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Effects of improved fat meat products consumption on emergent cardiovascular disease markers of male volunteers at cardiovascular risk

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Abstract

High meat-product consumption has been related to cardiovascular disease (CVD). However, previous results suggest the benefits of consuming improved fat meat products on lipoprotein-cholesterol and anthropometric measurements. Present study aims to assess the effect of consuming different Pâté and Frankfurter formulations on emergent CVD biomarkers in male volunteers at increased CVD risk. Eighteen male volunteers with at least two CVD risk factors were enrolled in a sequentially controlled study where different pork-products were tested: reduced-fat (RF), omega-3-enriched-RF (n-3RF), and normal-fat (NF). Pork-products were consumed during 4wk periods separated by 4wk washout. The cardiometabolic index (CI), oxidized low density lipoproteins (oxLDL), apolipoproteins (Apo) A1 and B, homocysteine (tHcys), arylesterase (AE), C-reactive Protein (CRP), tumor necrotic factor-alpha ($TNF\alpha$), lipoprotein (a) (Lp(a)) were tested and some other related ratios calculated. AE, oxLDL and Lp(a), AE/HDLc, LDLc/Apo B, AE/oxLDL rate of change were differently affected ($P<0.01$) by pork-products consumption. RF increased ($P<0.05$) AE, AE/HDLc and AE/oxLDL ratios and decreased $TNF\alpha$, tHcys; n-3RF increased ($P<0.001$) AE, AE/HDLc and AE/oxLDL ratios and decreased ($P<0.05$) Lp(a); while NF increased ($P<0.05$) oxLDL and Lp(a) levels. In conclusion RF and n-3RF products affected positively the level of some emergent CVD markers. The high regular consumption of NF-products should be limited as significantly increased Lp(a) and oxLDL values. The high variability in response observed for some markers suggests the need to perform more studies to identify targets for RF- and n-3RF-products.

Keywords: apolipoproteins, arylesterase; CVD; Frankfurters; homocysteine; lipids; lipoproteins; low-fat; omega-3; pâtés; pork; oxidized lipoproteins; CRP; $TNF\alpha$; high CVD risk-subjects.

List of abbreviations: AE, arylesterase; Apo A1, apolipoprotein A1; Apo B; apolipoprotein B; CI, cardiometabolic index; CRP, C-reactive protein; HDLc, cholesterol transported by high density lipoproteins; LDLc, cholesterol transported by low density lipoproteins; Lp(a), lipoprotein(a); oxLDL, oxidized LDL; tHcys, homocysteine; TG, triglycerides; TG/HDLc, triglyceride/cholesterol transported by high density lipoproteins ratio; $\text{TNF}\alpha$, tumor necrotic factor-alpha; W/H, Waist circumference/Height ratio.

INTRODUCTION

Alterations in lipoprotein lipids [3, 4, 42] are highly discriminant in assessing cardiovascular disease (CVD) risk. However, the use of emergent biomarkers would improve diagnostic, therapeutic and prognostic outcomes in cardiovascular medicine. Modifications in apolipoproteins (Apo) [1, 13, 23], oxidized low density lipoproteins (oxLDL) [35], arylesterase (AE) [11], plasma homocysteine (tHcys) [41], C-reactive protein (CRP) and the tumour necrosis factor alpha ($\text{TNF}\alpha$) [26, 46] are normally found in subjects presenting obesity and/or hypercholesterolemia. Increased lipoprotein (a) [Lp(a)] [22, 29] and tHcys, have been suggested to be independent risk factors for atherosclerosis [32, 41]. The ratio cholesterol transported by LDL (LDLc) to Apo B (LDLc/Apo B) and that of cholesterol transported by high density lipoproteins to Apo A1 (HDLc/Apo A1) have been used as markers of functionality and metabolism of LDL [12] and HDL [7], respectively. AE/HDLc, the AE/oxLDL, and the oxLDL/LDLc ratios have been proposed to help assessing the antioxidant availability of HDL and the capacity of AE to neutralize oxLDL [33]. In addition, the triglycerides to HDLc (TG/HDLc) molar ratio [9] and more recently the cardiometabolic index (CI), calculated as the product of the anthropometric marker waist to height ratio (W/H) and the triglyceride to cholesterol transported by high density lipoproteins ratio (TG/HDLc), have been suggested to be useful discriminator of CVD risk [44].

