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Experimental Study of the Effects of Three Types of Meat on Endothelial Function in a Group of Healthy Volunteers

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Abstract

Background: There is a relationship between atherosclerotic risk factors and increased vascular production of reactive oxygen species (ROS). Oxidized LDL and ROS may directly cause endothelial dysfunction by reducing endothelial nitric oxide (NO) bioavailability. The semi-essential amino acid L-arginine is the only substrate for NO synthesis in vascular endothelial cells. Therefore, this amino acid improves endothelial function and plays a role in the prevention and/or treatment of multiple cardiovascular diseases: atherosclerosis, hypertension, diabetes and so on. To determine the effects of three different protein matrices (250 g Fillet of Beef, FB; Chicken Raised on the Ground, CRG; Free-Range Chicken, FRC) with a known content of arginine on the cardiovascular workload, vascular compliance and urinary excretion of some parameters of endothelial function as TGF–Beta, NO (nitrate e nitrite) in a group of healthy volunteers.

Materials and methods: We enrolled 10 men to study the behavior of Systolic, Diastolic, Mean, and Pulse Blood Pressure, of Vascular Resistances, of Macro and Micro Vascular Elasticity, of urinary excretion of TGF-β and Nitric Oxide as ratio of creatinine before and after two hours of each meal. The cardiovascular parameters are determined by HDI/Pulse Wave CR 2000 (Hypertension Diagnostic Inc, Eagan, MN); TGF-β is analysed by Elisa method (R&D Systems) and NO by colorimetric method (Cayman).

Results and Conclusion: The protein meal packed with CRG causes a significant decrease in diastolic blood pressure mean pressure and vascular resistance in urinary excretion of TGF. FB resulted in a significant decrease in vascular resistance and urinary excretion of NO, while significantly increasing the Pulse Pressure, heart rate and urinary excretion of TGF-β. FRC resulted in a significant reduction of macrovascular elasticity; increase the urinary excretion of TGF and Pulse Pressure. We can conclude that CRG meat looks better both in terms of metabolic and cardiovascular load especially at endothelial level.

Keywords: Vascular compliance; TGF-β urinary excretion; L-Arginine; Beef; Chicken meal; FB: Fillet of Beef; CRG: Chicken Raised on the Ground; FRC: Free-range Chicken

Introduction

The L-arginine/nitric oxide (NO) pathway plays a critical role in maintaining normal endothelial function by causing blood vessel relaxation (vasodilatation). The semi-essential amino acid L-arginine is the only substrate for NO synthesis in vascular endothelial cells [1]. Therefore, this amino acid improves endothelial dysfunction and is expected to play a role in the prevention or treatment of multiple cardiovascular disease as diverse as atherosclerosis, hypertension, diabetes, to mention a few [2-5].

Reactive oxygen species which are mainly produced by vascular cells are implicated as possible underlying pathogenic mechanisms in a progression of cardiovascular diseases including ischemic heart disease, atherosclerosis, cardiac arrhythmia, hypertension, and diabetes and worsening endothelial function [6-8]. Nitric Oxide too prevents abnormal constriction of the arteries, which favours intraluminal clot formation, inhibits platelets aggregation expression of adhesion molecule at the surface of the endothelial cells, and adhesion and penetration of macrophages [9].

The process of arginine synthesis from dietary precursor is complex, involving at least eight enzymes directly and the intracellular and inter organs transfer of substrates [10,11] L-glutamine is a dietary precursor for L-arginine: the first step in L-arginine synthesis from glutamine is via glutaminase to produce glutamate and ammonia [12]. The intestinal conversion of glutamine leads to a release from a gut of citrulline, which, after its uptake from the blood stream, is converted by the kidneys into arginine [13].

High fat meals acutely impair endothelial function: several mechanisms have been proposed, but a likely candidate is a reduction in nitric oxide (NO) bioavailability resulting from decreased synthesis and/or enhanced degradation by reactive oxygen species (ROS). During postprandial lipaemia, the process of lipolysis increases chylomicron and degradation of NO with procoagulant and proinflammatory signaling pathways in the endothelium [5-8]. In support of these, it has been shown that co administration of antioxidant compounds prevents the postprandial increase in oxidative products, proinflammatory cytokines and impairment in endothelial function [14-20].

Several molecular processes have been implicated in regulation of vascular fibrotic process: activation of the renin-angiotensin-aldosterone system, induction of Transforming Growth Factor-β, oxidative stress, and endothelin-1.

Moreover increased activity of arginase competes with eNOS for the common substrate, arginine [14]; the augmented production of superoxide anions and thus the scavenging of NO leads to an increased presence of peroxynitrite [16].

