

## Comparison of Two Kinds of Red Meat Regarding Atherogenic Profile After Ingestion: A Crossover Study in Healthy Subjects

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### Abstract

**Background:** Some studies demonstrated higher levels of atherogenic biomarkers after red meat ingestion compared with other sources of protein, like fish or poultry. However, considering that different kinds of red meat contain varied levels of fat, few studies have compared different types of red meat regarding inflammation and atherosclerosis.

**Objective:** Compare 2 kinds of red meat in relation to atherosclerosis-related biomarkers.

**Design:** This was a double-blind, crossover, single-centre study. Healthy male subjects were enrolled in this study at the Heart Institute, Brazil. They ate 2 different diets in 2 simple meals 1 week apart. Meal 1 was composed of a balanced diet with rice, juice, and standard red meat. Meal 2 had the same composition as meal 1 but contained lean meat obtained by the cross between 2 breeds: Rubia Gallega and Nelore. Blood samples were obtained at baseline, 1 and 2 hours after ingestion of meal 1 (H1 and H2) and meal 2 (H3 and H4). Serum levels of IL-6, hs C-reactive protein (CRP), VCAM, ICAM, p-selectin, Apo-A1 and Apo-B were compared at these prespecified times.

**Results and Discussions:** Twenty healthy men participated in this study. Mean age was 30.5±2.89, and they had normal blood glucose and cholesterol levels. Apo-A1 mean levels (ng/mL) were higher at 1h after ingestion of meal 2 (p=0.010). Serum Apo B levels (ng/mL) were also higher 1 hour after ingestion of meal 1 (p=0.003). hs-CRP levels were lower at H3 and H4 compared with those at H1 and H2 (baseline:0.90; H1:0.93; H2:0.86; H3:0.59 and H4:0.58; p=0.031).

**Conclusion:** Lean red meat from a cross of Rubia Gallega and Nelore leads to a less atherogenic profile after ingestion than standard meat.

**Keywords:** Atherosclerosis; Inflammation; Biomarkers; Meat; Diet

### List of Abbreviations

Apo-A1: Apolipoprotein A1; Apo-B: Apolipoprotein B; Hs-CRP: High-Sensitivity C-Reactive Protein; ICAM: Intercellular Adhesion Molecule; IL-6: Interleukin 6; SFA: Saturated Fatty Acids; VCAM: Vascular Cell Adhesion Molecule

### Background

Meat is part of human diet since 2-6 million years ago [1],

and the inclusion of meat plays an important evolutionary role, explaining, in part, the large and complex human brain [2]. Red meat contains all 8 essential amino-acids for adults and 9 for children, constituting one of the main sources of protein in the Western diet. Besides, meat and meat products contribute to 21% of iron intake and 30% of vitamin D in adults [2]. However, its consumption is often associated with increased cardiovascular risk [3] and colorectal cancer [4]. This association is related to consumption of meat with large amounts of Saturated Fatty Acids (SFA), leading to an increase in postprandial inflammatory response and, consequently, endothelial dysfunction [5]. These changes, may increase the incidence of cardiovascular diseases

in the long term [6]. Intake of low-fat red meat could be associated with lower inflammatory activity and lipid levels [7]. Diets containing kangaroo or bison meat, when compared with meat traditionally sold in markets, resulted in lower levels of inflammatory biomarkers, such as high-sensitive CRP (hs-CRP) and Interleukin-6 (IL-6) [8,9].

To date, no study has compared postprandial inflammatory repercussion of 2 meats of the same species. Recently, a new beef developed by cross breeding Rubia Gallega and Nelore Cattle, resulted in a nutritional composition with lower amounts of fats and carbohydrates, compared with conventional red meat. The objective of this study was to compare the effects on postprandial inflammatory response of a diet containing meat originating from crossing of Rubia Gallega and Nelore breeds of cattle versus a conventional meat diet.

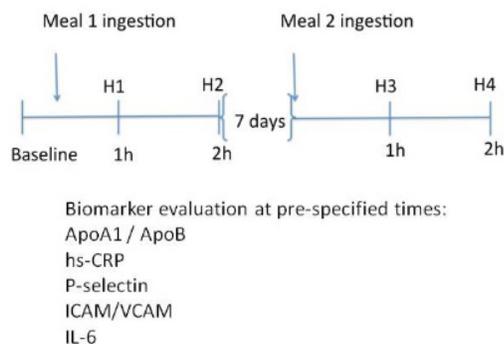
## Materials and Methods

### Design of the Study

This was a double-blind, crossover, single-centre study designed to compare 2 kinds of red meat in relation to atherosclerosis-related biomarkers.

