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Original/*Alimentos funcionales*

Impact of cooked functional meat enriched with omega-3 fatty acids and rosemary extract on inflammatory and oxidative status; A randomised, double-blind, crossover study

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Abstract

Background & Aim: n-3 fatty acid intake has been associated with inflammatory benefits in cardiovascular disease (CVD). Functionalising meat may be of great interest. The aim of the present study was to assess the effect of functional meat containing n-3 and rosemary extract on inflammatory and oxidative status markers in subjects with risk for CVD.

Methods and results: A randomised, double-blind, cross-over study was undertaken to compare the effects on the above markers of consuming functional or control meat products. 43 volunteers with at least two lipid profile variables showing risk for CVD were randomly assigned to receive functional meat (FM) or control meat (CM) over 12-weeks with a 4-week wash-out interval before crossover. Functional effects were assessed by examining lipid profile, CRP, PAI-1, *TNF-alpha, IL*-6, fibrinogen (inflammatory markers), and TBARS, FRAP and 8-iso-PGF$_{2\alpha}$ (oxidative status markers). 33 subjects (24 women) aged 50.7±8.8 years completed the study. In FM treatment, PAI-1, fibrinogen and 8-iso-PGF2α decreased significantly after 12 weeks, while FRAP significantly increased. In contrast, in CM treatment, a significant increase was seen in PAI-1, while FRAP significantly declined. Significant differences were also seen between the FM and CM treatments after 12 weeks in terms of the change observed in PAI-1, FRAP and 8-iso-PGF2α values. No significant differences were seen in anthropometric variables nor were adverse effects reported.

Conclusion: The consumption of FM containing n-3 and rosemary extract improved oxidative and inflammatory status of people with at least two lipid profile variables showing risk for CVD. The inclusion of such

IMPACTO DE LA CARNE FUNCIONAL COCINADA ENRIQUECIDA CON ÁCIDOS GRASOS OMEGA 3 Y EXTRACTO DE ROMERO SOBRE EL ESTADO INFLAMATORIO Y OXIDATIVO; UN ESTUDIO ALEATORIZADO, CRUZADO Y DOBLE CIEGO

Resumen

Objetivos: La ingesta de omega-3 se ha asociado con efectos antinflamatorios relacionados con la prevención de la enfermedad cardiovascular (ECV). Desarrollar productos cárnicos funcionales podría ser de gran interés para la población. El objetivo del presente estudio fue evaluar el efecto de una carne funcional con omega-3 y extracto de romero sobre marcadores de inflamación y oxidación en personas con riesgo cardiovascular.

Pacientes y métodos: Se diseñó un ensayo clínico cruzado y doble-ciego para estudiar el efecto del consumo de un producto cárnico funcional sobre marcadores de inflamación y oxidación. Se incluyeron 43 voluntarios con al menos 2 parámetros del perfil lipídico alterado, indicando riesgo de ECV. Fueron asignados aleatoriamente en 2 grupos que consumieron en cruzado carne funcional (CF) o carne control (CC) durante 12 semanas con un periodo de lavado de 4 semanas entre ellos. Al finalizar el estudio se evaluó: perfil lipídico, marcadores de inflamación (PCR, PAI-1, TNF-alpha, IL-6, fibrinógeno) y marcadores de oxidación (TBARS, FRAP, 8-iso-PGF2α).

Resultados: Completaron el estudio 33 personas (24 mujeres) con edad media de 50.7±8.8 años. Tras consumir CF durante 12 semanas se observó una disminución significativa del PAI-1, fibrinógeno y 8-iso-PGF2α, mientras que el FRAP incrementó significativamente. Sin embargo, con CC incrementó PAI-1 y disminuyó FRAP significativamente. Además se observaron diferencias significativas entre los cambios producidos tras consumir uno u otro producto de los marcadores PAI-1, FRAP y 8-iso-PGF2α. Al final de cada intervención no se observaron cambios en variables antropométricas ni efectos adversos.