Arterial stiffness is a major independent risk factor for cardiovascular morbidity and mortality; impaired arterial compliance is an independent predictor of early vascular damage and is related adverse cardiovascular outcome [21].

This study was designed to determine the effects of three different protein matrices with a known content of arginine on the cardiovascular impact, macro and micro vascular elasticity and urinary excretion of some parameters of endothelial function as TGF–Beta, NO (nitrate and nitrite) in a group of ten healthy volunteers.

Material and Methods

Bromatology analysis of the meats studied

The bromatology analysis shows that the meat of Fillet of Beef (FB). Compared to Chicken Raised on the Ground (CRG) and Free-Range Chicken (FRC). Has a greater amount of saturated fatty acids (Table 1):

Myristic acid (C14:0) 4.88 g (0.6 g FRC and CRG 1.13 g); Stearic acid (C18:0) 14.24 g (5.98 g FRC and 6.13 g CRG); Palmitic acid (C16:0) 27.38 g (24.13 g FRC and 22.44 g CRG). The Beeric acid (C22:0) is present with modest contribution (0.13 g) only FRC. Between monounsaturated fatty acids breathed oleic acid (C18:1) 39. 19 g (34.32 g FRC and 39.69 g CRG); palmitoleic acid (C16:1) 3.78 g (5.25 g FRC and 5.25 g CRG).

Polyunsaturated fatty acids most represented are: linolenic acid (C18:3) 0.56 g (0.64 g FRC and 1.51 g CRG). arachidonic acid (C20:4) 0.17 g FB (0.82 g FRC. 0.47 g CRG). eicosadienoic acid (C20:2) only present in poultry meat (0.28 g FRC and 0.27 g CRG).

From our bromatological data (Table 2) emerges that the content of L-arginine present in the three types of meat studied is significantly different. The meat richer in this semi-essential amino acid is FB (1.99 g/100 g) compared to FRC (1.84 g/100 g) and CRG (1.58 g/100 g).

Study population

We enrolled 10 healthy volunteers to study the behavior of some cardiovascular function before and after two hours a standardized protein meal with a known content of L-arginine. From January to June 2013, we studied 10 male adults with an average age of 53,5 years (range 40-60). Blood pressure measurements were obtained from the right arm of each subject in supine position, systolic and diastolic blood pressures were determined by automated oscillometric monitors [15]. Before and after the protein meal and urine's sample were collected.

Exclusion criteria

Females are excluded for the well-known effect of estrogen on the release of NO [16] and all known acquired cardiovascular, hepatic, renal or brain diseases, or gastrointestinal impairment like chronic inflammatory disease or endocrine disorders.

Study protocol

The subjects were studied in the morning between 11:00 and 12:00 AM; after 15 min of rest in a quiet, temperature-controlled room, blood pressure (BP) and pulse wave analysis (PWA) were performed three times at intervals of 5 minutes in recumbent position.

The urine is collected between 11 a.m. and 2 p.m. before and after the administration of the three types of meals. Each sample is immediately refrigerated and was frozen as soon as it reached the laboratory. TGF-β excretion was detected ELISA-Quantikine R&D Systems and Nitric Oxide as Nitrate/Nitrite by colorimetric method (Cayman), both parameters were corrected for urinary Creatinine concentration (by standard enzymatic method).

Dietary intervention

Each subject consumed the following three meals of meat cooked in standard mode:

- 250 g Fillet of Beef 15-18 months (FB)

- 250 g of Chicken Raised on the Ground (CRG)

- 250 g of Free-Range Chicken (FRC)

