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Arginine intake is associated with oxidative stress in a general population

Aline Martins de Carvalho M.Sc. [a], Antonio Anax Falcão de Oliveira B.Sc. [b], Ana Paula de Melo Loureiro Ph.D. [b], Gilka Jorge Figaro Gattás M.D., Ph.D. [c], Regina Mara Fisberg Ph.D. [a], Dirce Maria Marchioni Ph.D. [a,*]

[a] Department of Nutrition, School of Public Health, University of Sao Paulo, Sao Paulo, Brazil
[b] Department of Clinical and Toxicologic Analyses, Faculty of Pharmaceutical Sciences, University of São Paulo, Sao Paulo, Brazil
[c] Department of Legal Medicine, Bioethics and Occupational Health, Medical School, University of São Paulo, Sao Paulo, Brazil

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ABSTRACT

Objective: The aim of this study was to assess the association between protein and arginine from meat intake and oxidative stress in a general population.
Methods: Data came from the Health Survey for Sao Paulo (ISA-Capital), a cross-sectional population-based study in Brazil (N = 549 adults). Food intake was estimated by a 24-h dietary recall. Oxidative stress was estimated by malondialdehyde (MDA) concentration in plasma. Analyses were performed using general linear regression models adjusted for some genetic, lifestyle, and biochemical confounders.
Results: MDA levels were associated with meat intake (P for linear trend = 0.031), protein from meat (P for linear trend = 0.006), and arginine from meat (P for linear trend = 0.044) after adjustments for confounders: age, sex, body mass index, smoking, physical activity, intake of fruit and vegetables, energy and heterocyclic amines, C-reactive protein levels, and polymorphisms in *GSTM1* (glutathione S-transferase Mu 1) and *GSTT1* (glutathione S-transferase theta 1) genes. Results were not significant for total protein and protein from vegetable intake ($P > 0.05$).
Conclusions: High protein and arginine from meat intake were associated with oxidative stress independently of genetic, lifestyle, and biochemical confounders in a population-based study. Our results suggested a novel link between high protein/arginine intake and oxidative stress, which is a major cause of age-related diseases.

Introduction

Meat intake is especially high in developed and developing countries such as Brazil, where ~75% of the population consumes more than the daily meat recommendation [1].

High meat intake has been related to increased risk for chronic diseases, for example, cancer and cardiovascular diseases [2–4]. A possible explanation is that reactive oxygen species (ROS) might be formed after meat intake during its metabolism [5]. Furthermore, high-protein intake can cause oxidative stress, increasing risk for chronic diseases [6,7].

Accordingly, we hypothesized that amino acids present in meat in high amounts. Arginine (conditionally essential amino acid), is found in concentrations >1 g/100 g of meat and might be related to oxidative stress [8]. Arginine is a precursor of nitric oxide ($NO^{\bullet}$), a key signaling molecule, but high output of this amino acid can stimulate superoxide radical generation, leading to a state of oxidative stress [9]. On the contrary, high intake of antioxidants from fruits and vegetables, and the presence of detoxification enzymes, for instance, glutathione-*S*-transferases (GST), may provide protection against damage induced by excessive ROS production [10,11].

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* Corresponding author. Tel.: +55 11 3061 7856; fax: +55 11 3061 7856.
E-mail address: marchioni@usp.br (D. M. Marchioni).

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Although meat intake has been related to chronic diseases, to our knowledge, this is the first study to assess an association between protein and arginine and oxidative stress. In the present study, we tested this association in a general population, independently of the intake of energy, fruit and vegetables, polymorphisms in genes that codify GST enzymes, and other confounders.

Material and methods

Study population and data collection

Data came from the Health Survey of Sao Paulo (ISA–Capital), a cross-sectional population-based study, conducted in 2008–2009 in Sao Paulo, an important financial center in Latin America with ~11 million inhabitants as of 2008, with a multistage probability sample based on census tracts and households.

In 2008, the survey collected information on lifestyle and health at participants' homes from 2691 adolescents, adults, and older adults. After a few months, additional information on diet was collected from 1662 participants. One year later, new dietetic and sociodemographic information and blood samples drawn at participants' homes were collected from 750 participants. The loss of the study participants occurred due to refusal to participate, change in address/telephone, not being at home on multiple visits at different times during the week and on weekends, and repeated unanswered telephone contact. Of note, the loss of study participants was randomized among census tracts and sociodemographic features. All adolescents were excluded from the present study due to their age. Consequently, we used data only from adults (N = 549) who had genotyped DNA.