Conclusiones: El consumo de CF con omega-3 y extracto de romero mejora el estado inflamatorio y oxidativo de personas con al menos 2 parámetros del perfil lipídico alterado. La inclusión de estas CF en una dieta

functional meat in a balanced diet might be a healthy lifestyle option. ClinicalTrials.gov NCT0199088.

Abbreviations

CVD: Cardiovascular disease.
EPA: Eicosapentaenoic acid.
DHA: Docosahexaenoic acid.
CRP: C-reactive protein.
IL-6: Interleukin-6.
BMI: Body mass index.
TBARS: Thiobarbituric acid reactive substances.
F2α: 8-iso-PGF2α: 8-iso-prostaglandin.

Introduction

Improving consumer knowledge regarding the health benefits of different foods and their ingredients has triggered a transformation in the way food is perceived. An increasing number of consumers now associate food with health, and many rely on the consumption of nutritional supplements and fortified foods to prevent problems such as cardiovascular disease (CVD).

A number of reviews have reported omega-3 polyunsaturated fatty acids (n-3) to be effective at reducing the risk of CVD and fatal cardiovascular events[1], and there is strong evidence supporting the beneficial health effects of a diet with a good n-6/n-3 balance[2].

An association exists between several markers of inflammation and the risk of CVD in apparently healthy individuals. Similar associations may also be seen in patients that already have CVD or suffer heart failure[3]. Systemic biomarkers of early and late atherosclerosis are of great clinical interest given their potential in the identification of high risk patients. C-reactive protein (CRP), interleukin-6 (IL-6) and some cell adhesion molecules such as sVCAM-1 and sICAM-1 provide prognostic information beyond that obtained from clinical variables after acute coronary syndromes. Indeed, these seem to be powerful predictors of subsequent cardiovascular events[4]. Recently, the intake of marine-origin n-3 has been associated with reduced plasma concentrations of inflammatory markers[5].

Such findings have promoted the rapid development of functional foods enriched in n-3. Most of these new formulations are dairy products or margarines, etc.; few are based on meat products. Functionalising meat products is of great interest given the common presence of meat in the diet[6]. Meat and meat products are of high nutritional value, containing high quality proteins (including some 40% of all essential amino acids) vitamins and minerals. Their high fat content, however, has frequently been related to a higher incidence of chronic diseases[7]. In fact, conventional meat products have an n-6/n-3 ratio of >15 while healthy effects have been associated with ratios of <4[2]. One means of modifying their fat profile would, therefore, be to improve their n-6/n-3 ratio, replacing saturated fat with fish oil (which is high in eicosapentaenoic [EPA] and docosahexaenoic acids [DHA])[8]. Nevertheless, higher n-3 intakes can increase oxidative stress[9]. To minimize this problem, and to protect from autooxidation, the added n-3 should be combined with antioxidants[10]. The most interesting food-grade antioxidants are those obtained from natural sources, in particular from plants such as rosemary (*Rosmarinus officinalis*). Rosemary extracts obtained using supercritical fluid technologies are strongly antioxidant[11,12].

The Process for the Assessment of Scientific Support for Claims on Food (PASSCLAIM) insists that the beneficial effect of functional food consumption be demonstrated satisfactorily by means of appropriate intermediate biomarkers[13]. The aim of the present work was to assess the effect of a functional meat product containing n-3 and rosemary extract on inflammatory and oxidative markers in subjects with at least two lipid profile variables showing a risk for CVD.

Patients and methods

The present study was registered at http://clinicaltrials.gov with the number NCT0199088.

Sample Study

Forty-three men and women aged over 18 years, with at least two lipid profile variables (TAG ≥150 mg/dl and/or total cholesterol ≥200 mg/dl and/or LDL ≥130 mg/dl and/or HDL <40 mg/dl men *or* <50 mg/dl women) reflecting a risk for CVD[14], were recruited through the Vascular Risk Department and the Nutrition Department of the *Hospital Universitario La Paz*, Madrid (Spain). Subjects were excluded if they reported one of the following: body mass index (BMI) ≥35 kg/m^2, a diagnosis of diabetes mellitus, recent symptomatic heart disease including angina pectoris, a history of myocardial infarction or stroke, peripheral vascular disease, major surgery within the last three months, liver or kidney disease, the taking of antihypertension or

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cholesterol-lowering medication, the consumption of n-3 functional foods, the taking of fish oil or antioxidant supplements, or an inability to consume the test foods. All subjects gave their signed consent to take part in the study, which was approved by the Scientific Research and Ethics Committee of the *Hospital Universitario La Paz* and conformed to the ethical standards of the Declaration of Helsinki[15].

