The Antioxidant Status Response to Low Fat- and Walnut Paste-Enriched Meat Differs in Volunteers at High Cardiovascular Risk Carrying Different PON-1 Polymorphisms

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Running title: Meat consumption and PON-1 polymorphisms.

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Abstract

Background: Cardiovascular risk largely depends on diet, antioxidant status and gene polymorphisms. Low-fat meat (CM) and walnut enriched meat (WM) products may exert potential beneficial health effects with respect to conventional meat products.

Objective: To compare the effects of consuming WM *vs*. CM on reduced and oxidized glutathione, lipoperoxides, α- and γ-tocopherol levels and paraoxonase (PON-1), catalase (CAT), superoxide dismutase (SOD) activities in 22 volunteers (mean 54.8 years and BMI 29.6 kg/m^2) at high cardiovascular risk carrying different PON-1 192/55 polymorphisms.

Design: The study was a 5-week non-blinded, randomized, cross-over, controlled trial.

Results: In general term, WM *vs*. CM improved the volunteers' antioxidant status with several result modifications occurring after WM period. CM consumption increased oxidized glutathione and decreased PON-1 activity (at least P < 0.05). When WM *vs*. CM effects were compared SOD, CAT and PON-1 enzyme activities increased (at least P < 0.05) in PON-1 192QQ carriers. γ-tocopherol levels, SOD and PON-1 activities increased in PON-1 192QR+RR carriers besides the significant decrease of lipoperoxide levels. In PON-1 55LM+MM carriers, the intervention increased significantly all the investigated enzyme activities and glutathione levels while PON-1 55LL carriers increased their PON-1 activities.

Conclusions: WM consumption should be preferred to CM. The intake of WM *vs*. CM increased PON-1 but the effect upon other antioxidant enzymes and substrates varied depending on individual's PON-1 polymorphism. PON-1 192QR+RR carriers appear the targets for WM consumption as increased the enzyme activities and γ-tocopherol levels and decreased lipoperoxides.

Abbreviations: CHD = Coronary heart diseases; CM = Low-fat control meat; WM = Walnut paste-enriched meat, CAT = Catalase, LPO = lipoperoxides, SOD = Superoxide dismutase, GSH = Reduced glutathione, GSSG = Oxidised glutathione, PON-1 = Paraoxonase.

1. Introduction

Nut/tree nut consumption has been associated with decreased risk factors for coronary heart diseases (CHD) and Metabolic Syndrome (MS) [1]. Walnuts and other nut species present a favorable fatty acid profile [2] and contain several of bioactive compounds that are coupled with other nutrients such as vitamin E, vitamin C, selenium and act as antioxidants and anti-inflammatory factors [3-5]. Meat and meat products are essential components of diets in Western countries. In Spain, their average consumption is high (ca. 164 g/head and day) [6]. As epidemiological findings have related high-fat meat consumption to degenerative disease [7], efforts have been made to obtain low-fat meat and meat products. Restructured beef steaks and/or sausages formulated with walnut paste (WM) provide a unique combination of nutrients and phytochemicals [8] and display acceptable physiochemical and sensory properties [9]. Our research group previously found that volunteers at high risk of developing CHD who consumed walnut paste-enriched meat (WM) presented more favorable antioxidant status - measured as catalase (CAT), superoxide dismutase (SOD), and paraoxonase-1 (PON-1) activities, reduced (GSH) and oxidized glutathione (GSSG) levels in erythrocytes [10] and γ-tocopherol [11] and thrombogenic profiles [12] than those consuming low-fat meat (CM). However, data showed striking interindividual differences probably due to genetics.

Human paraoxonase (PON-1), a HDL-bound enzyme, is a pleiotropic enzyme involved in the protection against oxidative damage and detoxification of reactive molecules and/or xenobiotics [13-15]. More specifically, this enzyme is engaged in lipoprotein-phospholipid metabolism and may also inhibit lipid peroxide generation in LDL [7, 16,17]. When lipid peroxidation occurs in LDL, one of the first enzymes acting in order to preserve LDL and protect them against oxidation is PON-1 [18]. However, PON-1 can be inactivated by oxidative stress and oxidized lipids [19]. Martinelli et al. [20] hypothesized that a greater degree of severity of MS is associated with an increased oxidative stress which inactivates PON-1 function.