### Population and Compared Groups

Healthy male subjects were enrolled in this study at the Heart Institute (InCor – HCFMUSP), São Paulo, Brazil. They ate 2 different diets in 2 simple meals 1 week apart (Figure 1). Meal 1 was composed of a balanced diet with rice, juice, and standard red meat. Meal 2 had the same composition as meal 1 but contained lean meat obtained by the cross between 2 breeds: Rubia Gallega and Nelore (Table 1). Meat was served as hamburger obtained from the same cut of beef for both types of meat. Blood samples were obtained at baseline, 1 and 2 hours after ingestion of meal 1 (H1 and H2) and meal 2 (H3 and H4). Serum levels of IL-6, hs-CRP, VCAM, ICAM, p-selectin, Apo-A1, and Apo-B were compared during these prespecified times.



**Figure 1:** Design of the study.

Portion of 100g	Standard Red Meat	Comparative Red Meat
Calories	220 Kcal	141 Kcal
Carbohydrates	1.8 g	0.7 g
Proteins	24.9 g	22 g
Total Fat	13.8 g	5.8 g
Saturated Fat	5.5 g	2.8 g
Trans Fat	0.8 g	0.3 g
Fiber	0.8 g	0 g
Sodium	70.6 mg	55 mg

**Table 1:** Nutritional Facts for Both Kinds of Meat.

### Statistics

Baseline characteristics were summarized for all patients as percentages for categorical variables and as means with standard deviations for continuous variables. Comparisons between means of the groups used the Student *t* test for parametric and the Mann-Whitney test for nonparametric variables. The means of 3 or more groups were compared by 1-way ANOVA, followed by the Bonferroni multiple comparison test for parametric variables. For nonparametric variables, we used the Kruskal-Wallis test, followed by multiple comparisons based on Dunn's test. Tests were 2-sided. Values of  $P < 0.05$  were considered statistically significant. Statistical analysis was performed by using SPSS 21.0 for MAC.

### Ethics

Patients gave written informed consent and were randomly assigned to a specific group. The Ethics Committee of the Heart Institute (InCor) of the University of São Paulo Medical School in São Paulo, Brazil, approved the trial, and all procedures were performed in accordance with the Helsinki Declaration.

### Results

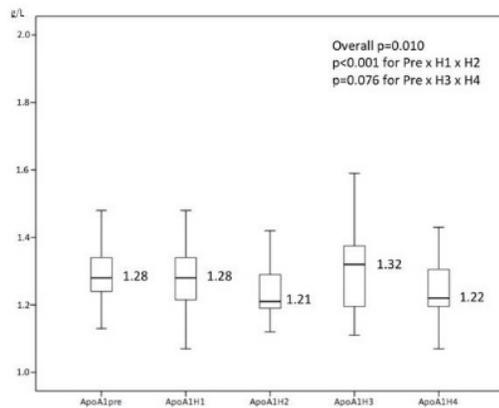
Twenty healthy men participated in this study. Mean age was  $30.5 \pm 2.89$ , and they had normal glucose blood levels ( $84.7 \pm 9.12$ ) and cholesterol levels (LDL  $113.1 \pm 27.15$ ; HDL  $44.6 \pm 10.3$ , and TG  $100.28 \pm 55$ ) (Table 2).

Characteristics	Mean	SD
Age (y)	30.55	2.89
Glucose (mg/dL)	84.79	9.12
Creatinine (mg/dL)	1.18	0.14

TC (mg/dL)	177.89	33.27
LDL (mg/dL)	113.11	27.15
HDL (mg/dL)	44.67	10.3
TG (mg/dL)	100.28	55
AST U/L	24.11	11.7
ALT U/L	34.95	8.69
Abbreviations: TC = Total Cholesterol; LDL = low-Density Lipoprotein; HDL = High-Density Lipoprotein; TG = Triglycerides; AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase.		

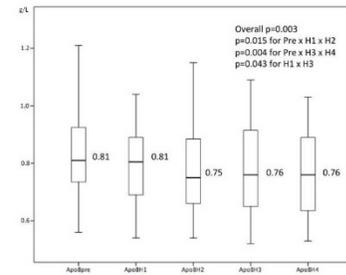
**Table 2:** Baseline Characteristics of Subjects.