Study Design

The study involved a 28-week, randomised, double-blind, crossover study to compare the effects of consuming functional (FM) and control meat (CM) products. Volunteers were randomly assigned by gender to follow one of two 12-week experimental treatments – the consumption of FM or CM - before swapping over. Neither the researchers nor the subjects knew to which treatment the latter had been assigned; the researchers were unblinded only at the end of the study. The two periods were separated by a 4-week wash-out interval during which subjects returned to their usual diet. During the FM period, volunteers consumed three 150 g servings of a functional meat product (based on cooked ham and cooked turkey breast) per week. During the CM period, volunteers consumed identical amounts of meat product with no functional ingredients. It was firmly recommended that all other meat and meat derivatives be excluded from the diet.

Functional meat product and rosemary extract

Pork and turkey meat for preparing cooked ham and cooked turkey breast were purchased by Grupo Frial

(Tres Cantos, Spain). Supercritical rosemary extracts were prepared by the UAM supercritical facilities (pilot plant scale) using the conditions described by Señorans et al. (2000)[16,17]. Food-grade vitamin E (BTSA, Madrid) and deodorized salmon oil (providing n-3) were supplied by Grupo Frial's habitual sources.

The FM (both cooked ham and turkey breast) was manufactured at Grupo Frial's facilities following their own quality control standards and patented formula (P200402755.2004). The amounts of rosemary extract, salmon oil (n-3) and vitamin E used were those necessary to achieve concentrations of 0.02% w/w, 0.6% w/w and 0.001% w/w respectively. The CM was the same cooked ham and turkey breast but without the above additives. More information about the preparation steps can be obtained from a previous paper[8]. Immediately after manufacture, the FM and CM were vacuum packed and refrigerated, and marked A or B to maintain the conditions of blinding. All samples were transported under refrigerated conditions. Table I shows the composition of the FM and CM meats.

Methods

Subjects attended appointments at the *Unidad de Ensayos Clínicos* at the *Hospital Universitario La Paz* in Madrid at baseline and at the end of the FM and CM experimental periods. At these times the following data were collected:

– *Dietetic data*. All food and beverages consumed were recorded in the week prior to each appointment using a food frequency questionnaire and a "3-day food and drink record" validated for the Spanish population[18]. This recorded all food

Table I
Meat compositions

		Functional Meat		Control Meat	
		Cooked Ham	Turkey Breast	Cooked Ham	Turkey Breast
Calories	(kcal/100 g)	113	106	113	106
Protein	(g/100 g)	19.00	19.00	19.00	19.00
Carbohydrates	(g/100 g)	0.50	1.00	0.50	1.00
Simple	(g/100 g)	0.50	0.60	0.50	0.60
Fat	(g/100 g)	3.50	2.70	3.50	2.70
SFA	(g/100 g)	1.10	1.00	1.10	1.00
MUFA	(g/100 g)	1.90	1.20	1.90	1.20
PUFA	(g/100 g)	0.80	0.50	0.80	0.50
Sodium	(g/100 g)	0.12	0.12	0.12	0.12
salmon oil (75% n-3)	(g/100 g)	0.90	0.90	-	-
Rosemary extracts	(g/100 g)	0.03	0.03	-	-
Vitamin E	(mg/100 g)	1.00	1.00	-	-

and drinks consumed at home and away for three days, including 2 weekdays and 1 weekend day. Subjects were instructed to record the weights of food consumed if possible and to use household measurements (spoonfuls, cups, etc) if not. The energy and nutritional content of the food consumed was then calculated using *Alimentación y Salud*® software (Food and Health, Nutrition Institute, University of Granada, Spain). The values obtained were compared to recommended values to determine dietary adequacy[19].