Despite current interest in this enzyme and numerous studies investigating its behavior, little is yet certain with regard to its biological function or the activity of its polymorphisms. PON-1 exists in two major polymorphic forms which include the replacement of glutamine (Q) by arginine (R) at position 192 [21], and that of leucine (L) by methionine (M) at position 55 [22]. Polymorphisms at 55 and 192 positions are associated with a 40-fold interindividual variability in PON-1 enzyme activity [23]. The

PON-1-192 polymorphism is associated with diminished PON-1 concentrations and an increased risk for CHD in RR-allele subjects [24]. It has been reported that PON-1 in HDL particles of 192R allele carriers offers significantly less protection against LDL peroxidation *in vitro* than in case of 192Q and 55L carriers [18]. Our group has analyzed the response of carriers of the major PON-1 polymorphisms to WM and CM with reference to arylesterase activity and lipoperoxides (LPO) [25] and other biomarkers for CHD, including PON-1, soluble vascular and intercellular adhesion molecules (sVCAM-1 and sICAM-1) and leukotriene B4 (LTB4) levels [26]. The positive effect of WM consumption was largely influenced by PON 1 polymorphisms [25,26].

Taking into account previous cited studies, the hypothesis of the present study is that WM is more effective than CM in reducing the *in vivo* oxidative stress in the erythrocytes of individuals at high risk for developing CHD with certain PON-1 gene alleles. Thus, the present trial aimed to analyze the effects of a 5-week consumption of WM and CM in volunteers carrying PON-1 55 and/or 192 polymorphisms on a) serum α-tocopherol and γ-tocopherol levels and PON-1 activity; b) erythrocyte reduced glutathione (GSH), oxidized glutathione (GSSG), and LPO concentrations and SOD and CAT activities.

2. Subjects and methods

2.1. Subjects

Eligibility and exclusion criteria have been already published [10,11]. Briefly, eligibility criteria for the study included a) age of men $\geq$45 years; age of women $\geq$50 years and postmenopausal, and b) BMI $\geq$25-<35 kg/m^2. In addition, one or more of the following criteria also had to be met: serum total cholesterol $\geq$5.69 mmol/l; smoking habit ($\geq$10 cigarettes per day); hypertension (systolic pressure $\geq$140 mmHg and/or diastolic pressure $\geq$90 mmHg). Subjects had to be frequent meat product consumers ($\geq$ 5 times/week). Exclusion criteria of the study included: a) familial hypercholesterolemia IIa and/or type I and II diabetes; b) use of vitamin or mineral supplements; c) hormone replacement therapy; d) regular use of aspirin and medications known to affect lipid absorption or metabolism and any chronic disease (e.g. antidiabetic, antiaggregant drugs); e) non-frequent meat products consumption (< 5 times/week). Four individuals taking medication for hypertension were initially excluded, but given the small number of subjects willing to participate, it was necessary to include them. Of the 144

candidates initially recruited through announcements in the media and in hospitals, twenty-five volunteers were finally chosen and started the trial. Three volunteers who did not complete the requisite blood extractions were also excluded from the trial; thus, twenty two volunteers, 12 males and 10 females, completed the intervention.

Procedures followed were in accordance with the standards of the Ethics Committee of the University Hospital of Puerta de Hierro (Madrid, Spain) and the Helsinki Declaration, as indicated in the guidelines of the Scientific Technologic Project AGL 2001-2398-C03. Informed consent was given by the participants prior to the start of the study.

