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Cardiovascular disease markers responses in male receiving improved-fat meat-products vary by initial LDL-cholesterol levels.

La respuesta de marcadores de enfermedad cardiovascular al consumo de cárnicos con composición grasa mejorada depende de los niveles iniciales de LDL-colesterol.

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Abstract
Objectives: Cardiovascular disease (CVD) is prevalent in people at high meat-product consumption. To study the effect of consuming different Pâté and Frankfurter formulations on clinical/emergent CVD biomarkers in male volunteers with different initial LDL-cholesterol levels (< and ≥ 3.36 mmol/L).
Method: Eighteen male volunteers with at least two CVD risk factors were enrolled in a sequentially controlled study. Pork-products were consumed during 4wk: reduced-fat (RF), omega-3-enriched-RF (n-3RF), and normal-fat (NF). Pork-products were separated by 4wk washout. Lipids, lipoproteins, oxidized LDL (oxLDL), apolipoproteins (apo) and their ratios, homocysteine (tHcys), arylesterase (AE), C-reactive protein (CRP), tumor necrotic factor (TNFα) were tested.
Results: The rate of change for AE, oxLDL, Lp(a), AE/HDL-cholesterol, LDL/apo B and AE/oxLDL ratios varied ($p<0.05$) among periods only in volunteers with LDL-cholesterol ≥3.36 mmol/L. TNFα decreased ($p<0.05$) among volunteers with low-normal LDL-cholesterol values while AE increased ($p<0.01$) in high LDL-cholesterol volunteers during the RF-period. AE increased while CRP decreased (both $p<0.01$) in low-normal LDL-cholesterol volunteers while AE ($p<0.001$) and apo B ($p<0.01$) increased in the high LDL-cholesterol group during the n-3RF-period. Total cholesterol ($p<0.05$) increased in the low/normal LDL-cholesterol group while tHcys decreased ($p<0.05$) in the high LDL-cholesterol group during the NF-period. Differences in response in volunteers with low-normal *vs.* high initial LDL-cholesterol levels to the n-3RF but not to the RF meat-products seem evident.
Conclusions: Subjects with high LDL-cholesterol seem target for n-3RF products while subjects with LDL-cholesterol <3.36 mmol/L were more negatively affected by NF-products. Any generalization about functional meat product or consumption should be avoided.

KEYWORDS
CVD risk markers; Frankfurters; Pâtes; low fat; omega-3; pork; high-CVD risk-subjects; LDL-cholesterol.

Resumen
Objetivos: La enfermedad cardiovascular (CVD) es prevalente en individuos con alto consumo de productos cárnicos. Estudiar los efectos del consumo de diferentes tipos de patés y salchichas Frankfurt sobre marcadores clásicos/emergentes de CVD en voluntarios con diferente concentración de LDL-colesterol (< y ≥ 3,36 mmol/L).
Métodos: Dieciocho hombres con al menos dos factores de riesgo de CVD se enrolaron en un estudio secuencial y controlado. Consumieron productos del cerdo reducidos en grasa (RF), RF-enriquecidos en omega-3 (n-3RF), y con contenido graso normal (NF) durante 4 semanas, separados por lavados de 4 semanas. Se determinaron lípidos, lipoproteínas, LDL oxidada (oxLDL), apolipoproteínas (apo) y sus cocientes, homocisteína (tHcys), arilesterasa (AE), proteína-C-reactiva (CRP), factor necrótico tisular (TNFα).
Resultados: La tasa de cambio para oxLDL, Lp(a), AE/HDL-colesterol, LDL/apo B y AE/oxLDL difirió ($p<0,05$) entre periodos solo en voluntarios con LDL-colesterol ≥3,36 mmol/L. En el periodo RF los valores de TNFα disminuyeron ($p<0,05$) entre los voluntarios con LDL-colesterol normal-bajo mientras que la AE incrementó ($p<0,01$) en aquellos con LDL-colesterol alto. La AE subió y la CRP bajó (ambas $p<0,01$) en los voluntarios con LDL-colesterol normal-bajo mientras que la AE ($p<0,001$) y apo B ($p<0,01$) incrementaron en los voluntarios con LDL-colesterol alto durante el periodo n-3RF. El colesterol total ($p<0,05$) subió en el grupo de LDL-colesterol

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normal-bajo mientras que la tHcys disminuyó (p<0,05) en el grupo con LDL-colesterol elevado durante el periodo NF. Las diferencias en respuesta en los voluntarios de los dos grupos fueron evidentes en el periodo n-3RF, pero no en el RF.
Conclusiones: Los sujetos con LDL-colesterol elevado resultaron ser los individuos diana para los productos n-3RF mientras que aquellos con niveles de LDL-colesterol <3,36 mmol/L se afectaron más negativamente por el de NF. Debe evitarse cualquier generalización sobre los beneficios del consumo de cárnicos funcionales.

