J Am Coll Nutr. 38:4: 334-341; **2019.**

COAGULATION, THROMBOGENESIS AND INSULIN RESISTANCE MARKER IN INCREASED CARDIOVASCULAR RISK SUBJECTS CONSUMING IMPROVED FAT MEAT PRODUCTS

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Short title: Improved meat products and cardiovascular risk

Abstract word count: 192

Word count from Introduction to References: 3154.

Abstract

Objectives- Cardiovascular disease (CVD) risk is prevalent in high meat-product consumers. The effect of consuming lipid-improved pâtés/frankfurters on plasma LDL-cholesterol, thromboxane A_2 (as TXB_2), prostacyclin I_2 (as 6-keto-$PGF_{1\alpha}$), activated partial thromboplastin time, fibrinogen, antithrombin and insulin-resistance/sensitivity markers in volunteers at high CVD risk was studied.

Subjects/methods- Eighteen male volunteers enrolled in a blind crossover-controlled study consumed improved products during three 4-wk periods: reduced fat (RF); n3-enriched-RF (n-3RF), and normal fat (NF), separated by 4-wk washouts.

Results- Fibrinogen and 6-keto-PG1α decreased ($P<0.05$) following the RF-period; LDL-cholesterol, TXB_2 and 6-keto-$PGF_{1\alpha}$ decreased ($P<0.05$) after the n-3RF-period, while LDL-cholesterol, fibrinogen, TXB_2, insulin and HOMA-IR increased (at least $P < 0.05$) while QUICKI decreased ($P<0.05$) during the NF-period. The rate of changes of fibrinogen, TXB_2 and 6-keto-$PGF_{1\alpha}$ and HOMA-IR differ between groups (repeated measures test $P<0.05$). Fibrinogen, insulin, and HOMA-IR differed significantly ($P<0.05$) between RF and n-3RF period *vs.* NF period while that of TXB_2 and 6-keto-$PGF_{1\alpha}$ differed between n-3RF *vs* NF periods ($P<0.05$).

Conclusions- The consumption of n-3RF meat-products followed by RF ones, partially reduced thrombogenesis, coagulation, and insulin-resistance markers. Thus, the inclusion of lipid-improved pâtés/frankfurters might be recommended into dietary strategies in at CVD-risk volunteers.

Keywords: modified fat; meat-products; coagulation; fibrinogen; prostacyclin; thromboxane; CVD-risk; insulin; HOMA-IR; controlled study

Abbreviations: AI, Atherogenic index; APTT, Activated Partial Thromboplastin Time; CVD, Cardiovascular disease; DHA, docasahexaenoic acid; EPA, eicosapentaenoic acid; HOMA-IR, Homeostatic Model Assessment–insulin resistance; MUFA, monounsaturated fatty acids; NF-products, normal fat-meat-products; RF-products, reduced fat-meat-products; n-3RF-products, n-3 enriched-reduced fat-meat products; PGI, prostacyclin; PUFA, polyunsaturated fatty acids; QUICKI, Quantitative Insulin Sensitivity Check Index; SFA, Saturated fatty acids, T2DM, Type 2 Diabetes Mellitus; TI, Thrombogenic index; TX, thromboxan..

INTRODUCTION

Large consumption of processed meats has been positively associated with the incidence of cardiovascular diseases (CVD) [1-4] and diabetes [1,3,5]. Despite the large number meat-based potentially functional foods developed using highly diverse strategies, most of them addressed to reduce their fat composition and atherogenic and thrombogenic indices [6-8], the effect of their consumption in humans has been scarcely investigated. In addition most studies tested the effect of meat consumption on classical cardiovascular disease markers [9-12] but very few refer on insulin resistance, coagulation, and thrombogenesis, all of them directly involved in the atherosclerosis and thrombotic event of CVD [13].

Despite numerous studies on cardioprotective effects of omega-3 polyunsaturated fatty acids (n-3 PUFAs), there is limited evidence for n-3 PUFA-enriched meat-based product consumption. In addition, to the best of our knowledge, non-studies have been performed on the effect of a meat-matrix with improved docosahexaenoic acid (DHA)-rich fat content on eicosanoid production and blood coagulation factors.

Taken into account that dietary intake of improved-fat pâtés and frankfurters in subjects with CVD risk exerted some benefits in lipid and anthropometric profile [14-16], we hypothesize that the consumption of improved-fat frankfurters and pâtés, formulated by replacing animal fat with a combination of olive, flax (refined linseed oil) and fish oils, has beneficial effects on thrombogenesis, coagulation, and insulin-resistance of volunteers at increased CVD risk. The aims of present paper were to ascertain the effect of different types of improved fat pâtés and frankfurters in participants at increased CVD risk a) plasma activated partial thromboplastin time, fibrinogen, and antithrombin; b) serum thromboxane and prostacyclin; c) plasma glucose, insulin and insulin resistance/sensitivity; d) plasma LDL-cholesterol, blood pressure and urine excretion were determined.

MATERIALS AND METHODS.