Bromatology Analysis			
MEAT AFTER STANDARDIZED COOKING	**CRG**	**FRC**	**FB**
Fatty Acids	g/100 g part of edible		
Capronic Acid (C6:0)	0.13	0	O
Caprilic Acid (C8:0)	0.51	0.58	0.49
Caprinic Acid (C10:0)	0.33	0.31	0.44
Lauric Acid (C12:0)	0.14	O	0.28
12-(CH₃) Tridecanoic (C14:0)	O	O	0.08
Miristic Acid (C14:0)	**1.13**	**0.61**	**4.88**
13-(CH₃) Tetradecanoie Acid (C15:0)	O	O	0.21
12-(CH₃)Tetradecanoic Acid (C15:0)	0	O	0.3
Miristoleic Acid (C14:1)	0.23	0.19	0.98
Pentadecanoic Acid (C15:0)	0.16	0.13	0.73
14-(CH₃) Pentadecanoic Acid (C16:0)	**O**	**0**	**0.31**
Palmitic Acid (C16:0)	22.44	24.13	27.38
15-(CH₃) Esadecanoic Acid (C17:0)	O	O	0.44
Palmitoleic Acids (C16:1) including isomers	5.25	6.46	3.78
14-(CH3) Esadecanoic Acid (C17:0)	0.05	O	0.69
Eptadecanoic Acid (C17:0)	0.23	0.14	1.2
Epatadecanoic Acid (C17:1)	0.18	0.06	0.63
Stearic Acid (C18:0)	**6.13**	**5.98**	**14.24**
Oleic Acids (C18:1) including isomers	39.69	34.32	39.19
Linoleic Acids (C18:2) including isomers	**20.63**	**24.83**	**2.94**
Arachico Acid (C20:0)	0.09	0.1	0.11
11-Eicosenoic Acid (C20:1)	0.41	0.26	0.16
Linolenic Acids (C18:3) including isomers	1.51	0.64	0.36
11.14-Eicosadienoic Acid (C20:2)	0.28	0.28	O
Beenic Acid (C22:0)	0	0.13	0
Arachidonic Acid (C20:4)	**0.47**	**0.82**	**0.17**

Table 1: Percentage composition of fatty acids in the three types of meat after cooking.

The chicken meat was made up of hip and leg, boneless and skinless: The cooking of the meat were carried out in a convection oven 180°C with the addition of 1 g of salt and a tablespoon of extra virgin olive oil. After the cooking is done the killing temperature to prevent bacterial growth. The day of administration of the meal the meat was heated with microwave for 2 minutes maximum power (900 watts) and was allowed only water ad libitum consumption.

Each meal was consumed by each subject at a distance of at least one week interval.

Assessment of arterial elasticity: The arterial waveform was measured in the no dominant arm with a cardiovascular profiling instrument (HDI/Pulse Wave CR-2000, Hypertension Diagnostics Inc, Eagan, MN). Briefly, the tonometer was applied to the patient's radial artery at the wrist overlying the radial bony prominence. The subject's arm was supported by a wrist stabilizer for optimal positioning and minimal movement during the measurements. The cuff for BP measurement was placed on the contra-lateral arm and inflated concurrently with the pulse waveform recording for calibration; 30 seconds of analogue waveforms were digitized at 200 samples/sec, and a beat marking algorithm determined the beginning of systole, peak systole, onset of diastole, and end of diastole for all beats in the 30 sec measurement period. The elasticity indices of the arteries (C1 and C2) were quantified during the diastolic portion of the heart cycle (mean recording time 30 sec). According to the modified Windkessel model of circulation, C1 is a marker of large artery elasticity and C2 is a marker of small artery elasticity. Heart rate (HR), mean arterial pressure (MAP), and stroke volume were also calculated from the radial pressure waveform using HDI/Pulse Wave CR-2000 software.

These hemodynamic parameters (i.e. MAP and stroke volume) are used in multivariate algorithms for determining C1 and C2. The full method has already been validated and described in detail [17,18].

Our Institutional Review Board approved this study. Informed consent was obtained from each subject after full explanation of the study before enrolment.

Statistical Analysis

All the variables were tested for normal distribution by the Kolmogorov-Smirnov test and for homoscedasticity by the Levene test. Descriptive statistics was expressed as mean and SD. 95% confidence intervals of sample means were also evaluated.

Multivariate analysis of variance (MANOVA) for repeated measures was used to evaluate the statistical significance of variable changes for both the within-subjects factors "diet" and "types of meat". One-way MANOVA was performed for between-subjects comparisons, while the multivariate test of Hotelling's Trace was used for within-subjects analysis which included interactions between factors. A statistical significance level of 95% ($p < 0.05$) was assumed in all statistical computations performed using SPSS software, version 10.

Results

Protein meal with CRG did not supply statistically significant differences ($p > 0.05$) in cardiovascular parameters under consideration (Table 3); with the exception of a significant reduction ($p < 0.05$) in Macrovascular Elasticity (Table 4) and urinary excretion of NO (Table 5). The CRG meat significantly increases the Pulse Pressure (Table 3) and urinary excretion of TGF-β.

The FB meat causes a significant reduction of BPD, VR (Table

3) and urinary excretion of NO (Table 5); the reduction of Macro and Microvascular Elasticity is not significant (Table 4). This meat significantly increases the Pulse Pressure, HR (Table 3) and urinary excretion of TGF-β (Table 5). The variations observed for the BPS, BPM are modest and not significant.

After the FRC meal we observed a significant reduction in BPD, BPM, VR (Table 3) and in the urinary excretion of NO, as shown in Table 5; the reduction of the BPS does not reach the statistical significance.