PALABRAS CLAVE
Marcadores de riesgo; cerdo salchichas tipo Frankfurt; patés; bajo-en-grasa; omega-3; alto riesgo cardiovascular; LDL-colesterol.

Abbreviations:

AE: arylesterase
apo A1: apolipoprotein A1
apo B: apolipoprotein B
Lp(a): lipoprotein(a)
CRP: C-reactive protein
tHcys: homocysteine
oxLDL: oxidized LDL

Introduction:

Despite the nutritional properties of meat and meat-products, concerns exist on their consumption, specifically at daily high amounts[1,2]. Consumption of pork-products with improved fat quality and content could impart health benefits[3,4]. Our group has demonstrated that the consumption of pâtés and frankfurters with improved fat quality and content affects positively some anthropometric measurements without significantly changing the consumption of macronutrients other than omega-3 PUFAs in male at-risk volunteers[5] and improved LDL-cholesterol and lipoprotein ratios in a sample of male and female volunteers[4], or of emergent cardiovascular disease (CVD) markers of males at cardiovascular (CVD) risk[6]. However, volunteer's dietary response displayed strinking interindividual variability. To this respect, Beynen *et al*.[7] classified individuals as hypo, normal and hyperresponders according to their response adjustments to expected equations. Furthermore, expected response could be different in volunteers presenting different basal concentrations of some disease biomarkers (e.g. cholesterol levels[8]), or gene polymorphisms[9], clearly suggesting that functional food should be exclusively or preferentially consumed by target individuals[10]. The present paper hypothesized that the effect of improved-fat pâtés and frankfurters on the lipoprotein profile, atherogenic lipoprotein and apolipoprotein (apo) ratios, and emergent CVD biomarkers (e.g. C-reactive protein (CRP), tumor necrotic factor alpha (TNFα), lipoprotein (a) (Lp(a)), homocysteine (tHycs), arylesterase (AE), oxidized LDL (oxLDL), LDL-c/apo B ratio) varies in volunteers at CVD risk presenting normal-low and high LDL-cholesterol levels (LDL-cholesterol < and $\geq$3.36 mmol/L respectively). Present study aims to identify differences in responses of volunteers within the low-normal or high LDL-cholesterol groups. This aim would be especially valuable when the effect on any biomarker of the whole volunteers' population studied appear as non-significant or non-relevant.

Materials and methods:

Subjects.

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[4-6]. Briefly, the selected subjects fulfilled the inclusion criteria: total cholesterol levels $\geq$5.2 mmol/L, LDL cholesterol levels $\geq$2.84 mmol/L), overweight (BMI, 25-34.9 kg/m^2) 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 helpings of 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. The study was approved by the Ethical Committee for Clinical Investigation of Hospital Universitario Puerta de Hierro-Majadahonda (Spain) (Acta nº 261, dated 20/ 12/ 2010) and the Bioethical Committee of the Spanish National Research Council.

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[11,12], 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. The non-randomization was due to limitations in the production of the meat products in the pilot plant. Each one consumed 200 g frankfurters and 250 g pâtés per week in each of three periods, separated by 4-week washout periods. During the washout phases the subjects followed their habitual diets.

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 around 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**.

Table 1: Main details of the assayed pork products

	Frankfurters			**Pâtés**		
	RF	n-3RF	NF	RF	n-3RF	NF
Macronutrients						
Energy (kcal)	213	217	239	203	212	346
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
Protein (g)	17.9	19.4	18.3	13.3	14.2	13.2
Cholesterol (mg)	49.8	41	51.4	138	129	147
Water (g)	66.8	65.2	63.7	69.2	64.8	50.7
Vitamins						
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
Vitamin A (Retinol Eq. µg)	0	0	0	11882	11882	11882
Minerals						
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

Modified from Celada *et al*.[5]

Statistical analysis.

Sample size calculation was performed on the basis of a mean value for 3.36 mmol/L LDL-cholesterol. A sample size of 18 subjects (SD = 0.41 mmol/L) (15.86 mg/dL) is necessary to obtain a 10% difference in LDL-cholesterol (0.36 mmol/L) (13.92 mg/dL) between two consecutive visits with 90 % power and an alpha error of 0.05. When population was classified according to two groups of 8-10 persons each (e.g. low-normal vs. high LDL-cholesterol values), the statistical power remained about 70%. The Kolmogorov-Smirnov test was used to assess normal value data distribution. The paired Student t-test was employed to determine the effect of the different meat product in each intervention period and to assess differences in response between both LDL-cholesterol groups. Rate of change differences [100* (Final value - Baseline value) / Baseline value] between periods were stated using the general linear model of repeated measures (GLM) followed by Least Standard Deviation (LSD) *post-hoc*, Significance was set at $p<0.05$. All statistics were performed

with the IBM SPSS statistics package v.22.0.