Participants

The study was approved by the Ethical Committee for Clinical Investigation of Hospital Universitario Puerta de Hierro-Madajahonda (Spain) (Acta nº 261, dated 20/12/2010) and the Bioethical Committee of the Spanish National Research Council. v The study was carried out according to the Helsinki Declaration (59ª General Assembly,

Seúl, Corea, October 2008) and the Good Clinical Practices. Study design and flow chart have been published in detail elsewhere [14,15]. Selected participants fulfilled the inclusion criteria: total cholesterol levels ≥ 5.2 mmol/L, LDL cholesterol levels ≥ 2.84 mmol/L), overweight (BMI, 25–34.9 kg/m^2) and willingness to consume 200g of frankfurters and 250g of pâté per week. Exclusion criteria were: use of drugs or plant sterol-enriched beverages/foods to control cholesterol levels, hypertension or obesity; regular consumption of n-3 enriched food products; intolerance of or allergy to any of the components of the meat products. Participants were requested to live as they did before the study, maintaining their normal pattern of activity, and were urged to replace meat and meat products in their habitual diet with the pork-products provided and to maintain a varied diet. All subjects gave their written informed consent after receiving oral and written information about the study.

Meat-products

Before each phase of the study, batches of 6 kg pâté and 6 kg frankfurters was developed in the Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN, CSIC, Madrid, Spain) following the standard procedure with slight modifications [17,18].

Three different type of pork-products were prepared: a) reduced-fat products (15% fat) (RF); b) reduced-fat (15% fat) n-3 enriched pork products (n-3RF) where pork backfat was replaced by a combination of olive, flax, and fish oils (composition percentages 44.39, 37.87 and 17.74, respectively); and c) normal-fat control-products (NF) (18% fat for frankfurters, 30% fat for pâtés).The resulting products were stored in refrigeration in plastic bags under vacuum till use. Volunteers received the corresponding meat-product every two weeks.

Intervention study design

Participants were enrolled in a non-randomized, crossover, controlled study of 5 months duration. The study was blind to volunteers, who did not know what kind of meat product they were eating. The study consisted in three experimental periods of 4 weeks. Each participant consumed 200g frankfurters and 250g pâtés per week in each of three experimental periods, separated by 4-week washout periods.

During the first dietary intervention volunteers consumed RF-products in which the fat source was 100% of animal origin. In the second dietary intervention they

consumed the RF-n-3-enriched frankfurters and pâtés, providing a total of 2g of n-3 fatty acids per day, of which 1.5 g was linolenic acid and 0.4 g eicosapentaenoic acid (EPA) plus DHA. During the third dietary intervention NF-frankfurters and pâtés were consumed. These NF-products were similar in fat content and composition to those usually found in the market.

Fatty acid composition of frankfurters and pâtés was determined by gas chromatography as reported by López-López *et al* [19]. Based on fatty acid composition, the atherogenic index (AI) and thrombogenic index (TI) of the control and improved meat-products were calculated according to to Ulbricht and Southgate [20].

AI= (C12:0 + 4*C14:0 + C16:0) / (n-3 PUFA + n-6 PUFA+ MUFA)

TI= (C14:0 + C16:0 + C18:0) / [0.5 n-6 PUFA + 3 n-3 PUFA + (n-3 PUFA / n-6 PUFA)]

Where MUFA are monounsaturated fatty acids and PUFA, polyunsaturated fatty acids.

Diet evaluation

Participants were interviewed by a dietician for 1 to 1.5-h. Food intake and food habits were registered. 72-h dietary register were collected at initial and at the end of the three experimental periods, thus a total of six measurements per volunteers were performed. Photographs of sample portions were used to estimate the serving size and volumes consumed when this information was not clearly available. The energy and nutrient intakes per person were calculated using a computer program (DIAL^{TM} Madrid, Spain) [21] to evaluate diet contribution to recommended energy and protein intakes and the diet quality consumed.

Biochemical analysis

Blood samples were collected by venepuncture after 12-h overnight fasting. Serum or plasma were obtained from blood or citrated blood, respectively by centrifugation at 2,500 rpm during 10 min at room temperature and maintained at -80ºC±5 until analysis. Six measurements were performed: Initial and at the end of the three experimental periods. In order to to minimize inter-day analytical variability, the six samples from each participant were analysed on the same day. LDL-cholesterol concentrations were assessed by enzymatic colorimetric kit BioSystems Direct LDL.

Platelet count was performed in a haemocytometer according to Canales *et al*. [22]. Citrated plasma was used for measuring the activated partial thromboplastin time (APTT) (Spinreact reagent products, Gerona, Spain), fibrinogen and Antithrombin III (radial immunodiffusion in EASY RID Liofilmchem (Teramo, Italy). Serum TXB_2 and serum 6-keto Prostaglandin (6-keto PGF1α), stable metabolites of TXA_2, and prostacyclin I_2 (PGI_2) respectively, were determined by enzyme immunoabsorbent assays (Cayman Chemical Company) according to the manufacturer's manual. Systolic and diastolic blood pressures were measured following WHO recommendations [23] with a CORYSAN CE-0398 sphygmomanometer (Barcelona, Spain).