– *Anthropometric measurements*. These were taken using standard techniques, adhering to international norms set out by the WHO[20]. All measurements were made by trained personnel, first thing in the morning, with the subject barefoot and wearing only underwear. Body weight was determined using a single frequency body composition analyser (TANITA BC-420MA, Biológica Tecnología Médica S.L. Barcelona, Spain). Height was determined using a height meter with an accuracy of 1 mm (range 80-200 cm). Subject BMI was calculated as weight (kg)/height^2 (m).

– *Health variables*. Information was collected on medical conditions and the consumption of medications. Blood pressure and heart rate were measured in the right arm using a Welch Automatic Monitor (Allyn Spot Vital Signs 420 series) (±5 mmHg). Three measurements were taken at 5 min intervals and means calculated.

— *Biochemical data*. Blood samples were taken early in the morning by the Extraction Unit of the Hospital Universitario La Paz after a 12 h overnight fast. Samples were kept at 4-6°C until analysis, which was always performed within 48 h. Biochemical lipid profiles (total cholesterol, HDL and LDL cholesterol, triglycerides) and glucose determinations were performed by enzymatic-spectrophotometric assay using an Olympus AU 5400 apparatus (Izasa. CA, USA). CRP concentrations were determined using a BNII nephelometer (Siemens Healthcare Diagnostics GmbH, Eschborn, Germany). Plasminogen activator inhibitor-1 (PAI-1), tumour necrosis factor-α (TNF-α) and IL-6 were determined using a Luminex®-100 multianalyte profiling system with commercially available immunoassay panels. Plasma fibrinogen was measured using the TOP 700 haemostasis testing system (Izasa. CA, USA). Total lipid peroxides in plasma were determined as an indicator of oxidative stress using the thiobarbituric acid reactive substances (TBARS) method[21]. The results were expressed as µmol MDAeq/mL. The plasma antioxidant capacity was analysed by FRAP assay[17], recording the results as µmol Trolox equivalent (Teq)/mL.

Finally, 8-iso-prostaglandin F2α (8-iso-PGF2α) was determined by competitive immunoassay using the Direct 8-iso-PGF2α EIA kit (Enzo Life Sciences AG, Lausen, Switzerland, following the manufacturer's instructions.

Compliance and adverse events

Subjects received the exact number of packages of FM or CM required for the FM or CM period at the initial visit of each (36 packages: 18 of cooked ham and 18 of cooked turkey breast). Subjects were asked to return any non-consumed meat or unopened packages, as well as their empty packages. Compliance was measured at the end of each experimental period by comparing the number of packages provided and the number of unopened packages returned. A subject was considered compliant when he/she consumed the contents of ≥70% of the packages provided (25 packages). Adverse events were recorded on the final visit of the FM and CM periods. An adverse event was defined as any unfavourable, unintended effect reported by a subject or observed by the investigator. All were recorded along with the symptoms involved (nausea, vomiting, diarrhoea, halitosis, constipation etc.).

Statistical analysis

Quantitative data are presented as means±standard deviation (SD). Qualitative data are presented as absolute frequencies and percentages. All data were tested for normal distribution and for homogeneity of variance using the Kolmogorov-Smirnov test and Levene's test respectively. Possible differences between baseline and final values in the intervention or control periods, as well as between the change in these values during these periods (with the starting values as covariates), were tested using repeated measures ANOVA. Significance was set at $p<0.05$. All calculations were performed using either SPSS 9.0 or SAS Enterprise Guide 3.0 software.

Results

Recruitment and study population

Forty-three volunteers (12 men, 31 women) were randomised for the study. Three subjects were lost to follow-up, and seven dropped out (4 during the I period, 3 during the C period) due to the taste of the product assigned. Thus, 33 subjects (9 men, 24 women) completed the 28-week study; only their data were included in analysis.

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Baseline characteristics

The mean age of the population was 50.7±8.8 years. The anthropometric, dietary and biochemical baseline characteristics of the members of the two randomised groups (FM/CM and CM/FM) were free of significant differences (Table II). The subjects' energy intake profiles were imbalanced: lipids and proteins provided more energy than recommended, and carbohydrate intakes were below those recommended (Table II).