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. LDL- and HDL-cholesterol (BioSystems LDL and HDL Direct), total cholesterol (TC) and triglyceride 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[13]. One unit of AE was defined as the phenol (mmoL) formed from phenyl acetate per min. oxLDL was determined using an ELISA test kit from Mercodia Laboratories (Uppsala, Sweden). tHcys was measured by fluorescence polarization immunoassay (Abbott Diagnostics, IL, USA). Serum TNFα 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.

TC/HDL-cholesterol, LDL-cholesterol/HDL-cholesterol, apo A1/apo B, HDL-cholesterol/apo A1, and LDL-cholesterol/apo B were calculated. AE/HDL-cholesterol, the AE/oxLDL, and the oxLDL/LDL-cholesterol ratios were determined to assess the antioxidant availability of HDL and the capacity of AE to neutralize oxLDL[14].

Results:

Table 2 shows non-significant differences between periods in the rate of change of any parameter tested among volunteers presenting initial LDL-cholesterol values < 3.36 mmol/L. However, rates of change for AE (p<0.001), oxLDL (p=0.049) and Lp(a) (p=0.049) differed significantly between periods in those volunteers with ≥3.36 mmol/L initial LDL-cholesterol values.

Table 2. Rate of change of cardiovascular disease markers to the three experimental pork products in subjects with initial low-normal/high LDL-cholesterol values

	Initial LDL-c[1]	Basal RF	Final RF	RF Rate of change	Basal n-3RF	Final n-3RF	n3RF Rate of change	Basal NF	Final NF	NF Rate of change	p+
Total cholesterol,	1	5.76±0.73	5.69±0.67	-0.9 (-9.0, 7.2)	5.74±0.79	5.70±0.63	0.1 (-9.9, 10.0)	5.49±0.92	5.92±0.85*	8.5 (0.4, 16.7)	0.22
mmol/L	2	6.05±0.28	6.13±0.27	1.3 (-1.9, 4.6)	5.84±0.46	6.23±0.58	7.1 (-0.6, 14.8)	6.21±0.74	6.01±0.51	-2.6 (-9.3, 4.0)	0.13
LDL-cholesterol,	1	2.89±0.34	3.04±0.66	4.6 (-8.5, 17.7)	3.14±0.52	2.93±0.41	-4.2 (-20.2, 11.8)	2.94±0.41	3.3±0.82	11.7 (-6.1, 29.6)	0.31
mmol/L	2	3.75±0.38	3.84±0.54	2.4 (-6.5, 11.3)	3.83±0.67	3.52±0.56**	-7.4 (-12.8, -2.1)	3.86±0.52	3.82±0.61	-0.1 (-11.6, 11.4)	0.24
HDL-cholesterol,	1	1.14±0.24	1.21±0.26	6.6 (-0.9, 14.1)	1.27±0.22	1.27±0.25	-0.3 (-10.1, 9.5)	1.22±0.2	1.20±0.26	-2.1 (-11.5, 7.4)	0.18
mmol/L	2	1.21±8.1	1.22±9.2	0.8 (-6.5, 8.1)	1.18±9.0	1.21±8.0	4.0 (-7.2, 15.1)	1.21±7.7	1.27±10.9	4.7 (-6.4, 15.7)	0.80
Triglycerides,	1	1.59±1.1	1.37±0.35	10.2 (-24.8, 45.2)	1.22±0.52	1.46±0.59	24.7 (-12.4, 61.7)	1.53±1.08	1.37±0.64	17.5 (-21.2, 56.3)	0.80
mmol/L	2	1.39±0.67	1.26±0.62	-3.1 (-30.7, 24.5)	1.40±0.57	1.57±1.06	7.5 (-16.0, 31.0)	1.55±0.83	1.28±0.48	-3.1 (-31.0, 24.7)	0.66
Arylesterase,	1	321±122	385±106	33.4 (-11.0, 77.7)	335±124	438±127**	37.6 (11.4, 63.7)	214±55.2	225±69.4	10.1 (-23.9, 44.2)	0.48
U/L	2	266±62.2	348±108**	29.9 (14.6, 45.2)[b]	240±109	397±119***	81.8 (46.8, 116.8)[a]	196±38.2	179±59.0	-7.7 (-31.1, 15.6)[c]	0.001
oxLDL,	1	53.7±12.2	49.6±7.9	-5.4 (-18.4, 7.6)	53.7±12.2	54.2±15.9	3.7 (-29.7, 37.1)	53.7±12.2	64.9±20.4	22.0 (-5.1, 49.1)	0.17
U/L	2	59.7±7.2	61.9±7.3	4.0 (-3.0, 11.1)[ab]	59.7±7.2	59.8±11.3	0.3 (-9.9, 10.5)[b]	59.7±7.2	65.8±9.1	11.3 (-1.8, 24.5)[a]	0.049
CRP[2],	1	4.0±5.9	3.0±3.2	43.7 (-46.1, 133.5)[a]	3.9±3.4	2.1±2.5**	-61.0 (-92.6, -29.3)[b]	5.4±5.1	5.2±6.8	30.9 (-103, 164)[ab]	0.14
µg/mL	2	1.8±2.2	1.5±1.0	92.4 (-68.8, 253.7)	1.8±1.4	2.4±3.1	183.8 (-131.5, 499)	1.7±0.8	2.4±2.1	39.8 (-14.4, 94.0)	0.46
TNFα,	1	26.4±10.4	17.9±6.9*	-27.1 (-49.9, -4.3)	20.8±9.0	16.8±11.6	-21.2 (-50.5, 8.0)	26.4±17.1	15.3±13.2	-17.8 (76.6, 40.9)	0.87
pg/mL	2	29.6±15.4	18.7±10.7	-24.5 (-55.1, 6.2)	16.1±12.2	17.9±5.2	140 (-93.3, 373)	21.6±12.7	24.4±15.5	39.2 (-45.0, 123.4)	0.19
apo A1,	1	1.45±0.23	1.47±0.26	1.3 (-2.1, 4.6)	1.47±0.23	1.53±0.28	4.7 (-7.3, 16.6)	1.47±0.24	1.43±0.28	-2.9 (-8.0, 2.2)	0.33
g/L	2	1.39±0.17	1.37±0.18	-1.3 (-5.6, 3.0)	1.36±0.2	1.35±0.17	-0.2 (-7.1, 6.7)	1.37±0.18	1.41±0.24	3.4 (-5.8, 12.7)	0.58
apo B,	1	1.08±0.26	1.04±0.22	-2.8 (-10.3, 4.6)	1.05±0.24	1.00±0.23	-4.4 (-17.7, 8.9)	1.03±0.37	1.04±0.26	3.4 (-7.6, 14.4)	0.51
g/L	2	1.15±0.12	1.15±0.12	0.2 (-4.2, 4.5)	1.10±0.14	1.17±0.14**	6.1 (1.3, 10.6)	1.15±0.11	1.13±0.13	-1.6 (-7.3, 4.2)	0.12
Lp(a),	1	78.5±95.9	84.7±102.8	7.9 (-15.4, 31.2)	78.1±92.5	74.3±93.5	-6.2 (-21.6, 9.2)	81.4±109.5	85.9±109.4	7.2 (-3.4, 17.8)	0.32
mg/L	2	112.1±111.5	98.1±92.4	-8.2 (-16.9, 0.5)[ab]	103.2±100.9	101.2±106	-6.8 (-15.5, 1.9)[b]	101.5±100.3	108.9±112.1	10.5 (-3.6, 24.5)[a]	0.049
Homocysteine,	1	13.2±1.8	12.5±1.7	-5.0 (-10.1, 0.9)[b]	12.7±1.5	13.0±1.8	3.2 (-3.4, 9.8)[a]	13.4±1.8	13.0±1.7	-1.9 (-12.7, 9.0)[ab]	0.29
µmol/L	2	13.4±2.9	12.8±2.8	-4.8 (-10.0, 0.4)	12.9±2.3	12.9±2.6	0.4 (-8.9, 9.7)	14.3±2.7	13.2±2.6*	-6.9 (-13.9, 0.1)	0.34