Serum glucose was determined according to the Glucose oxidase/peroxidase enzymatic method (Biosystems) in a Lambda 15 UV/VIS spectrophotometer, Perkin-Elmer, USA. Insulin concentrations were determined by electrochemiluminescence immunoassay (ECLIA) in an (Immunoanalyzer Elecsys y Cobas e, Roche Diagnostics (Basilea, Suiza).

All assays were calibrated using internal and external quality controls provided by the manufacturers. Intra- and inter-assay variation coefficients were 0.7 and 2% for LDL-cholesterol; 5 and 15% for fibrinogen and Antithrombin III; 6 and 6.8 % for TXB_2; 6.7 and 18.1 % for 6-keto $PGF_{1\alpha}$; 2 and 1.7% for glucose, 1.5 and 4.9% for insulin, respectively. APTT variant coefficients were not determined. Insulin resistance or sensitivity were tested using the Quantitative Insulin Sensitivity Check Index (QUICKI) [24], and HOMA-IR [25].

Statistical analysis

LDL-cholesterol change was considered as the major outcome. Sample size calculation was performed on the basis of a mean value for 3.36 mmol/L LDL-cholesterol. A sample size of 18 subject (SD=0.41 mmol/L) is necessary to obtain a 10% difference in LDL-cholesterol values (0.36 mmol/L) between two consecutive visits with 90% power and an alpha error of 0.05, considering a SD of 12% of LDL-cholesterol mean value.. The Kolmogorov-Smirnov test was used to assess normal value data distribution. TXB2 and 6-keto- PGF1α levels and responses were normalized by natural logarithm transformation. Spearman correlations were assessed. The paired Student *t*-test was used to determine the effect of the different meat products in each intervention period. The rate of change differences (%) [100* (Final value - Basal value)/Basal value] between periods was stated using the general linear model of

repeated measures (GLM)-followed by Least Standard deviation (LSD) *post hoc* test. The resultant changes of RF *vs.* NF or n-3RF *vs.* NF periods were also stated. The RF *vs.* NF periods comparison was performed after considering that the washout period was appropriated to delate the effect of the intermediate n-3RF period. . As smoking could potentially affect thrombogenesis and other CVD risk factors [26], the effect of smoking was also tested as covariable Significances were set at $P<0.05$ using the SPSS 22.0 package.

RESULTS

A detailed relation of baseline participant characteristics is shown in **Table 1**. Starting the study, and according to the cut-off point selected, 22% of participants were obese, 33% had increased waist circumferences, 72% presented hypercholesterolemia, 22% hypertriglyceridemia; 11% and 33% were hypertensive according to the systolic and diastolic blood pressures, respectively, 20% smoked between 10 and 20 cigarettes a day and 100% were sedentary. Their recommended energy intakes were higher (7.88± 5.89 %) than those of male adults of same age weighing 70 kg. Although none of participants followed hypocaloric diets, the energy intake was assessed to be about 10% lower than their respective theoretical energy expenditure [27]. Protein intakes were 72% higher than the protein recommended energy intakes for age matched individuals.

Baseline energy contribution (%En) for the RF-period from carbohydrates, protein, fat, and alcohol was 40.2 ± 8.4; 16.1 ± 3.3; 41.0 ± 9.2; and 10.0± 13.9, respectively. The energy contribution from SFA, MUFA, and PUFA was 14.1 ± 3.1; 18.3 ± 5.8, and 5.1 ± 1.6, respectively. Information for vitamins and minerals have been already reported [15]

Details of energy, macronutrient and fatty acid composition of the assayed pork products are shown in **Table 2**. NF pâtés were richer than NF frankfurters in energy, fat, cholesterol. RF and n-3RF presented lower energy, fat and SFA, MUFA and PUFA than NF-products, with n-3RF pâtés/frankfurters presenting, much higher total n-3 PUFA, EPA and DHA but a n-6/n-3 ratio 10-times lower than the other assayed meat-products. The IA and IT were lower in n-3RF products than in the RF and NF ones.

The energy contribution of lipids and fatty acids of diets has been included in Figure 1. The consumption of n-3RF products increased PUFA and n-3 PUFA %En in comparison with their respective basals.

Table 3 shows that the rate of changes of fibrinogen, TXB_2 and 6-keto-$PGF_{1\alpha}$ and HOMA-IR differ between groups according to GLM analysis ($P<0.05$). Fibrinogen and 6-keto-$PG_{1\alpha}$ levels decreased (both $P<0.05$) following the RF-period. After the n-3RF period, LDL-cholesterol ($P<0.05$), TXB_2 ($P<0.05$), and 6-keto-$PG_{1\alpha}$ ($P<0.001$) levels decreased while LDL-cholesterol ($P<0.05$), fibrinogen ($P<0.05$), TXB_2 ($P<0.01$), insulin ($P<0.05$), and HOMA-IR ($P<0.05$) increased during the NF-period. Following NF-period QUICKI decreased ($P<0.05$). The rate of changes of fibrinogen and HOMA-IR differed ($P<0.05$) between RF and n-3RF period *vs*. NF period while that of TXB_2 and 6-keto-$PGF_{1\alpha}$ differed between n-3RF *vs*. NF periods ($P<0.05$).