An assistant nurse measured body weight and height using a calibrated scale and portable stadiometer using the standardized protocol.

The School of Public Health Ethics Committee at University of São Paulo approved the project (approval number 275/09). Informed consent was obtained from all participants.

Assessment of dietary intake

The dietary intake was estimated by a 24-h dietary recall, administered by telephone using the Automated Multiple Pass method.

Each participant completed a detailed questionnaire containing information regarding their cooking preference for meat (pan-fried, grilled, boiled, baked, microwaved) and for levels of meat doneness (rare, medium, well done, very well done for beef; and medium, well-done, very well done for poultry and pork) to estimate the heterocyclic amine intake using the Computerized Heterocyclic Amines Resource for Research in Epidemiology of Disease database. Heterocyclic amine intake was estimated to adjust the final models.

Biochemical markers

Blood samples were obtained from venipuncture by an assistant nurse after a 12-h overnight fast. Samples were kept in a polystyrene box with ice packs and transported to the laboratory for centrifugation for 15 min at room temperature. After that, plasma samples were aliquoted and frozen at −80°C until analysis.

Based on a previous study, the concentration of malondialdehyde (MDA), a biomarker of oxidative stress, was measured from plasma after derivatization with thiobarbituric acid and quantification by high-performance liquid chromatography/diode array [12].

High-sensitivity C-reactive protein concentration (CRP) was measured by IMMAGE® assay (Beckman Coulter, Inc. Fullerton, CA, USA, cat no 474630). CRP was used as a covariate to minimize bias in the final models because it is a marker of inflammation and risk factor of chronic diseases, which could influence oxidative stress.

DNA extraction, SNP selection, and genotyping

Genomic DNA for genotyping was isolated from peripheral blood lymphocytes by a nonorganic DNA extraction procedure [13]. A single assay using a multiplex polymerase chain reaction (PCR) was performed for simultaneous gene amplification of the polymorphic alleles *GSTM1* (glutathione S-transferase Mu 1) and *GSTT1* (glutathione S-transferase theta 1). In the final models, the frequency of *GSTM1* and *GSTT1* deletion was used for adjustment as a dichotomous variable: either no deletion or deletion in one or both genes.

Statistical analysis

Multiple linear regression models were estimated to verify associations between MDA concentration and the following variables: meat intake, animal and vegetable protein, protein from meat, and arginine intake (continuous variables). We also used multiple regression models to verify the association between MDA and arginine from meat intake to test whether arginine in meat could lead oxidative stress or whether other components also could contribute to oxidative stress.

The models were adjusted for some genetic, biochemical, and lifestyle confounders to minimize bias. We performed two models: The first was adjusted for age, sex, and body mass index (BMI); and the second was further adjusted for smoking, physical activity, intake of fruit and vegetables, energy, and heterocyclic amines, CRP, and *GSTM1*/*GSTT1* deletions.

The statistical analyses were conducted using STATA package (Stata statistical software: Release 10, StataCorp 2011, College Station, TX, USA) and $P < 0.05$ was considered statistically significant.

Results

The sample comprised 38% men and 62% women. According to the MDA tertiles, the means and SDs of food intake, anthropometric, and sociodemographic information are presented in Table 1. Age, sex, and BMI were significantly associated with MDA (Table 1).

After adjustment for age, sex, BMI, smoking status, physical activity, CRP, *GSTM1*/*GSTT1* deletions, and intake of heterocyclic amines, fruit and vegetables, and energy, the tertiles of meat