2.2. Study Design

Volunteers were randomly assigned to follow a non-blinded, cross-over, controlled study, consisting of two 5-week experimental periods. The study design has been described previously [11], shortly participants followed their normal dietary habits during the 4 to 6-week wash-out interval that separated the two trial periods. During the WM period, volunteers consumed four 150 g restructured WM steaks and a 150 g ration of WM sausages per week, all containing 20% walnut paste. During the CM period, volunteers consumed four 150 g restructured CM steaks and a 150 g ration of CM sausages each week. The composition of the two types of meat studied is presented in **Table 1**. Information regarding the preparation of meat products (WM and LM) is available in Serrano et al. [9]. Study participants were strongly advised to avoid consuming any other meats or meat products in their diet during the trials.

2.3. Dietary assessment

Frozen CM and WM products were distributed to study participants on a weekly basis. Special emphasis was given to compliance and management of intake with regard to frequency, dates and numbers of steaks consumed. The substitution of conventional meat products by the experimental meat products in the framework of a mixed diet was confirmed and verified by regularly checking the volunteers' dietary records. Participants recorded the amount and kinds of food eaten every day to avoid any possible doubt regarding their diets. Food Composition Tables [27] were used to calculate the volunteers' dietary energy and nutrient intakes. Despite the peculiar taste of WM, most volunteers (80%) reported enjoying this type of meat. Forty percent of the participants, however, commented unfavorably on the low palatability of CM. In

164   addition, walnuts provide γ-tocopherol, which was used as a marker to assess

165   compliance by measuring volunteers serum concentrations after each trial period

166   [10,11].

167

168   *2.4. Sample collection and Preparation of hemolyzates*

169   Blood samples were collected and prepared for analysis by trained staff. Blood was

170   gently delivered in vacutainer tubes for PON-1 determinations while in citrate tubes for

171   SOD, CAST, GSH and GSSG analysis. Serum was separated at 1500 x *g* for 15 min at

172   4ºC while citrated blood was centrifuged at 1000 x *g* for 10 min at 4ºC, and the plasma

173   and buffy coat removed. Erythrocytes were washed with PBS (pH 7.00, containing 140

174   mM NaCl) three times and erythrocytes were hemolyzed with ice-cold distilled water.

175   Hemoglobin (Hb) content was determined by using the cyanmethemoglobin method

176   [28]. Hemolyzates were used to determine SOD and CAT enzymatic activities and GSH

177   and GSSG concentrations.

178

179   *2.5. Enzymes assays*

180   SOD activity (EC 1.15.11) was determined according to Marklund & Marklund

181   method [29], based on pyrogallol autoxidation. One unit of enzyme activity was defined

182   as 50% inhibition of the rate of pyrogallol autoxidation. Results were expressed as U/g

183   Hb. CAT (EC 1.11.1.6) activity was estimated according to Aebi method [30],

184   monitoring the rate of disappearance of hydrogen peroxide at 240 nm. CAT activity was

185   expressed as U/g Hb. PON-1 (EC 3.1.8.1) activity was determined in Tris/HCL buffer

186   (90mM Tris/HCl, 3.6mM NaCl and1.9mM $CaCl_2$) by measuring the rate of hydrolysis

187   of paraoxon in p-nitrophenol catalyzed by the enzyme at 37ºC and 405nm [31]. Frozen

188   aliquots of pool sera were used as the internal control. One unit of PON-1 activity was

189   defined as 1 μmol of p-nitrophenol formed per L per minute in sera.

190

191   *2.6. Glutathione status and Lipid Peroxide assay*

192   Total glutathione (GSH plus GSSG) was measured by fluorometry, according to

193   Hissin & Hilf method [32], using o-phthaldialdehyde. GSH and GSSG results were

194   expressed as μmol/g Hb. The redox index was calculated as the GSH/GSSG ratio.

195   Measurements of LPO in erythrocytes, based on determination of malonyldialdehyde

196 (MDA) and 4 hydroxyalkenals, was performed using the Bioxytech LPO-586 kit (Oxis

197 Research, Porland, USA).

198

199   *2.7. α-tocopherol and γ-tocopherol assays.*

200   α-tocopherol and γ-tocopherol in the serum of the volunteers were analyzed by

201 means of high performance liquid chromatography (HPLC). Methodological details

202 have been described elsewhere [11]. The HPLC method was validated throughout our

203 participation in the Fat-soluble Vitamins Quality Assurance Programme (for serum and

204 food samples) conducted by the National Institute of Standard and Technology (NIST,

205 USA). End data for α-tocopherol and γ-tocopherol correspond to the mean of analyses

206 on days 28 and 35 in each meat period.