Table 4 indicates the significant differences between NF and RF-periods and between NF and n-3RF periods. In comparison to NF products, n-3 RF products significantly reduced LDL (16%), TXB_2 (92.3%) and 6-keto PGF1α (59%). Net fibrinogen and HOMA-IR change between NF *vs*. RF and NF *vs*. n-3RF were significant (all P=0.05 and >20%).

DISCUSSION

To the best of our knowledge, there have been very few studies [28-30], other than those of our group [22], on n-3-enriched meat-products on eicosanoids and blood coagulation factors.

Diet at baseline for the RF-period were similar to those observed in other Spanish studies, with a low energy contribution from carbohydrates, a high to very high contribution from fat (41.0 ± 9.2 %En). Diets were also rich in SFA but moderate in PUFA [31].

As previously stated [15], except for n-3PUFA, the dietary energy contribution of macronutrients during the three intervention periods was similar. Therefore, participants seem to have kept their dietary habits and hence the intake of nutrients throughout the study [15]. The consumption of n-3RF products increased PUFA and n-3 PUFA %En in comparison with their respective basals (**Figure 1**). Similarly DHA, EPA, and linolenic acid consumption also increased (data not shown). Furthermore, in n-3RF period %En of lipid and SFA were significant lower while during NF-period, %En MUFA increased with respect to their respective basals.

The net difference in LDL-cholesterol change between NF and n-3RF periods found could entail an important reduction (more than 34%) in the combined end point

of death from cardiac disease, nonfatal myocardial infarction, and fatal myocardial infarction [32]. Results should be related to differences in the AI ratios and fat contents of n-3RF product vs. NF one. Different studies have demonstrated that low-density lipoprotein cholesterol (LDL-cholesterol) enhances platelet activation, leads to platelet hyperactivity, and subsequently increases the risk of arterial thrombosis [33]. Thus, high LDL levels would affect platelet thrombogenesis and in turn, blood coagulation, mainly through intrinsic pathways. However, the dietary interventions with improved meat-based foods were not able to modify APTT, which reflects the activities of several coagulation factors [34]. Four-week omega-3 PUFA supplementation reduced thrombotic potential in healthy subjects, as shown by reduced fibrin generation and peak thrombin. There was a greater effect on fibrin generation in healthy subjects compared with those with CVD [29]. Poreva *et al*. [30] observed that in patients with long-standing, well-controlled DM2 and atherosclerotic disease the treatment with a high dose of n-3 PUFAs (namely, 1 g/day of EPA and 1 g/day of DHA for 3 months) does not improve coagulation.

Insulin resistance is the major metabolic fact of Metabolic syndrome and type 2 Diabetes Mellitus (T2DM) and has been found to modify several CVD risk factors [35]. Moreover, blood coagulation and thrombogenesis are directly involved in the atherosclerotic and thrombotic events of CVD [12] [13] and in turn in Metabolic syndrome.

High fibrinogen levels are prevalent in obese and overweight subjects [36]. Fibrinogen significantly decreased following the RF period. Previously we observed a slight but significantly decrease in body fat mass (%) during the RF period [15], suggesting that the decrease in fibrinogen and fat mass were related. Following n-3RF period fibrinogen tended to decrease (8.1%, P=0.08). Moertl *et al*. [37] did not find any effects on fibrinogen after 4g/day n-3 fatty acid consumption. The net difference in fibrinogen change between NF *vs*. RF and NF *vs*. n-3RF, suggest that the production of this acute-phase reactant was decreased by the consumption of both improved meat-based products with respect to that of normal one. These results seem interesting as fibrinogen stimulates smooth muscle cell migration and proliferation and modulating platelet aggregation and blood viscosity [38].

Although available information on the effect of fatty acids on blood coagulation factors is scarce, several authors have reported that platelet phospholipids, their derived eicosanoids, and thrombogenesis are strongly affected by dietary fatty acid content [9-

12]. However, to the best of our knowledge, non-studies have been performed on the effect of a meat-matrix with improved DHA-rich fat content on eicosanoid production. Reductions observed in TXB_2 levels appeared related to the fatty acid composition of the meat products. SFAs have been found to promote thrombogenesis [10] by increasing TXB_2 [11,39] while n-3 fatty acids to reduce arterial thrombi production and synthesis of the series-2 eicosanoids (e.g. TXA_2) [12,22,40]. In a previous paper our group reported that the susbtitution of oleic acid enriched-sunflower oil by palmolein in the diet of postmenopausal women greatly increased the TXB_2 in urine [41]. In addition the consumption of a meat enriched in walnut (rich in linolenic acid) *vs.* a low-fat meat reduced the TXB_2 [22].