In the FRC the increase of Macrovascular Elasticity is not significant and also the Microvascular Elasticity augments to a lesser extent.

Discussion

The content of L-arginine in the three protein matrices (chicken raised on the ground, free-range chicken and fillet of beef) differs from the reports in the databases available at National and International level (IEO, INRAN, USDA).

The differences relate not only to the content of L-arginine (1.99 g/100 g for the fillet of beef, free-range chicken 1.84 g/100 g and 1.58 g/100g for chicken raised on the ground) but also for the quantity of amino acids and of the fatty acid composition, with particular reference to saturated fatty acids and L-glutamine.

These differences can interfere with the speed of absorption and the bioavailability of the amino acids, as well as on the load cardiovascular secondary commitment digestive of single meal [10-13].

The Free-Range Chicken Meat (FRG) reduces the Systolic, the Diastolic, the Mean Blood Pressure and the Vascular Resistances while the fillet of beef decreases Diastolic Blood Pressure and Vascular Resistance; we does not observe significant changes in Systolic Blood Pressure while significantly increases the heart rate.

The meat of Chicken Raised on the Ground (CRG) does not cause appreciable changes attributable to the parameters of cardiovascular function. The Pulse Pressure increases significantly after the meal with beef (FB) and chicken raised on the ground.

As regard the Arterial Compliance the two meats with higher content of L-Arginine (FRG and FB) increase the macro vascular elasticity but not significantly while the chicken raised on the ground (CRG) induces a statistically significant reduction of this parameter; the Microvascular Elasticity does not change. As regarding the urinary excretion of the TGF-β, cytokine able to modify and induce the inflammatory process, we observe a marked and significant increase after the beef's filet and meat's chicken raised on the ground, while the meat of the CRG chicken induces an increase of this cytokine but not statistically significant.

In agreement with these results, we can see a marked reduction in urinary excretion of Nitric Oxide, that correlates to increase of urinary excretion of TGF-β [22,23].

The changes to the above parameters may not depend only by the different amount of L-arginine present in the three types of the meats in our study. Bromatology analysis show that beef's meat contents more L-Arginine and so the significant decrease of the Systolic Blood Pressure could be dependent by the amount present in this type of meat of this semi-essential amino acid that nevertheless increases the heart rate, while the FRC chicken reduces the Systolic Blood Pressure and does not induce changes in heart rate. These data suggest that, although the higher content of L-Arginine, the beef' meat induces a greater

Bromatology Analysis			
MEAT AFTER STANDARDIZED COOKING	**CRG**	**FRC**	**FB**
AMINO ACIDS GROUP 1	g/100g part of edible		
Aspartic Acids	2.37	2.63	0
Glutammic Acid	**3.88**	**4.5**	**5.08**
Alanine	1.78	2.02	2.28
Arginine	**1.58**	**1.84**	**1.99**
Phenylalanine	1.16	1.31	1.44
Glycine	1.28	1.54	1.86
Hydroxyproline	0.248	0.363	0.473
Isoleucine	1.25	1.44	1.51
Histidine	0.96	1.15	1.01
Leucine	2.09	2.35	2.67
Lysine	2.1	2.47	2.8
Proline	1.1	1.27	1.48
Serine	1.12	1.25	1.36
Tyrosine	0.6311	0.739	0.803
Threonine	1.23	1.36	1.5
Valine	1.14	1.3	1.4
AMINO ACIDS GROUP 2			
Cysteina e Cystina	0.262	0.325	0.332
Methionine	0.616	0.777	0.76
AMINO ACIDS GROUP 3			
Tryptophan	0.192	0.239	0.225

Table 2: Percentage composition of amino acids in the three types of meat after cooking.

	SBP	DBP	HR	MBP	VR	PP
	119 ± 6.24 n.s.	68.9 ± 7.03 n.s.	59.4 ± 7.69 n.s.	85.20 ± 12.72 n.s.	1272.5 ± 174.59 n.s.	31.77 ± 3.54 n.s.
CRG	120.7 ± 7.67 n.s.	69.03 ± 6.04 n.s.	61 ± 6.69 n.s.	84.47 ± 11.64 n.s.	1285.83 ± 129.09 n.s.	33.17 ± 3.57.09 P< 0.05
	121.2 ± 4.68 n.s.	72.03 ± 6.62 n.s.	59.87 ± 7.9 n.s.	86.83 ± 11.79 n.s.	1320.63 ± 101.55 n.s.	31.27 ± 4.47 n.s.
FB	121.07 ± 7.29 n.s.	**68.27 ± 6.74 P< 0.05**	**64.7 ± 10.55 P< 0.05**	84.20 ± 10.35 n.s.	**1244.47 ± 86.76 P< 0.05**	**34.47 ± 2.55 P< 0.05**
	125.63 ± 8.82 n.s.	74.07 ± 7.36 n.s.	62.93 ± 11.18 n.s.	93.33 ± 7.63 n.s.	1371.2 ± 138.31 n.s.	32.40 ± 5.56 n.s.
FRC	121.5 ± 5.89 n.s.	**69.60 ± 4.59 P< 0.05**	66.57 ± 8.06 n.s.	**87.97 ± 4.62 P< 0.05**	**1265.63 ± 106.43 P< 0.05**	33.47 ± 4.81 n.s.