Dietary intake and compliance

No significant differences were seen in the number of meat packages consumed (FM 33.6±2.73, CM 34.1±2.63). n-3 and vitamin E intake were significantly higher with the FM than with the CM treatment (n-3 0.95±0.08 *vs*. 0.01±0.0 g/d, p<0.05; vitamin E 1.51±0.11 *vs* 0.11±0.01 mg/d, p<0.05). No significant differences were seen between the macronutrient and micronutrient intakes in either period when those provided by the FM or CM were left out of analyses.

Weight and BMI

Body weight did not significantly change in either treatment (FM from 73.08±13.92 kg to 72.51±13.26 kg at 12 weeks; CM from 72.85±14.11 kg to 72.24 ±13.22 kg at 12 weeks). Neither was any significant change seen in BMI (FM from 27.27±4.04 kg/m^2 to 27.07±3.88 kg/m^2 at 12 weeks; CM from 27.43±3.94 kg/m^2 to 27.22 ±3.93 kg/m^2 at 12 weeks).

Biochemical markers of CVD-related risk

Table III shows that the lipid profile remained unmodified over the intervention period.

In the FM treatment, PAI-1, fibrinogen and 8-iso-PGF$_{2\alpha}$ decreased significantly after 12 weeks (from 5.40±1.34 to 5.15±1.23 ng/mL [P<0.01], from 427.12±73.79 to 404.91±68.08 mg/dL [P<0.05], and from 26.62±7.48 to 21.51±7.24 ng/mL [P<0.05], respectively) while FRAP significantly increased (from 66.38±9.58 to 68.95±10.88 μmol Teq/mL [P<0.05]). In contrast, in the CM treatment, a significant increase was seen in PAI-1 (from 4.99±1.47 to 5.27±1.59 ng/mL [P<0.01]), while FRAP significantly decreased (from 70.32±11.16 to 67.13 ±9.63 μmol Teq/mL [P<0.01]). Significant differences were also seen between the FM and CM groups after 12 weeks in terms of the change in PAI-1 (-0.25±0.91 *vs*. 0.38±0.92 ng/mL, [P<0.01]), FRAP (2.57±8.11 *vs*. -3.19±8.09 μmol Teq/mL, [P<0.05]) and 8-iso-PGF$_{2\alpha}$ (-5.11±9.83 *vs*. 4.70±10.99 ng/mL, [P<0.05]) values (Table III).

Adverse events

No adverse reactions derived from the consumption of the FM or CM was reported.

Table II
Baseline characteristics of the FM/CM and CM/FM subjects

		FM/CM (n=17)	CM/FM (n=16)	P
Gender	(Female %)	76.5	62.5	NS
Age	(year) M±SD	52.5±8.3	48.7±9.2	NS
Body Mass Index	(kg/m^2)	26.5±3.4	28.5±4.4	NS
Cholesterol (total)	(mg/dL)	265.1±77.2	236.8±31.6	NS
HDL cholesterol	(mg/dL)	55.8±18.8	52.9±14.0	NS
LDL cholesterol	(mg/dL)	181.8±55.9	156.6±23.8	NS
Triglycerides	(mg/dL)	141.6±72.5	128.9±39.8	NS
Blood glucose	(mg/dL)	100.4±8.8	99.2±10.3	NS
Systolic pressure	(mm Hg)	119.6±12.5	113.4±10.3	NS
Diastolic pressure	(mm Hg)	75.3±8.2	70.8±7.8	NS
Energy intake (EI)	(kcal/d)	1766±365	1665±359	NS
Energy profile				
Proteins	(% EI)	22.6±7.3	22.7±3.2	NS
Carbohydrates	(% EI)	41.5±8.4	41.1±9.5	NS
Lipids	(% EI)	36.0±6.2	36.2±8.4	NS

NS not significant (p>0.1)

Table III
Biochemical markers at baseline and at the end of each treatment period[a].