Table 1
Descriptive characteristics of participants in ISA-Capital study

	Total		MDA level (in tertiles)						*P* value*
			Tertile 1		Tertile 2		Tertile 3		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
MDA (μmol/L)	0.73	0.34	0.37	0.15	0.71	0.08	1.09	0.24	<0.001
Individual characteristics									
Age (y)	55.3	19.1	52.3	20.9	53.4	18.4	60.1	16.9	<0.001
BMI (kg/m^2)	26.6	5.3	26.1	5.1	26.6	5.1	27.2	5.7	0.048
Sex (% men)	38.4		30.4		40.1		44.8		0.015
Smoking (%)	18.4		19		20.3		15.9		0.524
Physical activity (% active)	15.3		12.5		19.9		13.7		0.109
Diet									
Energy intake (kcal/d)	1558.5	708.7	1494	689	1634.9	777.7	1547	650.5	0.486
Protein intake (g/d)	69.7	37.4	66.3	36.6	71.2	41.2	71.9	34.2	0.163
Vegetal protein intake (g/d)	23.2	11.8	22.1	10.8	24	13.2	23.6	11.3	0.239
Animal protein intake (g/d)	46.4	33.4	44.1	32.8	47.1	37.5	48.2	29.6	0.252
Arginine (g/d)	3.9	2.3	3.7	2.3	3.9	2.5	4	2.1	0.207
Total meat (g/d)	128.9	116.6	123.6	119.4	133	129.2	130.1	99.7	0.604
Arginine from meat intake (g/d)	2.3	2.1	2.2	2.1	2.3	2.3	2.4	1.8	0.339

BMI, body mass index; MDA, malondialdehyde

* χ^2 for categorical variables; linear regression for continuous variables.

Table 2
Association between MDA levels, meat intake and its nutrients in ISA-Capital Study

	Model 1*		P_{trend}	Model 2†		P_{trend}
	β (SE)	*P* value		β (SE)	*P* value	
Protein intake						
Tertile 1	0	–	0.067	0	–	0.097
Tertile 2	0.01 (0.04)	0.873		0.01 (0.04)	0.742	
Tertile 3	0.07 (0.04)	0.068		0.09 (0.05)	0.093	
Animal protein intake						
Tertile 1	0	–	0.030	0	–	0.025
Tertile 2	0.03 (0.04)	0.423		0.04 (0.04)	0.294	
Tertile 3	0.08 (0.04)	0.03		0.10 (0.05)	0.026	
Vegetable protein intake						
Tertile 1	0	–	0.722	0	–	0.818
Tertile 2	−0.04 (0.04)	0.317		−0.03 (0.04)	0.468	
Tertile 3	0.01 (0.04)	0.738		−0.01 (0.05)	0.829	
Protein from meat intake						
Tertile 1	0	–	0.012	0	–	0.006
Tertile 2	0.02 (0.04)	0.664		0.04 (0.04)	0.399	
Tertile 3	0.10 (0.04)	0.012		0.12 (0.05)	0.008	
Meat intake						
Tertile 1	0	–	0.034	0	–	0.031
Tertile 2	0.02 (0.04)	0.527		0.04 (0.04)	0.397	
Tertile 3	0.08 (0.04)	0.035		0.10 (0.05)	0.037	

MDA, malondialdehyde; SE, standard error
* Model 1 adjusted for age, sex, and body mass index.
† Model 2 further adjusted for smoking, physical activity, C-reactive protein level, intake of fruit, vegetable, energy and heterocyclic amines, and polymorphisms in *GSTM1* and *GSTT1* genes.

intake were associated with MDA levels (*P* for linear trend = 0.031) (Table 2).

Significant relationships between animal protein intake and MDA levels (β = 0.052; *P* for linear trend = 0.025), and protein from meat intake and MDA levels were also found (β = 0.062; *P* for linear trend = 0.006), but the protein from vegetable intake was not significantly associated with MDA (β = −0.006; *P* for linear trend = 0.818) (Table 2). We also checked whether total protein intake also could increase MDA, but no significant association was observed (*P* > 0.05) (Table 2). Furthermore, arginine intake (Fig. 1A) and arginine from meat (Fig. 1B) was significantly associated with MDA.

Discussion

Data presented here suggest a relationship between plasmatic MDA levels and animal protein and arginine intake in a population-based study, which could reinforce the long-term debated hypothesis that high meat intake contributes to the development of chronic diseases by a mediating process such as oxidative stress [2,14].

Different explanations for the relationship between meat intake and chronic diseases have been described in the literature, especially via oxidative stress pathways. The present study investigated whether protein and arginine from meat could contribute to this association. First, we found that only protein from animal and meat intake was related to higher MDA levels, but protein from vegetables were not. Amino acids from these two different protein sources are different. Arginine, for example, is presented in high amounts in meat with >1 g/100 g of meat [8], contributing to oxidative stress generation as evidenced for increased MDA levels in individuals who consumed more arginine from meat, even after all possible adjustments (age, sex, BMI, smoking status, physical activity, *GSTM1*/*GSTT1* deletions, CRP, and intake of heterocyclic amines, fruit and vegetables, and energy).

