vegetarians and 41 omnivorous were studied in the current work.

The study protocol was approved by the research committee and the institutional review board of the Heart Institute. All participants provided informed consent to participate in the study. Blood samples were collected after a 10–12 h fasting. At the time of clinic attendance, venous blood samples were collected in EDTA tubes, immediately centrifuged and plasma samples were stored at −80 °C until used to measure biomarkers.

Plasma concentrations of MPO were measured using enzyme linked immunosorbent assay (R&D Systems, Inc., Minneapolis, MN). Plasma concentrations of MMP-9, MMP-2 (R&D Systems) and TIMP-1 and TIMP-2 (EMD Millipore, Billerica, MA, USA) were determined using a multiplexed bead immunoassay and measured with a Luminex MAGPIX instrument (Luminex) as following the manufacturer instructions.

2.2. Statistical analysis

Unpaired Student's *t*-test (or Mann Whitney *U* test) were used for testing differences for numerical variables. Pearson's (or Spearman's) correlation coefficients were used to study the relationship between the biomarkers and the clinical and biochemical parameters. A $P < 0.05$ was considered statistically significant.

3. Results

Table 1 shows clinical and biochemical characteristics of vegetarian and omnivore groups. Higher concentrations of BMI, waist/hip ratio, SBP, DBP, TC, LDL-C, TG, ApoB, fasting glucose, HbA1c, leukocytes and neutrophils counts were found in omnivores compared to vegetarian (all $P < 0.05$). Importantly, plasma concentrations of MPO, MMP-9, MMP-2 and MMP-9/TIMP-1 ratio are lowers in vegetarian compared to omnivorous ($P < 0.05$).

Table 2 shows correlations among biomarkers and clinical parameters. Regarding MMP-2 none correlations were found. We found strong positive correlations between the plasma MMP-9 concentrations and SBP, DBP (0.37, $P < 0.05$ and 0.53, $P < 0.05$ respectively) and TC (0.43; $P < 0.05$) in vegetarians. Absence of correlations were found among

Table 1
Clinical parameters in vegetarian and omnivorous subjects.

	Omnivorous	Vegetarians	*t-test* (*P* value)
Age (years)	46.5 ± 9.4	45.0 ± 7.3	NS
BMI (kg/m^2)	27.4 ± 4.8	23.1 ± 3	<0.01*
Waist/hip ratio	0.92 ± 0.1	0.87 ± 0.1	<0.01*
SBP (mmHg)	129.1 ± 15.1	119.3 ± 10.4	<0.01*
DBP(mmHg)	83.6 ± 10.3	75.7 ± 8.7	<0.01*
TC (mg/dl)	201.8 ± 34.3	178.5 ± 39.4	<0.01*
HDL-c (mg/dl)	45.3 ± 11.9	47.7 ± 9.7	NS
LDL-c (mg/dl)	128.1 ± 32.6	108.5 ± 32.0	<0.01*
TG (mg/dl)	142.3 ± 62.4	109.9 ± 71.5	<0.01*
ApoB (g/l)	1.0 ± 0.2	0.9 ± 0.3	0.02*
Fasting Glucose (mg/dl)	102.9 ± 13.3	94.8 ± 7.2	<0.01*
HbA1c (%)	5.5 ± 0.4	5.3 ± 0.3	0.02*
Hb (g/dl)	15.6 ± 0.8	15.7 ± 1.1	NS
T3 (ng/dl)	1.2 ± 0.2	1.2 ± 0.2	NS
T4 (ng/dl)	8.2 ± 1.5	8.3 ± 1.5	NS
Leukocytes (x10^3 cells/mm^3)	6.9 ± 1.9	5.9 ± 1.0	0.02*
Neutrophils (x10^{3}cells/mm^3)	4.4 ± 1.5	3.8 ± 0.8	0.02*
Biomarkers			
MPO (ng/mL)	23.1 ± 7.3	19.1 ± 5.3	<0.01*
MMP-9 (ng/mL)	249.6 ± 159.1	186.1 ± 122.0	0.04*
MMP-2 (ng/mL)	303.6 ± 120.8	249.9 ± 93.7	0.03*
TIMP-1 (ng/mL)	295.4 ± 49.7	310.3 ± 57.7	0.21
TIMP-2 (ng/mL)	131.9 ± 36.5	142.2 ± 45.5	0.26
MMP-9/TIMP-1 ratio	0.9 ± 0.6	0.5 ± 0.3	<0.01*
MMP-2/TIMP-2 ratio	2.4 ± 1.2	1.9 ± 1.0	0.11

SBP – systolic blood pressure; DBP – diastolic blood pressure; MPO – myeloperoxidase; MMP – metalloproteinase; TIMP –tissue inhibitor of metalloproteinase.
* $P < 0.05$ compared to omnivorous.

Table 2
Correlations among biomarkers and clinical/biochemical parameters in vegetarian and omnivorous.