			Baseline	Final	Change
Lipid profile					
t-Chol	(mg/dL)	FM	251.3±60.1	258.9±70.7	7.7±24.4
		CM	258.5±63.8	257.6±61.2	-0.9±28.3
LDL	(mg/dL)	FM	167.2±43.5	172.7±52.1	5.5±19.2
		CM	171.3±45.7	171.0±54.2	-0.3±23.0
HDL	(mg/dL)	FM	55.4±16.6	55.4±15.6	0.0±5.0
		CM	55.4±15.3	55.6±14.8	0.2±5.9
TG	(mg/dL)	FM	129.4±60.8	128.3±59.1	-1.1±47.7
		CM	147.9±79.6	128.2±60.2	-19.7±49.2
Inflammatory biomarkers					
CRP	(mg/dL)	FM	2.66±4.16	1.98±3.18	-0.69±5.27
		CM	2.18±2.15	1.91±2.29	-0.27±2.55
PAI-1	(ng/mL)	FM	5.40±1.34	5.15±1.23**	-0.25±0.91
		CM	4.99±1.47	5.27±1.59**	0.38±0.92##
TNF-alpha	(pg/mL)	FM	4.11±2.84	3.94±2.42	*-0.16±1.80*
		CM	4.38±3.20	4.62±4.21	*0.24±3.10*
IL-6	(pg/mL)	FM	1.49±0.94	1.44±1.00	*-0.05±0.88*
		CM	1.37±1.13	2.06±4.16	*0.67±3.37*
Fibrinogen	(mg/dL)	FM	427.1±73.8	404.9±68.1*	*-17.0±80.2*
		CM	400.6±55.5	416.8±54.8	*16.2±53.1*
Oxidative stress status biomarkers					
TBARS	(μmol MDAeq/mL)	FM	66.8±19.7	58.1±17.5	*-8.7±16.9*
		CM	62.3±17.7	58.2±21.1	*-2.2±23.2*
FRAP	(μmol Teq/mL)	FM	66.4±9.6	69.0±10.9*	*2.6±8.1*
		CM	70.3±11.2	67.1±9.6**	*-3.2±8.1#*
8-iso-PGF$_{2\alpha}$	(ng/mL)	FM	26.6±7.5	21.5±7.2*	*-5.1±9.8*
		CM	21.8±7.6	26.5±12.4	*4.7±11.0#*

t-Chol: total cholesterol; TG: triglycerides; CRP: C-reactive protein; PAI-1: plasminogen activator inhibitor-1; TBARS: thiobarbituric acid reactive substances; 8-iso-PGF2α: 8-iso-prostaglandin F2α; a Data are expressed as means±SD.
Significantly different compared to the start value of each treatment (*P<0.05. **P<0.01)
Significantly different for the comparison of mean changes after the intervention between the two treatments with baseline values as covariate (#p<0.05. #.# p<0.01)

Discussion

To our knowledge, this is the first study to examine the effect of the intake of FM enriched in n-3 (from salmon oil) and rosemary extract on inflammatory and oxidative status in subjects at some potential risk of developing CVD. The consumption of three servings/week of FM reduced markers of inflammatory and oxidative status after 12 weeks of intervention.

Despite recommendations that the consumption of meat products be reduced while fish, vegetable and fruit consumption be increased[22], people seldomly rectify their diets sufficiently. This study involves the consumption of a number of portions of meat per week consistent with current recommendations in Western countries[23]. The idea of adding salmon oil to the FM was to promote an increase in n-3 consumption. This increased the contribution of n-3 to the coverage of to-

tal energy by 0.49±0.04%; this would help in covering the 1-2% recommended in Spanish nutritional objectives[24].

Although some studies in rodents and humans have indicated that the consumption of n-3 may help reduce obesity[25], the increase in the consumption of n-3 with the FM led to no change in either body weight or BMI.