207

208   *2.8. Serum lipids and glucose.*

209   Serum cholesterol, HDL cholesterol and triglycerides were determined by standard

210 enzymatic analysis (Boehringer, Manheim, Germany). Cholesterol transported by LDL

211 was calculated using the Friedewald et al formula, where LDL cholesterol = Total

212 cholesterol – (HDL cholesterol + Triglycerides /5), all parameters in mg/dl.

213

214   *2.9. Paraoxonase genotyping*

215   DNA was extracted from peripheral blood cells using the Ultraclean Bloodspin kit

216 (MoBio Laboratories Inc, Carlsbad, California, USA). PON-1 genotyping was carried

217 out by a multiplex PCR assay [33]. Amplification of 111 and 144 pb fragments was

218 performed using a standard polymerase chain reaction (PCR) for PON-1-Q192R and

219 PON-1-L55M, respectively. Reagents were purchased from Promega (Madison, USA)

220 and PCR was performed using a DNA thermocycler (Mastercycler-ep380®, Eppendorf,

221 Hamburg, Germany).

222

223   *2.10. Statistical Analyses*

224   Data are presented as means ± SD. The Kolmogorov-Smirnov test was used to test

225 for the normal distribution of data. Although the study was designed having serum

226 cholesterol as the primary outcome variable, the present paper displayed a statistical

227 power higher than 85% to detect a 30% relative difference (15 U/L) between the

228 responses in PON-1 activity of volunteers who consumed the two different meats,

229 considering an alpha level of 0.05. A pooled SD of 25% (12 U/L) for the change from

230 baseline PON-1 activity was assumed for this calculation. The statistical power for

231 individuals with different PON-1 polymorphisms (group comparison of 11 *vs*. 11 or 8

232 *vs*. 14) was 79% and 76%, respectively. Repeated measures analysis of variance was

233 used to compare the effect of WM *vs*. CM on the variables studied in carriers of each

234 PON-1 allele. A post hoc study was performed in each polymorphism by repeated

235 measures analysis of variance. The net change (%) mean and 95%CI for each variable

236 was calculated taken into account end and baseline of both periods as follow:

237 Net change (%) = $(WM_{end}-WM_{baseline})-(CM_{end}-CM_{baseline})*100/CM_{baseline}$

238

239 **3. Results**

240 From the twenty two participants, 12 were males and 10 females. The average age

241 of them was 54.8 years with no-significant age gender differences (p>0.1). Mean BMI

242 was 29.6 kg/m^2 (55% overweight and 45% obese). Genotype frequencies at both

243 restriction enzyme sites (55 and 192) were in Hardy-Weinberg equilibrium (p > 0.05).

244 **Table 2** shows the daily intake of macronutrients, fiber, and cholesterol during both

245 experimental periods in PON-1 192QQ and QR+RR carriers and in PON-155 LL and

246 LM-MM carriers, respectively. Volunteers followed isocaloric diets during both

247 intervention periods. The significant differences found in energy and macronutrient

248 intakes during WM and CM periods involved fat, PUFA, SFA, total tocopherol and the

249 omega-6/omega-3 ratio. No significant differences were found between QQ and

250 QR+RR carriers and between LL and LM+MM carriers. **Table 3** shows some

251 anthropometrical and clinical data of volunteers carrying the PON-1 192 and PON-1 55

252 polymorphisms during both study periods. The level of triglycerides were differently

253 affected (p=0.024) in PON-1 192QQ *vs*. QR+RR carriers. Triglycerides, the systolic and

254 diastolic blood pressures were differently affected (at least p=0.033) in PON-1 55LL *vs*.