Values are means ± SDs; [1]1, LDL-cholesterol <3.36 mmol/L, 2, LDL-cholesterol ³3.36 mmol/L; [2]CRP, C-reactive protein. RF, reduced fat product; n-3RF, n-3 enriched reduced fat product; NF, normal fat product; *p<0.050, **p<0.010, ***p<0.001 with respect to its respective 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 (a > b > c, repeated measures followed *post-hoc* LSD, at least p<0.05) indicate significant differences.

Table 2 also shows that during the RF-period, TNFα decreased (p<0.05) among volunteers with initial low-normal LDL-cholesterol values while AE increased (p<0.01) in volunteers with high LDL-cholesterol values. During the n-3RF-period, AE increased (p<0.01) while CRP decreased (p<0.01) in low initial LDL-cholesterol volunteers while AE (p<0.001) and apo B (p<0.01) increased in the high LDL-cholesterol group. During the NF-period TC (p<0.05) increased in the low/normal initial LDL-cholesterol group while tHcys decreased (p<0.05) in the high LDL-cholesterol group.

Table 3 shows that only the rates of change of the TC/HDL-cholesterol (p=0.064) and AE/oxLDL (p=0.079) ratios tended to be affected in volunteers with LDL-cholesterol < 3.36 mmol/L initial values. In volunteers with initial LDL-cholesterol ≥3.36 mmol/L, the rates of change for AE/HDL-cholesterol (p<0.001), LDL-cholesterol/apo B ratio (p=0.007)

and the AE/oxLDL ratio (p=0.004) differed significantly between periods.