In conditions such as starvation, injury, or stress, arginine becomes essential amino acid, but in healthy adult individuals, arginine is a semi-essential amino acid [15]. Thus, there is no minimum or maximum dietary arginine recommendation, but a previous study estimated a daily arginine mean from meat intake of ∼2 g/d [16], similar to the mean that we found. Although some studies have reported the use of arginine supplementation (from 3 to 15 g/d) to improve health conditions among patients with chronic diseases, there are no conclusions about use, especially because some studies have reported no benefits or worsening of the health state [17,18].

Contribution in protein synthesis, and a precursor of molecules involved in different physiological processes, including synthesis of urea, ornithine, NO•, and agmatine are some of the roles of arginine [15,19]. For enzymatic synthesis of NO•, L-arginine is basically oxidized by NO synthase (NOS) into L-citrulline and NO• in the presence of oxygen and nicotinamide adenine dinucleotide phosphate (NADPH) [20,21]. Actually, the chemical aspects behind the synthesis of NO• from endothelial NOS (eNOS) are complex. Already known that beyond the availability of arginine and cofactors (flavin adenine dinucleotide, flavin mononucleotide, and calmodulin), the tetrahydrobiopterin (BH4) is necessary and rate limiting for eNOS activity [22,23]. This endogenous production of NO• is crucial for the maintenance of a variety of biological functions, including vascular tone regulation, leukocyte adhesion, platelet aggregation, and neurotransmission. In fact, due to the

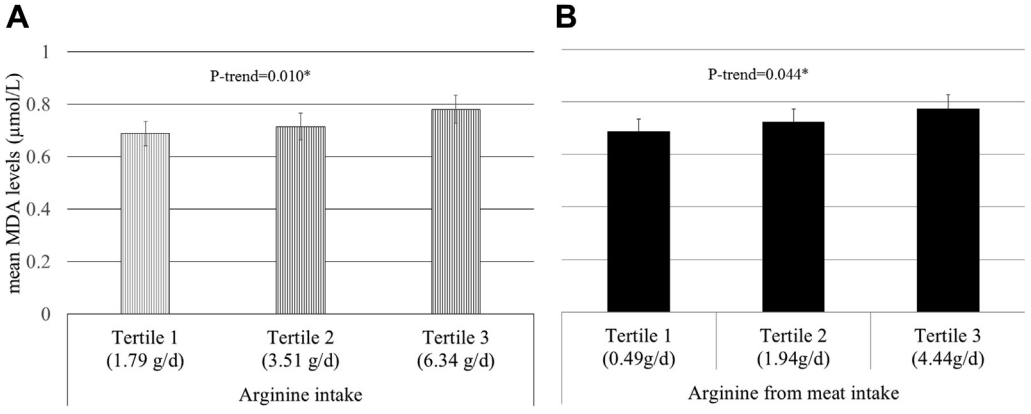

Fig. 1. Means and 95% confidence intervals from MDA levels in plasma according to arginine intake and arginine from meat intake (in tertiles). (A) Arginine intake; (B) Arginine from meat intake. *Models adjusted for age, sex, BMI, smoking, physical activity, intake of fruit and vegetables, energy and heterocyclic amines, C-reactive protein, and polymorphisms in *GSTM1*/*GSTT1* genes. BMI, body mass index; MDA, malondialdehyde.