Intake of an unbalanced diet, rich in saturated fat and cholesterol and with a high omega-6/omega 3 ratio, has been strongly associated with the development of CVD [45]. Pork is a widely-consumed food in Spain (about 29 g/day/head) [2, 14] and is a good source of many nutrients. It has been suggested to reduce meat-products consumption on account of epidemiological evidences linking meat consumption with increased prevalence of degenerative diseases [16]. However, several studies have shown the benefits of frequent consumption of meat and optimized meat and meat-products in different populations [10, 30, 31]. Our group has demonstrated that the consumption of pâtés and frankfurters with optimized fat content decreased waist circumference, body fat mass and the waist/hip ratio in male at increased risk volunteers [15] and improved cholesterol transported by low density lipoproteins (LDLc) and lipoprotein ratios in a sample of male and female volunteers [17]. However, the effects that these functional foods have on emergent CVD risk factors or biomarkers have not been reported. The present paper hypothesized that the consumption of different improved-fat pâtés and frankfurters: reduced in fat (RF) and reduced-fat but n-3 enriched pork-products (n-3RF) exert benefits on the level of emergent CVD biomarkers: CI, Apo A1, Apo B, CRP, Lp(a), tHycs, AE, oxLDL, LDLc/Apo B, HDLc/Apo A1 and TG/HDLc) in volunteers at increased CVD risk. Thus, present study aims a) to study the effect of consuming different types of improved-fat pâtés and frankfurters on some relevant emergent CVD risk factors in volunteers at increased CVD risk; and b) to assess which type of those meat-products should be preferentially consumed according to the obtained data.

MATERIALS AND METHODS

Subjects.

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. Forty-eight subjects were

interested and contacted through advertisements in various universities, research centres and several notice boards. Study design and enrolment inclusion and exclusion criteria have been published in detail elsewhere [15, 17]. Briefly, the selected subjects fulfilled the inclusion criteria: total cholesterol levels ≥ 5.2 mmol/L (200 mg/dL), LDL cholesterol levels ≥ 2.84 mmol/L (110 mg/dL), overweight (BMI, 25 - 34.9 kg/m²) and willingness to consume 200 g of frankfurters and 250 g of pâté per week. The 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. Volunteers were requested to live as they did before the study, maintaining their normal patterns 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. Information on all biomarkers tested was available for a total of eighteen male volunteers who completed the three experimental periods of the study. **Figure 1A** summarizes volunteers' flow chart.

Meat products.

Frankfurters and pâtés were designed and developed in the pilot plant of the Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN, CSIC, Madrid, Spain). Following slight modifications of the standard procedure [18, 19], three different batches of each type of pork-products were produced: a) reduced-fat-(15% fat)-products (RF); b) reduced-fat (15% fat) n-3 enriched pork-products (n-3RF) where pork backfat was replaced by a combination of olive, linseed 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).

Intervention study design.

Volunteers were enrolled in a non-randomized, sequentially controlled study of 5 months duration (**Figure 1B**). The non-randomization was due to limitations in the production of the meat products in the pilot plant. Nonetheless, in a previous work [15] we proved that diets consumed by the volunteers through the different periods showed high similarity in energy, fiber, macro and most micronutrients, permitting to associate possible changes in the biochemical profiles of volunteers only with the nutritional input from the meat-product consumed, thus, discarding potential confounding factors (e.g. seasonal impact). The study consisted in three experimental periods of 4 weeks each separated by 2 washout periods of 4 weeks. During the experimental periods each volunteer consumed 200 g frankfurters *plus* 250 g pâtés per week. During the washout phases the subjects followed their habitual diets. The weekly amount consumption of NF products implied 111g fat *per* week while that of n3-RF or RF products just 65 g fat *per* week, supporting the use of “reduced fat-products” term.