Apo-A1 mean levels (ng/mL) varied after ingestion of meal 1 but not after meal 2 (baseline: 1.28; H1:1.28; H2: 1.21; H3:1.32 and H4:1.22; overall  $p=0.010$ ;  $p<0.001$  for baseline versus H1 and H2;  $p=0.076$  for baseline versus H1 and H2) (Figure 2).



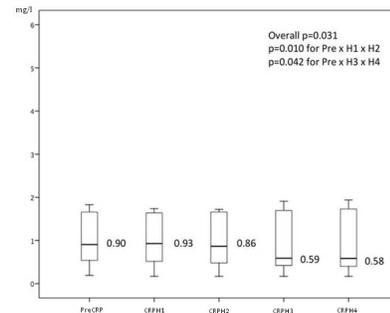
**Figure 2:** Levels of ApoA1 (g/L) at baseline, 1h and 2h after ingestion of meal 1 (H1 and H2) and meal 2 (H3 and H4).

Serum Apo B levels (ng/mL) were also different from baseline for meal 1 and 2 (baseline: 0.81; H1:0.81; H2:0.75; H3:0.76; H4:0.76; overall  $p=0.003$ ). Differences were observed for both meals compared with baseline ( $p=0.015$  for baseline versus H1 and H2;  $p=0.004$  for baseline versus H3 and H4). Additionally, we observed lower levels of Apo-B 1h after ingestion of meal 2 compared with meal 1 ( $p=0.043$  for H1 versus H3) (Figure 3).



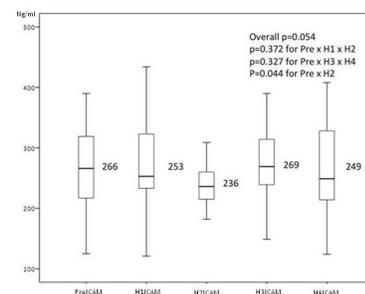
**Figure 3:** Levels of ApoB (g/L) at baseline, 1h and 2h after ingestion of meal 1 (H1 and H2) and meal 2 (H3 and H4).

Levels of hs-CRP were lower after ingestion of meal 2 compared to meal 1 (baseline: 0.90; H1:0.93; H2:0.86; H3:0.59; and H4:0.58; overall  $p=0.031$ ) (Figure 4).

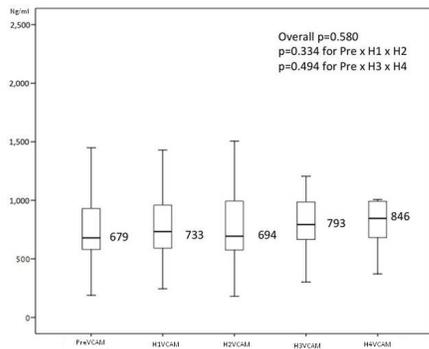


**Figure 4:** Levels of Hs-CRP (mg/L) at baseline, 1h and 2h after ingestion of meal 1 (H1 and H2) and meal 2 (H3 and H4).

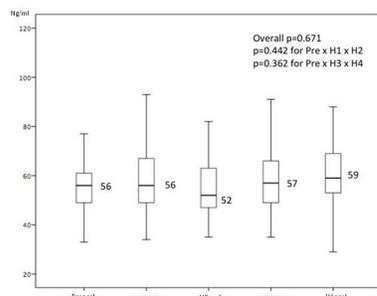
No differences were observed between meal 1 and meal 2 regarding ICAM, VCAM, p-selectin, or IL-6 serum levels ( $p=0.054$ ,  $p=0.580$ ,  $p=0.671$ , and  $p=0.938$  respectively) (Figures 5-8).



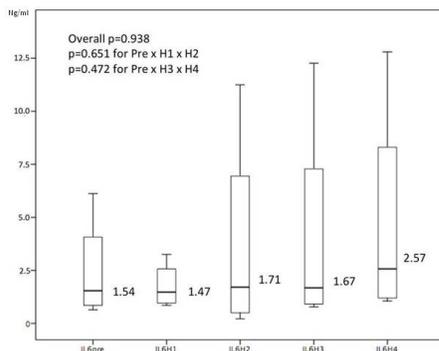
**Figure 5:** Levels of ICAM (ng/mL) at baseline, 1h and 2h after ingestion of meal 1 (H1 and H2) and meal 2 (H3 and H4).