Table 3. Rate of change of cardiovascular disease ratios to the three experimental pork products in subjects with initial low-normal/high LDL-cholesterol values

	Initial LDL-c[1]	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 +
TC/HDL-cholesterol	1	5.3±1.7	4.9±1.3	-6.3 (-17.4, 4.8)[b]	4.6±1.1	4.7±1.1	0.5 (-4.7, 5.6)[ab]	4.6±1.3	5.2±1.4	12.8 (-5.6, 31.1)[a]	0.064
	2	5.1±0.8	5.2±0.9	1.5 (-6.9, 9.8)	5.1±0.8	5.3±1.1	4.5 (-6.1, 15.1)	5.3±1.1	4.9±1.0	-5.8 (-14.5, 2.9)	0.22
LDL-cholesterol/	1	2.6±0.7	2.6±0.8	-9.6 (-16.2, 14.3)	2.5±0.6	2.4±0.5	-4.4 (-12.8, 4.0)	2.5±0.6	2.9±1.1	16.5 (-10.1, 43.0)	0.11
HDL-cholesterol	2	3.2±0.5	3.3±0.7	2.7 (-9.9, 15.3)	3.3±0.6	3.0±0.5*	-9.4 (-18.5, -0.4)	3.3±0.7	3.1±0.8	-3.6 (-14.8, 7.6)	0.24
AE/	1	281±101	318±65.2	24.3 (-14.4, 63.0)	264±95.2	353±89*	40.0 (7.8, 72.3)	176±36.3	189±76	12.0 (-20.1, 44.1)	0.48
HDL-cholesterol	2	220±54.4	286±94.5**	30.1 (12.0, 48.2)[b]	203±76.6	328±110.3***	74.8 (48.6, 101.1)[a]	162±43.5	141±47.1	-11.6 (-33.1, 9.9)[c]	<0.001
apo A1 / apo B	1	1.4±0.4	1.5±0.4	4,7 (-0,7, 10,1)	1,5±0,4	1.6±0.5	11.8 (-8.2, 31.7)	1.5±0.5	1.4±0.4	-4.9 (-15.9, 6.2)	0.19
	2	1.2±0.2	1.2±0.2	-1.1 (-7.3, 5.1)	1.3±0.3	1.2±0.3	-5.7 (-12.6, 1.2)	1.2±0.2	1.3±0.3	5.6 (-5.1, 16.3)	0.12
HDL-cholesterol/	1	0.3±0.03	0.3±0.03	5.4 (-2.1, 12.9)	0.3±0.02	0.3±0.03	-4.3 (-10.2, 1.7)	0.3±0.1	0.3±0.03	1.2 (-9.3, 11.6)	0.18
apo A1	2	0.3±0.04	0.3±0.04	2.7 (-7.3, 12.6)	0.3±0.03	0.3±0.05	4.0 (-2.9, 10.9)	0.3±0.04	0.3±0.04	1.3 (-5.4, 8.0)	0.86
LDL-cholesterol/	1	1.1±0.2	1.1±0.2	7.6 (-2.6, 17.7)	1.2±0.2	1.2±0.3	1.0 (-12.8, 14.9)	1.2±0.3	1.2±0.2	9.5 (-13.4, 32.4)	0.70
apo B	2	1.3±0.2	1.3±0.1	2.2 (-5.3, 9.8)[a]	1.3±0.1	1.2±0.2***	-12.5 (-18.2, -6.8)[b]	1.3±0.2	1.3±0.1	1.4 (-7.9, 10.8)[a]	0.007
LDL-cholesterol/	1	1525±269	1626±265	7.6 (-2.6, 17.7)	1667±217	1689±368	1.0 (-12.8, 14.9)	1680±421	1757±315	9.5 (-13.4, 32.4)	0.70
apo B[2]	2	1808±218	1837±208	2.2 (-5.3, 9.8)[a]	1910±212	1670±238	-12.5 (-18.2, -6.8)[b]	1859±270	1863±184	1.4 (-7.9, 10.8)[a]	0.007
AE/oxLDL	1	6.0±2.8	7.6±2.3*	39.9 (-2.0, 81.9)[a]	6.2±2.3	8.4±2.5*	47.4 (5.7, 89.0)[ab]	4.1±1.6	3.7±1.5	-2.7 (-35.1, 29.8)[b]	0.079
	2	4.5±1.1	5.6±1.3**	25.7 (9.8, 41.6)[b]	4.0±1.8	6.8±2.1**	87.8 (39.5, 136.1)[a]	3.3±0.6	2.7±0.8*	-17.3 (-35.2, 0.7)[c]	0.004

Values are means ± SDs; [1]1, LDL-cholesterol <3.36 mmol/L, 2, LDL-cholesterol ³3.36 mmol/L; [2]Molar ratio; RF, reduced fat product; n-3RF, n-3 enriched reduced fat product; NF, normal fat product; *p<0.050, **p<0.010, ***p<0.001 with respect to its respective baseline; Rate of change (%). 100*(mean (CI 95%) of RF or n-3RF or NF - baseline/baseline). p +, probability obtained by the general linear model (GLM) of repeated measures. Different letters in the same row (a > b > c, repeated measures followed *post-hoc* LSD, at least p<0.05) indicate significant differences.