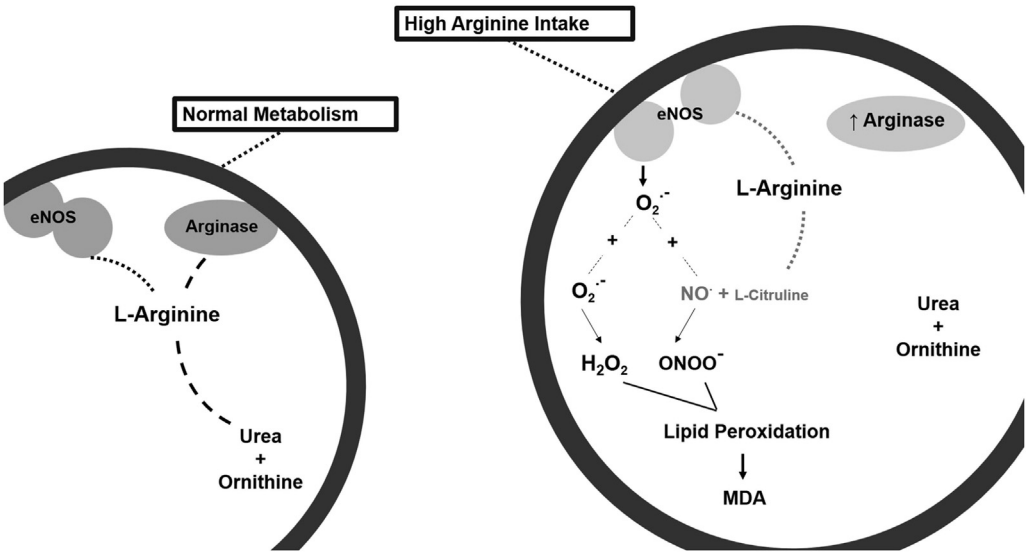

Fig. 2. Metabolism linking arginine and oxidative stress. Under normal arginine intake, L-arginine is used as a substrate for both arginase and eNOS. However, if arginine intake is increased, there is an induction of arginase activity in such way that L-arginine is less available for NO• synthesis by eNOS, promoting eNOS uncoupling. Then, eNOS starts to produce superoxide that in turn generates hydrogen peroxide and peroxynitrite. These reactive oxygen and nitrogen species can increase lipid peroxidation, assessed by MDA quantitation in plasma. eNOS, endothelial nitric oxide synthase; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; NO•, nitric oxide; $O_2^{\bullet-}$, superoxide; $ONOO^-$, peroxynitrite.

lack of NO•, the endothelial dysfunction is linked to development of cardiovascular diseases [24,25].

The catabolism of arginine also involves degradation to ornithine and urea, mediated by arginase enzymes (types 1 and 2), which compete for arginine with eNOS. Under high arginine concentration or low eNOS/arginases molar ratio, arginase activity exceeds eNOS activity because the maximal catalytic activity of arginases is higher than eNOS [20,26]. It has been shown that high arginine levels induce arginase expression. Increased arginase activity decreases arginine availability for NO• production and leads to an uncoupling of eNOS. Consequently, uncoupled eNOS starts to produce superoxide by transferring the electron from NADPH to molecular oxygen, rather than using the electron for arginine oxidation. Superoxide can, in turn, react with NO• to produce peroxynitrite or can also form hydrogen peroxide. In both cases, damage to biomolecules can be induced, for example, membrane lipids may undergo lipid peroxidation [9,27,28] (Fig. 2). In individuals who consume higher arginine from meat, the process of catabolism by arginases could be a plausible hypothesis to explain the association with higher plasmatic MDA levels observed in this study.

The relationship between arginine and oxidative stress has been addressed before in vitro. In aqueous solution, arginine is able to generate hydroxyl radicals, possibly linked with the pathogenesis of hyperargininemia [29]. Arginine at high concentrations in vitro, as well as other guanidine compounds, induced oxidative stress in brain tissue by impairing the total antioxidant capacity and inhibiting the activity of catalase and glutathione peroxidase [30]. High concentration of homoarginine was shown to enhance lipid peroxidation and decrease thiol content and catalase activity in both plasma and kidney in vitro [31].

This study has some limitations. First, we used one 24-h dietary recall, a method that is adequate to estimate mean intake, but one that can promote a wide distribution, which can contribute to a reduction of statistical power for detecting associations [32]. Despite that, we had a moderate sample size (N = 549), which remedied loss of power, and we found a significant relationship, meaning that association could be stronger if we have had an adjusted distribution. A causal relation between meat intake and oxidative stress could not be inferred from a cross-sectional design study. Second, unmeasured or unknown confounders might exist, although we carefully adjusted for many diet and lifestyle factors. Third, we were not able to quantify arginine and NO• levels in the plasma of the individuals in the present study. Furthermore, we could not control for diseases that could influence oxidative stress, but the models were adjusted for BMI and PCR, a marker of inflammation and chronic diseases. Nonetheless, this is a plausible hypothesis to explain the link involving high meat intake and oxidative stress, and to the best of our knowledge, this is the first work to suggest that arginine and protein from high meat intake can be associated with MDA in a population based-study.

Conclusions

Our results indicated that protein from meat intake and arginine from meat intake were associated with higher levels of a marker of oxidative stress independently of genetic, lifestyle, and biochemical confounders in a population-based study. These results suggested a novel link between high-protein/arginine intake and oxidative stress, a major cause of age-related diseases and cancer.

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