		MPO (ng/mL) (r; *P-value*)	MMP-9 (ng/mL) (r; *p-value*)	MMP-2 (ng/mL) (r; *P-value*)
BMI (kg/m^2)	Omn	0.00; NS	0.09; NS	0.12; NS
	Veg	−0.05; NS	0.31; NS	−0.02; NS
Waist/hip ratio	Omn	0.04; NS	0.19; NS	−0.01; NS
	Veg	0.06; NS	0.21; 0.17	−0.03; NS
SBP (mm Hg)	Omn	0.31; NS	0.17; NS	0.10; NS
	Veg	0.14; NS	0.37; 0.02*	0.05; NS
DBP (mm Hg)	Omn	0.16; NS	0.00; NS	0.04; NS
	Veg	0.19; NS	0.53; 0.00*	−0.10; NS
TC (mg/dl)	Omn	0.29; NS	0.01; NS	0.03; NS
	Veg	0.30; NS	0.43; 0.00*	−0.22; NS
LDL-c (mg/dl)	Omn	0.26; NS	-0.02; NS	0.04; NS
	Veg	0.19; NS	0.32; NS	0.07; NS
TG (mg/dl)	Omn	0.19; NS	0.00; NS	−0.06; NS
	Veg	0.35; NS	0.30; NS	−0.05; NS
Fasting Glucose (mg/dl)	Omn	0.07; NS	-0.06; NS	0.19; NS
	Veg	0.11; NS	0.30; NS	−0.14; NS
Leukocyte (x10^3 cells/mm^3)	Omn	0.19; NS	0.39; 0.01*	−0.33; NS
	Veg	0.42; 0.00*	0.56; 0.00*	−0.18; NS
Neutrophils (x10^3 cells/mm^3)	Omn	0.28; 0.09	0.46; 0.00*	−0.35; NS
	Veg	0.28; 0.08	0.64; 0.00*	−0.24; NS

Omn – omnivorous; Veg – vegetarian; SBP – systolic blood pressure; DBP – diastolic blood pressure; MMP – metalloproteinase.
* $P < 0.05$.

biomarkers and BMI, waist-hip ratio, LDL-C, TG and fasting glucose in vegetarian and omnivorous (all $P > 0.05$). As the main source of MMP-9 and MPO is the leukocytes, we correlated biomarkers concentrations with leukocytes and neutrophils. Importantly, strong positive correlations (all $P < 0.05$) were found between leukocyte/neutrophils and MMP-9 both in vegetarians and omnivorous. Moreover in vegetarians positive correlation was found between leukocytes and MPO.

4. Discussion

Weshowed that vegetarian diet was associated to lower concentrations of MPO, MMP-9, MMP-2 and MMP-9/TIMP-1 ratio when compared to omnivorous in apparently healthy subjects. The lower concentrations of MMP-9/TIMP-1 ratio and MPO may be related to higher intake of vegetables and fruits (plant-based antioxidants) [5] and with the reduced number of leukocytes and neutrophils in vegetarian in comparison to omnivorous, since these are the major sources of MMP-9 and MPO. Higher concentrations of leukocytes may implicate in more incidence of metabolic syndrome and diabetes [16], which are associated with increased MMP activity and cardiovascular dysfunction and remodeling. Interestingly, corroborating with our results in omnivorous group, Snitker et al. [17] reported a significant and positive correlation between leukocyte and MMP-9 concentrations in a healthy population, suggesting that leukocytes (mainly neutrophils) are an apparent source of MMP-9.

The lower concentrations of MMP-2 in plasma of vegetarians may contribute to protect them to develop many cardiovascular diseases, in which MMP-2 concentrations in plasma and vascular tissues are increased. Increased MMP-2 activity contributes to maladaptive cardiovascular remodeling by degrading many extra- and intracellular proteins, and these effects may precede the development of cardiovascular diseases. Lower MMP-2 concentrations in vegetarians may be related to higher consume of vegetables and fruits, which are very rich in polyphenols. In fact, many vegetables as kale and broccolis, and fruits, such as apple and grape, contain quercetin. Studies have shown that treatment with quercetin in rodents ameliorates reperfusion-induced cardiac dysfunction and prevents the development of aneurysm, by reducing ROS and increased MMP-2 expression and activity [18,19].

Furthermore, diet-induced atherosclerosis significantly contributes to an inflammation process in the vasculature, with neutrophils infiltration, which then release both MPO and MMP-9. MMP-9 in turn contributes to the proteolysis of several extracellular matrix components, such as arterial collagen and elastin. This proteolytic effect of MMP-9 results in chronic vascular remodeling and plaque rupture, which may precede several other cardiovascular diseases such as stroke and myocardial infarction [20].

Although this study presents limitations about its cross-sectional design, that may limit the causal inference, and the relative restricted number of individuals, it is interesting to observe that the concentrations of MMP-9, MMP-2, MMP-9/TIMP-1 ratio and MPO follow the same reduced patterns of other cardiovascular biomarkers already seen in the vegetarian studies.

5. Conclusion

A vegetarian diet is associated with a healthier profile of cardiovascular biomarkers when compared to omnivorous diet even in apparently healthy men. As consequence, vegetarians may present reduced risks to develop cardiovascular diseases. The implications of this study may orientate primary prevention of cardiovascular disease with great impact in term of public health economy and life quality as suggested by Benzie and collaborators [21].

Acknowledgements

This study was funded by the Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq), and the Fundação o de Amparo a Pesquisa do Estado de São Paulo (FAPESP-Brazil).

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