**Figure 6:** Levels of VCAM (ng/mL) at baseline, 1h and 2h after ingestion of meal 1 (H1 and H2) and meal 2 (H3 and H4).



**Figure 7:** Levels of p-selectin (ng/mL) at baseline, 1h and 2h after ingestion of meal 1 (H1 and H2) and meal 2 (H3 and H4).



**Figure 8:** Levels of IL-6 (ng/mL) at baseline, 1h and 2h after ingestion of meal 1 (H1 and H2) and meal 2 (H3 and H4).

## Discussion

This single-centre randomized study evaluated the atherogenic profile after ingestion of 2 different kinds of meat of the same species and found a most favourable profile of genetically selected meat compared with standard meat. Lower levels of ApoB and CRP after ingestion corroborated this fact. Metabolically triggered inflammation has been traditionally associated with

modifications in levels of apolipoproteins, hs-CRP, and other inflammatory mediators immediately after ingestion of a meal. Thus, the degree of inflammation triggered by a specific kind of food could be objectively measured. A more atherogenic profile has been associated with higher levels of inflammatory mediators (such as hs-CRP, IL-6, VCAM, ICAM, and p-selectin) besides higher levels of ApoB, and lower levels of ApoA1. In fact, non-healthy food ingestion has been related to a more atherogenic profile [10].

Ingestion of red meat is traditionally associated with unhealthy habits, and some diets, such as the Mediterranean Diet, restrict its ingestion favouring intake of fish or poultry [11]. However, some previous trials have demonstrated that red meat is not uniform in terms of atherogenic profile after ingestion. Type of meat and amount of evident fat are factors that could interfere with the release of inflammation mediators after ingestion of meat. It is important to point out that different kind of red meat contains different proportions of saturated fat [12]. Emerson et al. in a systematic review found consistent evidence for postprandial elevation of IL-6 after ingestion of a high-fat meal but not for other markers of inflammation [13].

Recently, Bergeron et al. [14] conducted a randomized controlled trial to test whether levels of atherogenic lipids and lipoproteins differed significantly following consumption of diets with high red meat content compared with diets with similar amounts of protein derived from white meat or nonmeat sources, and whether these effects were modified by concomitant intake of high compared with low SFA. They found that LDL cholesterol and apoB were higher with red and white meat than with nonmeat, independent of SFA content. However, levels of LDL cholesterol, apoB, small + medium LDL, and total/HDL cholesterol ratio did not differ significantly between red and white meat. Besides, independent of protein source, high compared with low SFA increased LDL cholesterol ( $p = 0.0003$ ), apoB ( $p = 0.0002$ ), and large LDL ( $p = 0.0002$ ). These findings confirm that most differences after ingestion of source of proteins are related to SFA content.

Comparing different kind of meat in relation to race, Arya and colleagues found that postprandial levels for 1 and 2 h of TAG, IL-6 and TNF- $\alpha$  were significantly higher after eating wagyu meat compared with kangaroo, concluding that the meta-inflammatory reaction to ingestion of a ‘new’ form of hybridized beef (wagyu) is indicative of a low-grade, systemic, immune reaction compared with lean game meat (kangaroo). Noteworthy is the fact that not only the kind of meat tested was different but also the amount of fat in both tested meats were very different; wagyu is one of the fattiest meats currently available, and even the visible fat of kangaroo meat was removed in this study [8].

In fact, the meat derived from crossing of Rubia Gallega and

Nelore breeds has fewer calories, total and saturated fat, and sodium compared with standard meat. Thus, differences in postprandial biomarkers could be explained not only by the kind of meat itself but also by its nutritional content. Some final considerations must be made. Different from previous trials with a similar methodology, our study compared 2 kinds of bovine red meat from the same beef cut offered as hamburger in a double-blind approach. Thus, differences observed between them would be explained by the origin of the meat and its constitution in terms of fat and sodium. However, the current study has limitations. Blood samples were collected just after a single meal for each kind of meat, and not after a period of daily ingestion of the meats. Therefore, impact of daily ingestion of red meat was not assessed in this study.

## Conclusions

In conclusion, lean red meat from a cross of Rubia Gallega and Nelore leads to a less atherogenic profile after ingestion than standard meat, regarding to Apo-A1, Apo-B, and hs-CRP levels. These findings are in keeping with the understanding that even when red meat is obtained from the same species and the same cut, a breed containing lean meat could be associated to lower inflammation after ingestion.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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