The net difference in TXB_2 rate of change between NF and n-3RF periods (ca. 90%) seems important and highlights the effect of n-3RF products on thrombogenesis. These results are relevant as a relationship between high LDL-cholesterol and platelet activation or thrombogenesis has been reported [42]. Besides the decrease in TXB_2, the intake of n-3 fatty acids during the n-3RF period reduced 6-keto-$PGF_{1\alpha}$ levels. A significant positive correlation was observed throughout the present study between the rate of changes in TXB_2 and 6-keto-$PGF_{1\alpha}$, ($r=0.365$; $P<0.01$). Chan *et al*. [43] found a reduction in the synthesis of both TXA_2 and PGI_2, after substituting an oil mixture with a high-linoleic/linolenic ratio with an intermediate- or low-linoleic/linolenic acid mixture. The n-3RF product contained significant amounts of EPA [17], and EPA is known to be a poor substrate for cyclo-oxygenase; resulting in reduced levels of both TXB_2 and PGI_2. Furthermore, the high level of oleic acid in the meat-products consumed during the n-3RF period could also be responsible for this effect, as it has been demonstrated by Sanchez-Muniz *et al*. [38] that the platelet TXB_2 production after ADP activation and TXB_2 in urine was lower following high oleic sunflower oil diet *vs*. palm olein diet. Chicken-meat naturally enriched with algae-sourced omega-3-PUFA in healthy volunteers increased amount of plasma omega-3-PUFA and diminished systolic blood pressure and urinary thromboxane values [28]. Differences in the TXB_2 rate of change between periods have to be related to differences on TI index of meat based foods (1.17 in n-3 RF and 0.18 in NF) and in the amount of fat of those products (almost twice higher in the NF than in the RF one). Thus, the consumption of NF products came to 111g fat per week and that of n-3RF or RF products to 65g fat per week, supporting a “reduced fat-products” European nutritional claim [44].

The consumption of n-3RF products *vs.* that of NF shows net benefits on insulinemia and HOMA-IR. Nonetheless, none of the participants showed glycemia ≥7 mmol/L, and initial HOMA-IR values were much lower than those observed in T2DM patients, a fact that could limit the effects of the meat-products tested on insulin markers in some way. Low carbohydrates/SFA ratios have been related to insulin-resistance [11]. An increase in the carbohydrate/SFA ratio was observed during the RF and n-3 RF periods *vs.* the NF one. Previously we have reported that the consumption of RF and n-3RF products increased PUFA intake in comparison with their respective basal values [15]. In addition, the decrease in the dietary n-6/n-3 ratio observed and the change in the carbohydrate/SFA ratio mainly in the n-3RF period could also affect HOMA-IR [11,45]. The absence of significant changes on blood pressure has to be related to the relatively low n-3 PUFA consumed [43].

Although active/first-hand smoke, passive/second-hand smoke exposure and residual tobacco smoke contaminant that remains after a cigarette is extinguished are known to increase the risk of coronary thrombosis [26], the low prevalence of smokers and the relatively low number of cigarettes smoked a day explain, at least in part, why smoking or of its interaction with diet did not influence our results.

Our study has some limitations: a) The study intervention period order was not randomized; b) the amount of meat-products consumed, although compatible with normal feeding, was relatively high; b) only pâtés and frankfurters were tested; c) only males at CVD risk were studied; and d) the number of volunteers studied was relatively small. Any generalization about consumption of improved fat-functional meat-products should be avoided as the observed effects were not of same magnitude for the markers studied. However, the study has the strength of being the first to consider the response of selected eicosanoids, coagulation factors and insulin resistance markers to fat-improved pork-products in subjects at high risk of cardiovascular disease. In addition, it was blinded to the participant. The assayed n-3RF products meet the criteria for normal cardiac function of the European Food Safety Authority (EFSA) for n-3 fatty acid health claims [41].

CONCLUSIONS

In view of the reductions observed in fibrinogen, LDL-cholesterol, TXB_2, and insulin resistance/sensitivity markers the consumption of RF and n-3RF products seems preferable to that of NF products in those at CVD-risk participants. As improved meat-

derivate partially blocked the modifications on thrombogenesis, coagulation and insulin resistance promoted by NF products, the regular and moderate consumption of those improved meat-derivate by individuals at CVD-risk is highly recommended.

ACKNOWLEDGEMENTS

This research was supported by AGL 2014-53207-C2-1R and 2014-53207-C2-2R and Intramural CSIC: 201470E056 projects. We are grateful for the voluntary participation and the statistical study assessment carried out by Laura Barrios from the Subdirección General de Apoyo a la Investigación of CSIC.

Conflict of interest: None

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Footnotes of Figure 1

Figure 1. Contribution of protein, carbohydrates, lipids, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), n-6PUFA and n-3PUFA to total energy (%Energy) during the n-3RF period. ■ Meat and meat-products; □ Improved meat products; ▥ Rest of the diet. Basal refers to data obtained the previous week before starting any respective experimental period. *P<0.05, **P<0.01 and ***P<0.001 significant differences between basal and final values.