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 2 g of n-3 fatty acids per day, of which 1.5 g was linoleic acid and approximately 0.4 g eicosapentaenoic *plus* docosahexaenoic acids. Finally, during the third dietary intervention normal-fat frankfurters and pâtés (NF) were consumed. These NF-products were similar in fat content to those usually found in the market. The composition of meat products is summarized in **Table 1**. Pâtés were richer than frankfurters in energy, fat, cholesterol, most minerals and vitamins. RF and n-3RF presented lower energy, fat and SFA, MUFA and PUFA than NF-products, with n-3RF pâtés/frankfurters registering a n-6/n-3 ratio 10-times lower than the other assayed meat products.

In order to avoid any possible dietary misunderstanding, volunteers recorded the amount and type of food consumed on a daily basis. Compliance was also weekly assessed asking volunteers about product taste and consumption. All volunteers declared the complete

consumption of the different meat-products through the study periods. Seventy percent of them enjoyed more pâtés than frankfurters.

Analyses

Determination of anthropometric measurements

Anthropometric measurements were performed in each period. Body weight and height were measured in fasting conditions to the nearest 0.1 kg and 0.1 cm respectively using an electronic digital scale (Omron BF400 Body Fat Monitor with scale). Body Mass Index (BMI) was calculated as body weight (kg) divided by the square of body height (m²). Waist circumference was measured to the nearest 1 mm midway between the lowest rib margin and the iliac crest. Circumference was measured with the subject standing with weight distributed equally between the two legs and feet about 12-15 cm apart [15].

Determinations of Lipids, Lipoproteins, AE, and other CVD risk

All samples from each volunteer were analysed on the same day to minimize inter-day analytical variability. LDLc and HDLc (BioSystems LDL and HDL Direct), TG concentrations were assessed by enzymatic colorimetric kit (BioSystems Cholesterol and BioSystems Triglycerides Glycerol phosphate oxidase/peroxidase, respectively). Serum apo A, apo B and Lp(a) were measured by an immunoturbidimetric method in Tina-quant® Roche Diagnostics (Basel, Switzerland).

Serum oxLDL levels were quantified by an ELISA system (Mercodia AB, Uppsala, Sweden). Serum AE activity was measured using serum mimetic as a buffer [34]. One unit of AE was defined as the phenol (mmol) formed from phenyl acetate per min. tHcys was measured by fluorescence polarization immunoassay (Abbott Diagnostics, IL, USA). Serum TNF α was determined using enzyme-linked immunoabsorbent assay (ELISA) (Human TNF alpha ELISA, Diaclone, France) according to the manufacturer's manual. Serum CRP was measured using

enzyme immunoassay for the quantitative high sensitive determination of C-Reactive Protein a (hsCRP ELISA, Kiel, Germany) according to the manufacturer's manual and the colour intensity evaluated using a microplate reader (LT-4000, Labtech International Ltd., United Kingdom). All assays were properly calibrated and performed under internal and external quality controls provided by the manufacturers and Sociedad Española de Química Clínica (SEQC) respectively.

The Apo A1/Apo B, HDLc/Apo A1, AE/HDLc, AE/oxLDL, LDLc/Apo B, and TG/HDLc were calculated. The CI [44] was calculated according to the following formula:

$$\text{Waist circumference (cm)/Height (cm)} * \text{TG (mmol/L)} / \text{HDLc (mmol/L)}$$

Statistical analysis.

The Kolmogorov-Smirnov test was used to assess normal value data distribution. A square root-transformation rather than a log transformation for normalizing the distribution of Lp(a) levels and responses was performed. The paired Student *t*-test was employed to determine the effect of the different meat-products in each intervention period. Rate of change differences [100* (Final value - Baseline value) / Baseline value] between periods were stated using the general linear model of repeated measures (GLM). To ascertain differences between specific groups, the GLM analysis was followed by Least Standard Deviation (LSD) *post-hoc test*. Significance was set at $P < 0.05$. All statistics were performed with the IBM SPSS statistics package v.22.0.

RESULTS

Baseline characteristics of volunteers are shown in **Table 2**. All volunteers were considered at CVD risk as presented both elevated serum cholesterol and body weight. A more detailed scrutiny indicates that according to the BMI, 22% were obese while 33% exceeded the waist circumference cut-off points of 102cm. In addition 72% of volunteers presented cholesterol levels

≥ 220mg/dL, 22%, triglycerides ≥ 150mg/dL and 11% systolic blood pressure ≥ 130mmHg and 33% diastolic blood pressure ≥ 85mm Hg. Twenty eight percent of volunteers smoked between 10 and 20 cigarettes a day.