Figures 1-3 summarize significant differences between rates of change occurring in volunteers with low-normal *vs.* high initial LDL-cholesterol values. No significant differences (p>0.05) were found for any parameter or ratio following the RF-period between both groups of volunteers. Following the n-3RF-period differences between groups were observed for AE (p<0.05) and opposite tendencies in rates of change were observed for apo A1/apo B, LDL-cholesterol/apo B and HDL-cholesterol/apo A1 ratios. In the NF-period, differences in the rates of change of TC and TC/HDL-cholesterol (p<0.05) were observed between both groups of volunteers.

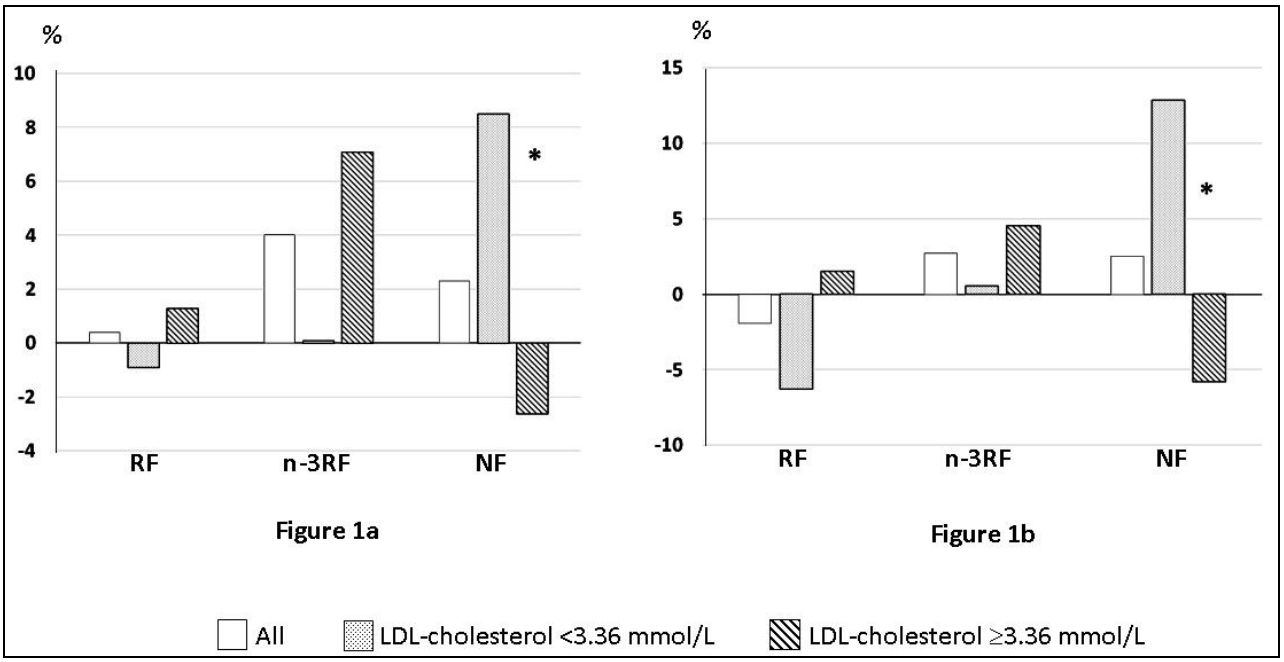

Figure 1. Rate of change (%) for total cholesterol **(Fig. 1a)** and the total cholesterol/HDL-cholesterol ratio **(Fig. 1b)** following the reduced-fat (RF), the n-3 reduced-fat (n-3RF) and normal-fat (NF) periods in volunteers classified according to initial LDL-cholesterol values, <3.36 mmol/L or ≥3.36 mmol/L. *p<0.05, low-normal LDL-cholesterol *vs.* high LDL-cholesterol values.

Discussion:

In order to identify targets for these meat products, volunteers were classified according to their initial LDL-cholesterol levels. n-3RF-products affected volunteers' variables more than the other two products. Results during the n-3RF-period in volunteers with high initial LDL-cholesterol levels were notable, with LDL-cholesterol and LDL-cholesterol/HDL-cholesterol values diminishing and AE and apo B increasing. LDL-cholesterol reduction has been defined as a central fact for CVD risk improvement[(15)]. According to present results this reduction should be significant, thus the CVD risk, only in high-LDL-cholesterol individuals. However, as each LDL contains only one molecule of apo B100[(16)], the apo B increase found during the n-3RF-period suggests that more LDLs were present; in addition, the LDL-cholesterol/apo B ratio

indicated that these LDLs were denser and smaller. Nonetheless, the observed increase in AE activity (AE, AE/oxLDL, AE/HDL-cholesterol) would be enough to keep oxLDL values low in these theoretically more atherogenic LDL particles. No clear hypothesis can be drawn for the increase in LDL numbers; however, it has been reported that LDL particles enriched with n-3 PUFAs are poorly recognized by LDL receptors in some people[17].