Table 1. Baseline characteristics of the volunteers at the start of the intervention study

	Baseline levels	**Cut-off point**
	Mean ± SD	
Age (years)	44.9 ± 10.3	
BMI (kg/m^2)	28.6 ± 2.5	≥30 kg/m^2†
Weight (kg)	84.8 ± 10.3	
Waist circumference (cm)	100.3 ± 7.1	≥102 cm†
Body Fat Mass (%)	29.2 ± 4.0	≥20%
Total cholesterol (mmol/L)	5.93 ± 0.53	≥5.69 mmol/L†
Triglycerides (mmol/L)	1.48 ± 0.86	≥1.69 mmol/L†
Glucose (mmol/L)	4.97 ± 0.93	≥6.67 mmol/L†
Systolic blood pressure (mmHg)	120.3 ± 10.5	≥130 mmHg†
Diastolic blood pressure (mmHg)	76.6 ± 9.6	≥85 mmHg†
Alcohol consumption	7 never 9, sometimes (1-2 drink/week) ǂ 2, daily (1 drink)	
Smoking	13, non-smokers 5, smokers (10-20 cigarettes/day)	
Actual Energy expenditure (TEE)*	0.92 ± 0.60 (5, 0.90-0.95 TEE; 6, >0.90 TEE)	
Physical activity	100% sedentary**	

Values are means and standard deviations for eighteen male volunteers).
†According to NCEP-ATP III [47]
ǂOne alcohol ration corresponds to 15g alcohol.
*With respect to recommended energy for 70 kg age active matched men [27]
**According to FAO/WHO/UNU [27].

Table 2. Energy and macronutrients (composition per 100 g wet matter), percentage of total fatty acids, atherogenic and thrombogenic indices of the assayed pâtés and frankfurters

Meat products	**Pâtés**			**Frankfurters**		
	RF	**n-3RF**	**NF**	**RF**	**n-3RF**	**NF**
Energy (kcal)	203	212	346	213	217	239
Protein (g)	13.3	14.2	13.2	17.9	19.4	18.3
Carbohydrates (g)	1.3	1.4	1.3	0	0.09	0
Total fat (g)	15.2	15.5	30.8	15.3	15.1	18
SFA (g)	4.7	3.1	9.5	5.7	2.8	6.7
MUFA (g)	8.4	6.3	17.1	7.2	6.7	8.5
n-6 PUFA (g)	1.1	1.6	2.4	1.3	1.1	1.6
n-3 PUFA (g)	0.2	3.6	0.2	0.2	3.7	0.2
Dietary fibre (g)	0	0.29	0	0	0.31	0
Cholesterol (mg)	138	129	147	49.8	41	51.4
% of total fatty acids						
SFA	32.3	21.3	32.3	39.4	19.3	39.4
Myristic acid	1.1	1.1	1.1	1.16	1.11	1.16
Palmitic acid	20.5	13.6	20.5	23.4	11.4	23.4
Stearic acid	9.9	6.0	9.9	13.8	5.4	13.8
Arachidic acid		-		0.3	0.4	0.3
Other SFAs		0.6		0.8	1.1	0.8
MUFA	58.3	42.5	58.3	49.5	46.9	49.5
Palmitoleic acid	2.1	2.0	2.1	1.8	1.6	1.8
Oleic acid	50.5	37.4	50.5	42.5	42.5	42.5
Vaccenic acid	3.9	2.8	3.9	3.6	2.3	3.6
Eicosanoic acid	1.4	0.3	1.4	1.2	0.4	1.2
Other MUFAs	0.5	-	0.5	0.5	-	0.5
PUFA	9.0	35.6	9.0	10.6	33.6	10.6
Linoleic acid	7.4	11.6	7.4	8.6	10.8	8.6
Linolenic acid	0.5	17.5	0.5	0.6	17.7	0.6
Eicosadienoic acid	0.5	-	0.5	0.5	-	0.5
Arachidonic acid		-		0.4	-	0.4
Eicosapentaenoic acid	-	2.7	-	-	2.6	-
Docosapentaenoic acid	-	0.5	-	-	0.4	-
Docosahexaenoic acid	-	1.8	-	-	1.7	-
Other PUFAs		1.5		0.5	0.5	0.5
PUFA/SFA	0.3	1.7	0.3	0.3	1.7	0.3
∑n-3 PUFA	1.2	24.0	1.2	1.04	22.8	1.04
∑n-6 PUFA	7.9	11.6	7.9	9.6	10.8	9.6
n-6/n-3	6.58	0.48	6.58	9.23	0.47	9.23
AI	0.37	0.23	0.37	0.47	0.2	0.47
TI	0.86	0.20	0.86	1.17	0.18	1.7

Modified from Delgado *et al* [17,18]. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. AI, atherogenic index, TI, thrombogenic index

Table 3. Blood coagulation factors, plasma eicosanoids, insulin resistance markers, systolic and diastolic blood pressure values response after 4-wk consumption of reduced fat (RF); n-3RF enriched reduced fat (n-3RF) and normal fat (NF) products