Table 3 shows that the rate of change for AE ($P<0.001$), oxLDL ($P=0.037$) and Lp(a) ($P=0.008$) differed significantly between periods. There was a strong increase of AE activity during the RF (31.4%; $P<0.01$) and n-3RF (62.1%; $P<0.001$) periods. oxLDL and Lp(a) increased significantly (16.1% and 10.8%, respectively; both $P<0.05$) after consumption of the NF-products. TNF α and tHcys levels decreased significantly (25.6% and 4.9% respectively; both $P<0.01$) during the RF-period. **Table 3** also shows significant differences in rates of change between phases for AE/HDLc ($P<0.001$), AE/oxLDL ($P=0.001$) and the LDLc/Apo B ratio ($P=0.049$). AE/HDLc and AE/oxLDL ratios increased significantly during the RF-period (both $P<0.01$) and the n-3RF-period (both $P<0.001$). No significant changes were observed in any ratio during the NF period.

DISCUSSION

Present results clearly suggest that the regular high consumption of the three meat-products distinctly affected the emergent CVD markers of volunteers. Thus, following consumption of RF pork products, AE, AE/oxLDL increased, and TNF α and tHcys levels decreased; after that of n-3RF, AE, AE/HDLc and AE/oxLDL increased, while the consumption of NF products increased Lp(a) and oxLDL levels.

In a previous paper [15] we reported that with respect to their respective basal values, the intake of SFA decreased during the RF and n-3RF periods but increased during NF period. Moreover, the intake of n-3PUFA increased during the n-3RF period, decreasing, by 49.4%, the n6/n3 ratio.

Apo A1 and Apo B levels fail to change significantly during the three studied periods.

Although dietary SFA slow the clearance of ApoB100 and A-I from the circulation, contributing to increase Apo B and Apo A1 levels [28], it has to be pointed out that in our study changes in SFA intake with respect to their respective basal values were modest in all periods, explaining, at least in part, present Apo results. In addition, Skulas-Ray et al [40] found that different doses of n-3 fatty acids were unable to modify Apo A1 concentrations, whereas only high daily doses of EPA+DHA (3.4 g) decreased Apo B values. In our study volunteers received much lower amount of these long chain fatty acids.

The increase observed in AE during both RF and n-3RF periods suggests improvement of the lipoprotein antioxidant status. AE, one of the enzymatic activities of paraoxonase-1, plays a protective pleiotropic role against peroxidation of LDL and other lipoproteins [5, 11]. Paradoxically during these periods oxLDL levels were kept at basal values. However, during the NF period, oxLDL increased due, at least in part, to the absence of changes in AE activity. We have not a clear explanation for these results but AE has been defined as a suicide enzyme [6, 43], which presents a systemic action assuring the continued antioxidant activity of other enzymes. Sánchez-Muniz et al [37] found that paraoxonase activity response to restructured meat consumption was highly modulated by the presence of PON-1 polymorphisms. This hypothesis should be tested in future studies.

Differences in rates of change in Lp(a) seem to be important as this lipoprotein-fraction has been found to be an independent risk factor of CVD [27] and suggest increased CVD risk, linked to Lp(a), after the consumption of NF-products. Changes in oxLDL and Lp(a) are apparently non-related; however, Lp(a) has been suggested to be an important carrier of oxidized-phospholipids [42]. The mechanisms by which macronutrient compositions influence Lp(a) levels are unknown. Although several studies have suggested little or no response of Lp(a) to dietary changes [24], Shin et al. [39] observed increases in Lp(a) after a high-fat low-carbohydrate diet as compared to

a low-fat high-carbohydrate diet. Berglund et al [8] reported a slight increase in Lp(a) when replacing saturated fat with monounsaturated fat, which is consistent with our present results.