Table 3: Means and SD (N=10) of Systolic (SBP). Diastolic (DBP) Mean (MBP). and Pulse (PP) Blood Pressure (mm/Hg). Heart rate (beats/min) and Vascular Resistances (dynes/s xcm-5) before and after the 3 types of meals; CRG: Chicken Raised on the Ground; FB: Fillet of Beef. FRC: Free-Range Chicken.

	C1	C2
CRG	17.97 ± 2.61 n.s.	7.82 ± 2.36 n.s.
	16.35 ± 3.04 P< 0.05	8.1 ± 2.14 n.s.
FB	16.81 ± 2.51 n.s.	8.62 ± 2.28 n.s.
	17.46 ± 2.43 n.s.	7.89 ± 2.65 n.s.
FRC	15.56 ± 2.26 n.s.	7.65 ± 2.77 n.s.
	16.55 ± 3.36 n.s.	7.86 ± 1.85 n.s.

Table 4: Means and SD (N=10) of Macrovascular Elasticity (C1 in ml/mm Hg x 10). and of Microvascular Elasticity (C2 in ml/mm Hg x 100) before and after the 3 types of meals; CRG: Chicken Raised on the Ground; FB: Fillet of Beef. FRC: Free-Range Chicken.

	TGF	NO
CRG	8.32 ± 4.26 n.s.	240.83 ± 101.48 n.s.
	11.21 ± 5.25 P< 0.05	**180.49 ± 73.31 P< 0.05**
FB	11.44 ± 6.05 n.s.	209.22 ± 76.94 n.s.
	14.23 ± 6.84 P< 0.05	**180.15 ± 68.67 P< 0.05**
FRC	7.71 ± 4.51 n.s.	216.47 ± 82.12 n.s.
	7.99 ± 3.1 n.s.	**155.57 ± 38.93 P< 0.05**

Table 5: Means and SD (N=10) of Urinary Excretion of TGF-β (picograms/mg/ creatinine) and Nitric Oxide (NO/mg/creatinine) before and after the 3 types of meals; CRG: Chicken Raised on the Ground; FB: Fillet of Beef. FRC: Free-Range Chicken.

cardiovascular workload than the meal cooked with CRG chicken' meat.

The interpretation of these data is not easy because the structure of the three meats is different especially for the content of saturated fatty acids: as known the Palmitic and the Stearic acids are able to slow the speed of intestinal absorption of individual amino acids [15,16].

Also the different content of L-Glutamine may interfere in some fashion on the gut's synthesis of Citrulline and of L-Arginine thus interfering on endothelium-mediated response [12,13].

To resolve these complex interrelationships between glutamine, arginine and saturated fatty acids and enteral absorption of amino acids would be necessary to monitor the plasma concentrations of L-Arginine and Nitric Oxide Synthase that are not part of this section of the study. Nevertheless we must stress the increased urinary excretion of TGF-β after meal with filet beef of our study: it is, in some way, correlated to the induced cardiovascular workload. Also the chicken raised on the ground significantly increases the excretion of TGF-β but this is not accompanied by an increased cardiovascular load.

In opposite ways, the FRG chicken significantly reduces indexes of cardiovascular commitment (BPS, BPD, BPM, VR) but did not induce a statistically significant increase in urinary excretion of TGF-β.

This indicates that the composition of Free-Range Chicken meat is able to have a better impact both on some of the main parameters of cardiovascular function (BPS, HR, and Macrovascular Elasticity) and on the indexes of endothelial involvement as the urinary excretion of TGF-β and NO.

Our data, still in progress, and the small sample size, it will help to clarify the issues raised to date the origin of the present study because it is not possible to determine:

1) How individual amino acids are absorbed in the intestine;

2) Such as fatty acids and L-glutamine interfere on their intestinal absorption;

3) The influence of these nutrients on cardiovascular workload induced by their metabolites.

We can conclude that the Free-Range Chicken meat looks better both in terms of metabolic and cardiovascular workload especially at endothelial level.

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