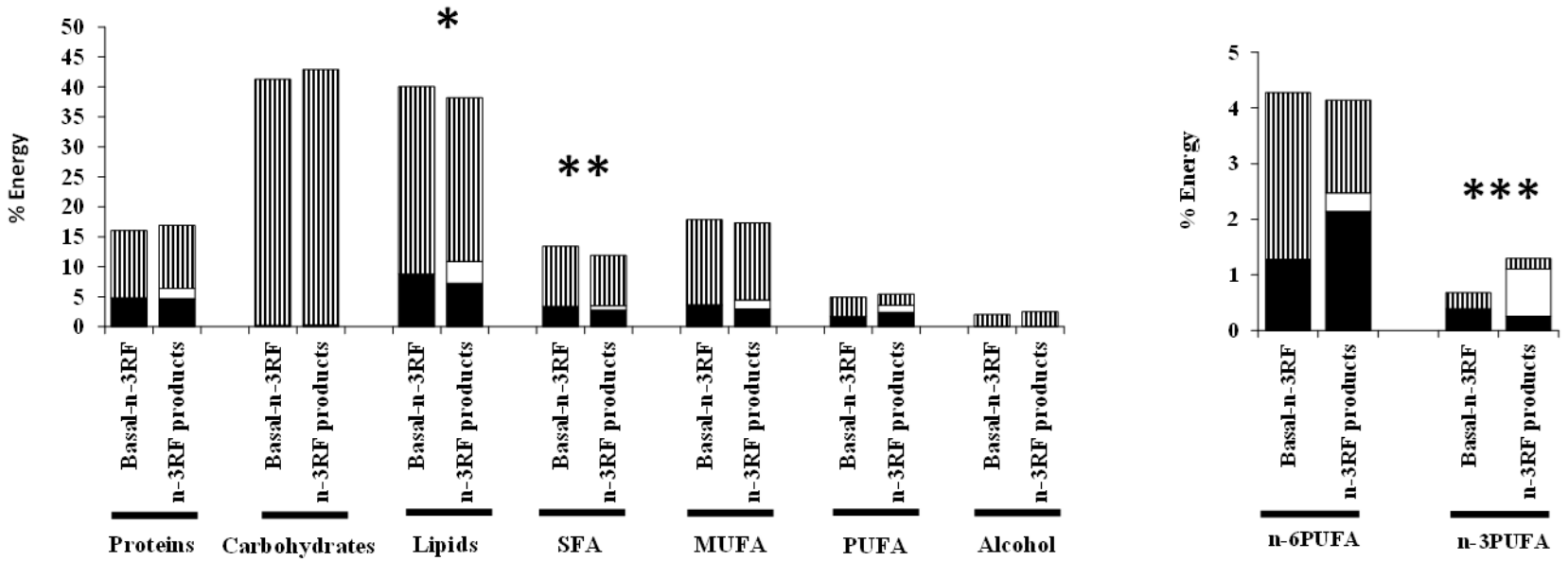
	RF			n-3RF			NF			
	Basal	**Final**	**Rate of Change (%)**	**Basal**	**Final**	**Rate of Change (%)**	**Basal**	**Final**	**Rate of Change (%)**	***P* GLM**
LDL-cholesterol (mmol/L)	3.4 ± 0.6	3.5 ± 0.7	3.4 (-3.4, 10.1)	3.5 ± 0.7	3.3 ± 0.5*	-6 (-11.9, 0.01)	3.4 ± 0.7	3.7 ± 0.7*	10 (0.3, 20.7)	0.08
Platelets (x1000/µL)	196.6 ± 43.9	195 ± 40.3	0.3 (-6.3, 5.7)	189.9 ± 44.4	191.7 ± 36.1	3.7 (-5.3, 12.6)	214.8 ± 47.2	220.1 ± 41.1	4 (-3.7, 11,7)	0.71
APTT (sec)	35.4 ± 4.4	34.3 ± 6.1	-2.7 (-11, 5.7)	39.3 ± 8.5	35.5 ± 4	-6.9 (-15.1, 1.2)	33.8 ± 4.5	35.1 ± 3	5.2 (-0.3, 10.6)	0.09
Fibrinogen (mg/dL)	374 ± 66	327 ± 213*	-11.6 (-21.2, -2.0)^{b}	331 ± 60.6	298 ± 56.9	-8.1 (-17.8, 1.6)^{b}	319 ± 73.6	348 ± 52.4*	13.7 (-0.01, 27.4)^{a}	0.008
Antithrombin (mg/dL)	32.5 ± 5.7	30.2 ± 5.8	-4.6 (-16.0, 6.8)	31.4 ± 5.7	31.6 ± 6.4	3.3 (-10.4, 17)	34.8 ± 7.2	36.6 ± 3.6	9.4 (-2.9, 21.7)	0.29
TXB_2 ‡(pg/mL)	354 ± 197	324 ± 155	-8.2 (-30, 13.6)^{ab}	309 ± 158	254 ± 75.7*	-18.9 (-38.4, 0.6)^{b}	289 ± 119	451 ± 261**	73.4 (34.9, 112)^{a}	0.037
6-keto $PGF_{1\alpha}$ ‡(pg/mL)	832 ± 493	689 ± 417*	-14.2 (-28.5, -0.2)^{ab}	747 ± 452	563 ± 336***	-24.1 (-31.9, -16.4)^{b}	723 ± 400	781 ± 450	34.8 (-10.7, 8.2)^{a}	0.048
Glucose (mmol/L)	5 ±0.9	4.9 ±0.5	0.9 (-7.8, 9.7)	5.1 ±0.8	5.1 ±0.8	0.9 (-7.5, 9.2)	5.1 ±0.6	5 ±0.6	-0.2 (-9.6, 9,2)	0.98
Insulin (µIU/mL)	11.1 ±4.6	11 ±4.6	-2.1 (-15.6, 11.4)	10.8 ±5.8	10 ±5.5	1 (-17.9, 19.9)	10.4 ±5.5	11.9 ±8.9*	20.1 (10.4, 29.9)	0.09
HOMA-IR	2.5 ±1.3	2.4 ±1.2	-0.4 (-13.3, 12.5)^{b}	2.5 ±1.5	2.6 ±1.7	2.9 (-11.3, 17)^{ab}	2.4 ±1.2	2.7 ±2.1*	24.2 (8.3, 40.2)^{a}	0.041
QUICKI	0.339 ±0.03	0.343 ±0.03	1.1 (-1.6, 3.8)	0.342 ±0.03	0.347 ±0.03	1.8 (-2.1, 5.7)	0.342 ±0.03	0.336 ±0.02*	-1.8 (-3.7, 0,02)	0.23
Systolic blood pressure (mmHg)	120 ± 10.5	118 ± 10	-1.3 (-5.2, 2.5)	119 ± 11	119 ± 11	0.8 (-4.2, 5.8)	125 ± 13.8	125 ± 14.6	0.7 (-3.2, 4.5)	0.76
Diastolic blood pressure (mmHg)	76.6 ± 9.6	76.1 ± 11.1	-0.1 (-5.8, 5.6)	77.8 ± 9.3	75 ± 7.9	-3.1 (-7.4, 1.3)	80 ± 10.7	77.5 ± 13.6	-3 (-9.1, 3)	0.69