Hyperhomocysteinemia has been associated with increased risk of degenerative diseases like CVD and renal disease and neurodegenerative processes [38, 41]. tHcys decreased significantly during the RF-period, being significantly higher the rate of change in RF-period than in the NF-period. tHcys levels are strongly influenced by the consumption and interaction of vitamin B₁₂, vitamin B₆ and folates [21]. In our study vitamin B₁₂ intake increased in the three experimental periods due to the meat-product consumption; however, significant changes in folate were observed only during the RF-period [15]. Other factors (e.g. body fat mass, waist circumference and waist/hip ratio) may possibly be implicated in this reduction of CVD markers, since tHcys levels have been found to be lower in lean than in obese subjects [38]. RF- but not n-3RF-products were able to reduce body mass fat, explaining, at least in part tHcys results.

Atherosclerosis is considered an endothelial inflammatory phenomenon, and so reducing pro-inflammatory TNF α cytokines should slow down the development of coronary disease [36]. TNF α is recognized as an inflammation marker amply related to body mass fat [26]. Results for TNF α also suggest the importance of RF-product consumption, as according to Celada et al. [15] during the RF-period in same volunteers a reduction of body mass fat was registered. However, as indicated, body fat did not change during the n-3 RF-period [15], explaining at least in part present results.

Despite changes observed in same volunteers in LDLc and previously reported [17], none of the product interventions produced any significant change in the Apo A1/Apo B ratios. The LDLc/Apo B ratio has been found to be an accurate marker of LDL size and atherogenicity [12]; suggesting lower ratios the presence of small and oxidizable LDL. During the n-3RF-period the rate of change of the LDLc/Apo B ratio was significantly lower than that in the RF one, suggesting that LDL particles in the n-3RF period became more oxidizable LDL [12]. This change was

probably due to n-3 enrichment and relatively poor recognition by Apo E/B100 receptors [25]. This increase was presumably responsible for the increase of AE expression, and hence for the observed increase (62%) in AE activity. The level of AE/HDLc also increased much in this period (59%), supporting the protective role of AE on LDL and HDL [6, 11, 29]. Changes in the TG/HDLc molar ratio also inform of potential changes in LDL size and oxidizability [9]; however, changes and differences between periods were not significantly different; suggesting that changes in LDLc/Apo B and TG/HDLc do not always overlap.

Although in same volunteers the W/H ratio significantly decrease in the NF period [15] the CI was not significantly affected. This CI show a broad variability [39], explaining the lack of significance in the present study.

Although results in some emergent CVD are significant, the study has some limitations: a) the daily amount of meat products to be consumed, although compatible with normal feeding, is relatively high; b) only two types of meat derivate, pâtés and frankfurters, were tested; c) the study focused on CVD markers; c) only males at CVD risk were studied. Nonetheless, the study has the strength of being the first to consider the response of several CVD markers and ratios in people at high risk of CVD to pork-products formulated with an improved fat profile.

CONCLUSIONS

High regular consumption of the three assayed meat products impact differently upon the emergent CVD markers selected. RF increasing AE, AE/HDLc, and AE/oxLDL and decreasing tHcys and TNF α , and n-3RF increasing AE, AE/HDLc, and AE/oxLDL should be preferred to NF-meat as its high consumption tended to impair concentrations of some relevant markers. However, striking interindividual variability was found, suggesting that more studies should be performed to find out target people for those potential functional meat-products and to clarify mechanisms involved.

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Footnote of figure 1. A) Study flow diagram. Data obtaining, recruitment and exclusion reasons are detailed. **B) Experimental design.** Volunteers were enrolled in a non-randomized, sequentially controlled study of 5 months duration. The study consisted in three experimental periods of 4 weeks each separated by 2 washout periods of 4 weeks. During the experimental periods each volunteer consumed 200 g frankfurters *plus* 250 g pâtés per week. During the washout phases the subjects followed their habitual diets.

Table 1: Main details of the assayed pork products. Composition per 100 g wet matter.