The main dietary guidelines recommend restricting total and saturated fat intake as a means of managing lipid profiles in the control of CVD risk. These recommendations usually involve reducing the consumption of red meats, sausages and *charcuterie*, replacing them with white meats[23,26]. Certainly, the consumption of the present CM and FM, i.e., cooked ham and cooked turkey breast (which commercially might be considered *charcuterie* products) within the context of current recommendations[27], had no negative effect on lipid profiles. Other intervention studies have reported

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positive effects of functional foods (especially dairy products) containing EPA and DHA on lipid profiles, especially the reduction of triglycerides[28]. Indeed, a meta-analysis of 36 randomised, controlled, cross-over trials in which 3–4 g of EPA + DHA were consumed showed prominent triglyceride reductions when baseline values were >177 mg/dL[29]. In the present work, however, the subjects had a lower mean baseline triglycerides value (135.5±40.9 mg/dl); this might explain why no such reduction in triglycerides etc. was seen.

The FM treatment led to a significant reduction in plasma PAI-1 and fibrinogen concentrations over the 12 week period. A significant difference was also seen between the FM and CM treatments in terms of the change in PAI-1. The anti-inflammatory effects of n-3 are well known[30]. Some authors also suggest that rosemary extract has an effect on systemic inflammation[31,32]. This was recently confirmed in a study of 19 healthy young volunteers that received oral supplementation with this extract (77.7 mg/d) for 21 days. As in the present study, the authors observed a significant reduction in PAI-1, but no significant effect on lipid profile or inflammation markers[33]. These findings are important since high PAI-1 is one of the most commonly studied emerging cardiovascular risk factors. The reduction of PAI-1 levels may prevent atherosclerosis and its complications[34]. The potential use of PAI-1-reducing medication in treating CVD would appear quite promising[34].

A consensus is emerging that the proportion of n-3 in the diet should be increased. It is also recognized, however, that the propensity of such fatty acids for oxidation could lead to potentially harmful levels of hydroperoxides, so higher *relative* proportions of n-3 are now often advised[35]. The addition of bioactive antioxidants to n-3-containing food matrices might therefore be an optimal design for a healthy functional food. Rosemary is widely accepted as having some of the highest of all antioxidant activities[36]. Results obtained in animal models by our group indicate rosemary extract to have a moderate antioxidant effect in heart tissue. In the hippocampus, reactive oxygen species were significantly reduced[37]. In the present work, the change in values for two of the plasma markers of oxidative stress (FRAP and 8-iso-PGF$_{2\alpha}$) suggest that the consumption of the present FM might modulate oxidative stress.

A study involving 22 overweight/obese volunteers at high risk of CVD showed a significant increase in plasma antioxidant markers (glutathione, catalase, superoxide dismutase and paraoxonase enzyme [PON-1]) after providing 5 servings/week of 150 g of restructured meat enriched with 20% nut paste (rich in linoleic acid and polyphenols) during 5 weeks[38]. In another crossover, controlled trial comparing the effects of a low fat meat (LFM) and a walnut-enriched meat (WM) (4 x 150g servings/week for 5 weeks) in volunteers at high risk of CVD, it was observed that the intake of WM increased PON-1. The effect on other antioxidant enzymes and substrates varied, however, depending on the individual's PON-1 polymorphism[39].

The measurement of 8-iso-PGF$_{2\alpha}$ has emerged as one of the most reliable ways of determining oxidative stress status *in vivo*, providing an important means of exploring the role of this stress in the pathogenesis of human disease[40]. The present study is the first to use this biomarker to evaluate the antioxidant effect of a functional meat enriched in n-3 and rosemary extract on oxidative status. Mas et al.[41], who studied subjects with moderate dyslipidemia consuming 4 g/day of EPA or DHA or olive oil capsules (control group) for 6 weeks, recorded a significant reduction in 8-iso-PGF$_{2\alpha}$ (24% and 14% in each group respectively). It is important to note that these subjects also made lifestyle changes (diet, physical activity, alcohol consumption)[41]. Nalsen et al. recorded a significant reduction in isoprostanes in a randomised, multicentre study involving healthy subjects receiving a supplement of 3.6 g of n-3/day Supplementation with n-3 may therefore lead to a reduction in isoprostanes in both healthy and diseased populations[42].

Conclusions

The consumption of functional meat products enriched with n-3 and rosemary antioxidant within the context of a balanced diet, can improve the oxidative and inflammatory status of people with at least two markers of risk for CVD. The inclusion of such functional meat in a balanced diet might be a healthy lifestyle option.

Acknowledgments

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