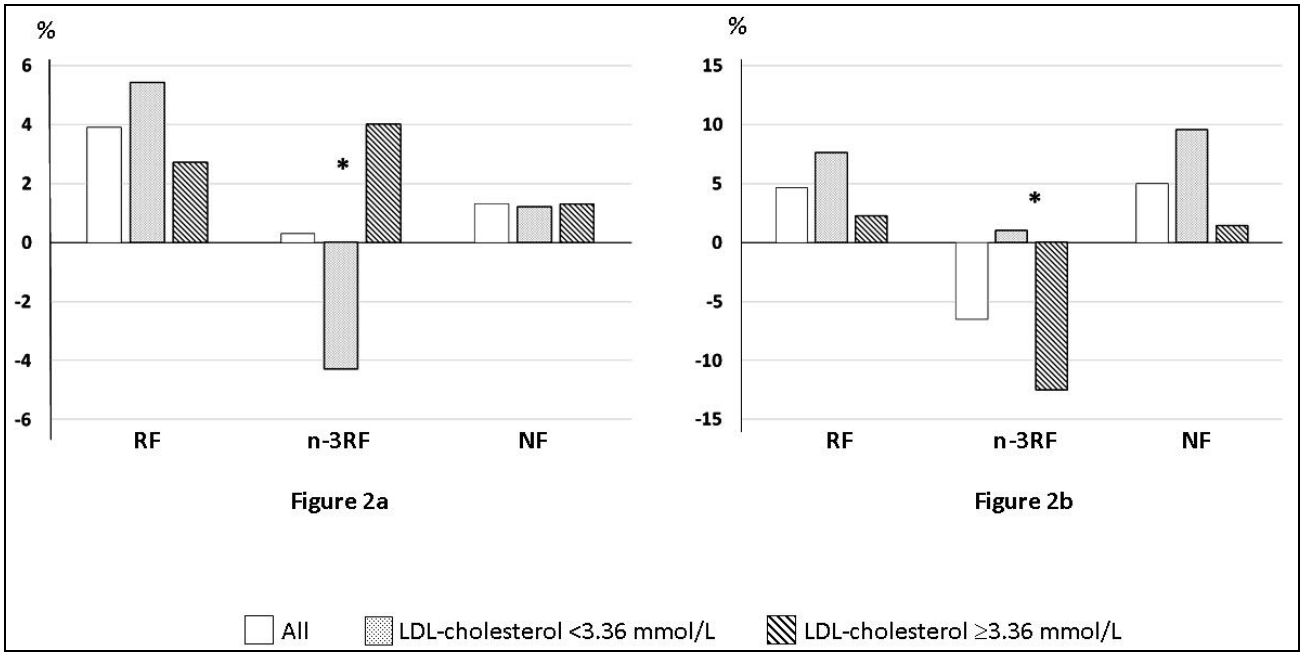

Figure 2. Rate of change (%) for HDL-cholesterol/apo A1 ratio **(Fig. 2a)** and LDL-cholesterol/apo B ratio **(Fig. 2b)** following the reduced-fat (RF), the n-3 reduced-fat (n-3RF) and normal-fat (NF) periods in volunteers classified according to initial LDL-cholesterol values, <3.36 mmol/L or ≥3.36 mmol/L. *p<0.05, low-normal LDL-cholesterol *vs.* high LDL-cholesterol values.