	Frankfurters			Pâtés		
	RF	n-3RF	NF	RF	n-3RF	NF
Macronutrients¹						
Energy (kcal)	213	217	239	203	212	346
Protein (g)	17.9	19.4	18.3	13.3	14.2	13.2
Carbohydrates (g)	0	0.09	0	1.3	1.4	1.3
Dietary fibre (g)	0	0.31	0	0	0.29	0
Total fat (g)	15.3	15.1	18	15.2	15.5	30.8
SFA (g)	5.7	2.8	6.7	4.7	3.1	9.5
MUFA (g)	7.2	6.7	8.5	8.4	6.3	17.1
PUFA (g)	1.5	4.8	1.8	1.3	5.2	2.6
PUFA/SFA	0.3	1.71	0.27	0.28	1.68	0.27
[PUFA+MUFA]/SFA	1.5	4.11	1.54	2.06	3.71	2.07
n6/n3 PUFA	6.5	0.47	7.5	5.5	0.46	11
Cholesterol (mg)	49.8	41	51.4	138	129	147
Water (g)	66.8	65.2	63.7	69.2	64.8	50.7
Minerals²						
Ca (mg)	4.7	7.1	4.8	30.2	32.4	30.7
Fe (mg)	1.3	1.2	1.3	6.4	6.4	6.5
I (mg)	4.1	2.5	4.5	10.6	9	12.4
Mg (mg)	21.2	23.7	21.2	21	23.3	21.7
Zn(mg)	1.6	1.5	1.6	2.8	3.7	2.9
Se (mg)	9.1	9.2	9	21.5	21.6	21.7
Na (mg)	909	823	927	909	827	1004
K (mg)	198	211	199	218	231	232
P (mg)	116	117	116	192	194	199
Vitamins²						
Vitamin D (µg)	0	0	0	0.47	0.47	0.47
Vitamin E (mg)	0.017	0.75	0.02	0.22	0.89	0.24
Vitamin B ₁ (mg)	0.54	0.54	0.54	0.28	0.27	0.29
Vitamin B ₂ (mg)	0.14	0.14	0.14	1.1	1.1	1.1
Niacin Eq. (mg)	8.8	8.3	8.9	9.5	9	10.2
Vitamin B ₆ (mg)	0.36	0.31	0.37	0.34	0.3	0.4
Folates (µg)	3.9	6.8	4	46.7	49.3	47.2
Vitamin B ₁₂ (µg)	2	2	2	13.5	13.5	13.5
Vitamin C (mg)	0	0.04	0	7.7	7.7	7.7
Retinol Eq. (µg)	0	0	0	11882	11882	11882

Source Celada et al [15]. This table is reproduced with permission of the Nutrición Hospitalaria Journal.

Table 2. Baseline characteristics of the volunteers (n=18) at the start of the intervention study

	Baseline levels
Age (years)	44.9±10.3
BMI (kg/m ²)	28.6±2.5
Weight (kg)	84.8±10.3
Waist circumference (cm)	100.3±7.1
Fat Body Mass (%)	29.2±4.0
Total cholesterol (mg/dL)	229.5±20.5
Triglycerides (mg/dL)	131.5±76.9
Glucose (mg/dL)	89.4±16.7
Blood pressure (mm Hg)	
Systolic	120.3±10.5
Diastolic	76.6±9.6
Alcohol consumption	7 never
	9 sometimes (1-2 drink/week)
	2 daily
Smoking	13 non-smokers
	5 smokers (10-20 cigarettes/day)

Values are mean ± SD of 18 volunteers. To transform mg/dL of cholesterol, triglycerides and glucose in mmol/L divide them by 38.68, 89, and 18, respectively.

Table 3. Biomarkers, lipoprotein and apolipoprotein ratios response after 4week consumption of reduced fat (RF); n-3 enriched reduced fat (n-3RF) and normal fat (NF) products.