Values are means and standard deviations for eighteen male volunteers. RF, reduced fat product; n-3RF, n-3 enriched reduced fat product; NF, normal fat product; *P<0.05, ** P<0.01, *** P<0.001 with respect to its respective basal. Change (%) = 100*mean of RF or n-3RF or NF - basal/basal (CI 95%). *p*, probability obtained after comparing changes (%) of the three experimental periods by the general linear model (GLM) of repeated measures. Different letters in the same row indicate significant differences (a > b > c, repeated measures followed *post-hoc* LSD, at least P<0.05). ‡P values were calculated after natural logarithm values transformation.

Table 4. Net rate of change in blood coagulation factors, plasma eicosanoids, insulin resistance markers, systolic and diastolic blood pressure values after 4-wk consumption of reduced fat (RF); n-3RF enriched reduced fat (n-3RF) and normal fat (NF) products

	RF *vs.* NF Change (%)	**n-3RF *vs.* NF Change (%)**
LDL-cholesterol (mmol/L)	-6.6 (-15.6, 2.4)	-16 (-26, -5)*
Platelets (x1000/µL)	-3.7 (-12, 4.6)	-0.3 (-12.3, 11.6)
APTT (sec)	-7.8 (-18.1, 2.5)	-12.1 (-21.9, -2.3)
Fibrinogen (mg/dL)	-25.3 (-44.9, -5.7)*	-21.8 (-38.8, -4.8)*
Antithrombin (mg/dL)	-14 (-31.8, 3,8)	-6.1 (-27.9, 15.7)
TXB_2 ‡ (pg/mL)	-81.6 (-153.6, -9,6)	-92.3 (-160, -24.6)*
6-keto $PGF_{1\alpha}$ ‡ (pg/mL)	-49 (-111.8, 13.7)	-59 (-118.8, 0.9)*
Glucose (mmol/L)	1.2 (-12.9, 15.2)	1.1 (-11.2, 13.3)
Insulin (µIU/mL)	-22.2 (-41.8, -2.6)	-19.1 (-38.5, 0.1)
HOMA-IR	-24.6 (48.3, -1)*	-21.3 (-42.4, -0,2)*
QUICKI	2.9 (-1.1, 6.9)	3.6 (-0.7, 8)
Systolic blood pressure (mmHg)	-2 (-7, 3)	0.1 (-7.8, 8.1)
Diastolic blood pressure (mmHg)	2.9 (-6.5, 12.3)	0 (-7, 6.9)

*Significant differences ($P<0.05$) between RF *vs.* NF change (%) or between n-3RF *vs.* NF change (%)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Proteins
Carbohydrates
Lipids
SFA
MUFA
PUFA
Alcohol
n-6PUFA
n-3PUFA

Figure 1