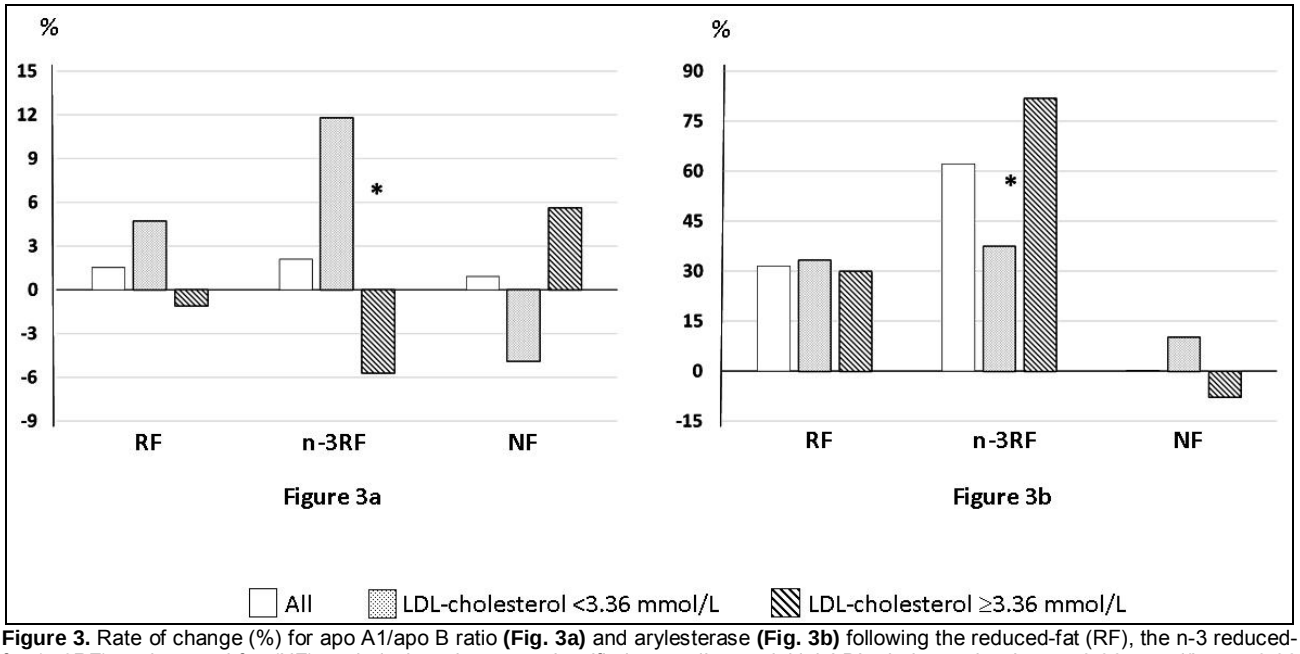

Figure 3. Rate of change (%) for apo A1/apo B ratio **(Fig. 3a)** and arylesterase **(Fig. 3b)** following the reduced-fat (RF), the n-3 reduced-fat (n-3RF) and normal-fat (NF) periods in volunteers classified according to initial LDL-cholesterol values, <3.36 mmol/L or ≥3.36 mmol/L. *p<0.05, low-normal LDL-cholesterol *vs.* high LDL-cholesterol values.

The 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[18], tending to decrease in both LDL-cholesterol groups during the n-3RF-period but to increase in the NF-period. Although publications on dietary effects on plasma Lp(a) levels are scarce[19], Shin *et al.*[20] reported that Lp(a) increases after a high-fat low-carbohydrate diet as compared to a low-fat high-carbohydrate diet. Furthermore, a slight increase in Lp(a) when replacing saturated fat with monounsaturated fat has been published (Berglound *et al.*[21]). In relation to the previous discussed parameters, Lp(a) has been suggested to be an important carrier of oxidized-phospholipids[22]. The tendency for Lp(a) to decrease in those volunteers during the n-3RF-period may also have counterbalanced the modifications reported in the LDL fraction. Some differences were observed during the three intervention periods in the rates of change in volunteers with low-normal or high initial LDL-cholesterol levels. Both groups

responded similarly to the RF, while AE activities and HDL-cholesterol/apo A1 ratios increased in volunteers with high initial LDL-cholesterol, and apo A1/apo B and LDL-cholesterol/apo B ratios decreased after the n-3RF-period. These results, all together, suggest that these products could exert some double-edged effects. In fact, the observed decrease in the LDL-cholesterol/apo B ratio suggests the presence of smaller and more oxidized LDL[16], while the HDL-cholesterol/apo A1 ratio suggests that the HDL particles were metabolically less active[23]. Thus, the increased AE activity appears to be responsible for blocking, at least partially, the increased oxidability of LDL and HDL during this period, which was characterized by high n-3 fatty acid consumption. Finally, subjects with low-normal LDL-cholesterol levels were more negatively affected by the consumption of NF-products, as reflected by a greater increase in TC and the TC/LDL-cholesterol ratio.

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, was relatively high; b) only two types of meat derivate, pâtés and frankfurters, were tested; c) the study focused on CVD markers; d) only males at CVD risk were studied, and e) a low number of volunteers were studied in both LDL-cholesterol groups. Nonetheless, the study has the strength of being the first to consider the response of several CVD markers and ratios in subjects at high risk of CVD differing in their LDL-cholesterol levels to pork-products formulated with an improved fat profile. The present publication also deals with the JONNPR objectives to inform about negative and inconclusive results[24].

Conclusions:

Differences in response between volunteers with low-normal *vs.* high initial LDL-cholesterol levels were evident in the n-3RF but not in the RF period. The relevant increase of AE, AE/HDL-cholesterol, AE/oxLDL in both LDL-cholesterol groups following the n-3RF-product and mainly in volunteers with ≥3.36 mmol/L LDL-cholesterol was a remarkable fact. Subjects with high LDL-cholesterol seem target for n-3RF products while subjects with LDL-cholesterol <3.36 mmol/L were more negatively affected by that of NF-products. Any generalization about fat-improved functional meat-products consumption should be avoided as the effect is not equivalent for all major markers and appears related to LDL-cholesterol levels of the volunteers.

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Conflict of interest:

None.

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