	RF-basal	RF	RF Rate of change	n-3RF-basal	n-3RF	n-3RF Rate of change	NF-basal	NF	NF Rate of change	P +
Apo A1, g/L	1.42±0.19	1.41±0.22	-0.2 (-2.8, 2.4)	1.41±21.6	1.43±0.24	1.9 (-3.9, 7.8)	1.41±20.1	1.42±0.25	0.6 (-4.7, 5.9)	0.75
Apo B, g/L	1.12±0.19	1.10±0.18	-1.2 (-4.8, 2.5)	1.08±0.18	1.09±0.2	1.4 (-4.8, 7.7)	1.01±0.26	1.09±0.2	0.6 (-4.6, 5.9)	0.79
Arylesterase, U/L	290±95.5	365±106**	31.4 (12.8, 50.1) ^a	282±122	415±120***	62.1 (38.9, 85.4) ^a	204±45.9	199±66.3	0.2 (-17.9, 18.3) ^b	0.001
oxLDL, U/L	57.0±9.9	56.4±9.7	-0.2 (-6.7, 6.4) ^b	57.0±9.9	57.3±9.9	1.8 (-12.0, 15.6) ^{ab}	57.0±9.9	65.4±14.7*	16.1 (3.5, 28.7) ^a	0.037
CRP, µg/mL	2.8±4.3	2.2±2.3	70.8 (-18.6, 160.1)	2.8±2.7	2.3±2.8	75.0 (-96.6, 246.6)	3.3±3.8	3.6±4.8	35.8 (-22.0, 93.7)	0.76
TNFα, pg/mL	28.2±13.1	18.3±8.9**	-25.6 (-43.4, -7.9)	18.2±10.8	17.4±8.4	68.1 (-57.1, 193.4)	23.7±14.6	20.4±14.8	12.4 (-36.5, 61.2)	0.18
Lp(a) ¹ , mg/L	97.1±103.3	92.1±94.4	-1 (-11.8, 9.7) ^a	92±95.3	89.3±99.7*	-7.9 (-16.7, 1) ^a	92.5±102	98.7±108.3*	10.8 (1.1, 20.6) ^b	0.008
Homocysteine, µmol/L	13.3±2.4	12.6±2.3**	-4.9 (-8.3, -1.4)	12.8±1.9	13.0±2.3	1.6 (-3.8, 7.0)	13.9±2.3	13.1±2.2	-4.7 (-10.3, 0.9)	0.12
Apo A1/Apo B	1.3±0.3	1.3±0.4	1.5 (-2.6, 5.5)	1.3±0.3	1.4±0.4	2.1 (-7.4, 11.6)	1.4±0.4	1.3±0.3	0.9 (-6.4, 8.3)	0.94
HDLc/Apo A1	0.3±0.04	0.3±0.04	3.9 (-2.0, 9.7)	0.3±0.03	0.3±0.04	0.3 (-4.3, 5.0)	0.3±0.04	0.3±0.04	1.3 (-4.0, 6.5)	0.58
LDLc/Apo B	1.2±0.2	1.2±0.2	4.6 (-1.0, 10.2) ^a	1.3±0.2	1.2±0.2	-6.5 (-13.4, 0.4) ^b	1.3±0.2	1.3±0.2	5.0 (-5.1, 15.2) ^{ab}	0.049
AE/oxLDL	5.2±2.1	6.5±2.1**	32.0 (13.8, 50.3) ^b	5.0±2.3	7.5±2.3***	69.8 (38.9, 100.7) ^a	3.7±1.2	3.1±1.2	-10.8 (-26.5, 5.0) ^c	0.001
AE/HDLc	246±81.2	299±81.6**	27.5 (10.1, 45.0) ^b	231±101	335±98.1***	59.4 (38.2, 79.5) ^a	167±39.8	161±64.8	-1.1 (-18.6, 16.3) ^c	<0.001
TG/HDLc (molar)	1.4±1.1	1.2±0.7	-0.7 (-19.3, 18)	1.1±0.6	1.3±0.9	15.4 (-7.3, 38.1)	1.4±1.1	1.2±0.7	7.8 (-16.9, 32.5)	0.468
Cardiometabolic index	0.8±0.6	0.7±0.4	-1.3 (-20.1, 17.8)	0.7±0.4	0.8±0.6	15.5 (-7.3, 38.2)	0.8±0.7	0.7±0.4	7.6 (-17.3, 32.3)	0.440

Values are means ± SD of 18 volunteers. ¹Lp(a) values were statistically compared after their square root normalization. +*P*<0.1, marginally significant; **P*<0.050, ***P*<0.010, ****P*<0.001 with respect to its response to baseline; Rate of change (%). 100*(mean (CI 95%) of RF or n-3 RF or NF - baseline/baseline). +*P*, probability obtained by the general linear model (GLM) of repeated measures. Different letters in the same row indicate significant differences for periods using the post-hoc Least Standard Deviation (LSD) test (a > b > c, at least *P*<0.05). For abbreviation see the abbreviations' list.