255 LM+MM. The net effect of the intervention (WM *vs*. CM) only affected the diastolic

256 pressure in PON-1 192QQ and QR + RR carriers, although some significant effects

257 were observed for total cholesterol (PON-1 192QR +RR), HDL cholesterol (PON-1 192

258 QQ), diastolic blood pressure (PON-1 192QQ) during the CM period and for HDL

259 cholesterol (PON-1 55LM+MM), systolic and diastolic blood pressures (PON-1

260 192QR+RR and PON-1 55LL) during the WM period.

261 In the volunteers, and without considering polymorphisms, a significant net increase

262 (% mean, 95%CI) of CAT (28.5, 8.1 to 49.0 U/g Hb), SOD (16.7, 4.6 to 28.7 U/g Hb)

263 and PON-1 (62.2, 31.8 to 100.5 U/L) activities was obtained by the global intervention

264 (WM *vs*. CM) (data not shown). CAT, SOD, and PON-1 activities and GSH, GSSG,

265 and γ-tocopherol levels increased significantly ($p < 0.05$) in the total of the 22

266 volunteers after 5 weeks of consumption of WM diet but not in CM diet. PON-1 activity

267 decreased ($p < 0.05$) following CM diet period. Thus, results following the WM were

268 not strictly similar to those found after the WM *vs*. CM comparison (data not shown).

269   The redox index ($p = 0.044$) and LPO concentrations ($p = 0.045$) were affected by

270 the PON-1 192 genotype*treatment interaction (**Table 4**). In comparison to CM, WM

271 increased GSH ($p = 0.046$) and GSSG ($p = 0.001$) levels in PON-1 192QQ while in

272 QR+RR carriers increased γ-tocopherol levels ($p = 0.037$) and decreases LPO levels ($p

273 = 0.009$) (Table 4). WM *vs*. CM consumption leads to an increase of CAT ($p = 0.014$),

274 SOD ($p = 0.029$), PON-1 ($p = 0.001$) and PON-1/HDL cholesterol ($p = 0.05$) activities

275 in PON-1 192QQ while in QR+RR carriers increased SOD ($p = 0.015$), PON-1 ($p =

276 0.037$) and PON-1/HDL cholesterol ($p = 0.045$) activities (**Table 5**).

277   In PON-1 192QQ carriers consuming WM, CAT ($p < 0.01$), SOD ($p < 0.001$), and

278 PON-1 ($p < 0.01$) and PON-1/HDL cholesterol ($p < 0.05$) activities and levels of GSH

279 ($p < 0.01$) GSSG ($p < 0.001$) and γ-tocopherol levels ($p < 0.05$) increased significantly,

280 while the redox index ($p < 0.05$) decreased. In PON-1 192QR+RR carriers consuming

281 WM, LPO values ($p < 0.01$) significantly decreased, while GSH ($p < 0.05$), GSSG ($p <

282 0.001$) and γ-tocopherol ($p < 0.05$) levels increased. In PON-1 192 QQ carriers

283 consuming CM, GSSG values ($p < 0.01$) increased significantly while PON-1 activity

284 decreased ($p < 0.05$) (Tables 4 and 5). In PON-1 192QR+RR, CM decreased PON-1

285 activity ($p < 0.05$) (Tables 4 and 5).

286   A significant interaction between the PON-1 55 polymorphism and meat type was

287 found for GSH levels ($p = 0.047$) (Table 4). In comparison to CM, WM increased GSH

288 ($p = 0.001$) and GSSG ($p = 0.001$) levels in PON-1 55 LM+MM (Table 4). WM *vs*. CM

289 consumption increased CAT ($p = 0.007$), SOD ($p = 0.003$), PON-1 ($p = 0.009$) and

290 PON-1/HDL cholesterol ($p=0.048$) activities while in LL carriers increased PON-1 ($p =

291 0.002$) activities (Table 5).

292   In PON-1 55LL carriers, WM intake increased SOD ($p < 0.05$) and PON-1 ($p <

293 0.05$) and PON-1/HDL cholesterol ($p < 0.05$) activities and GSSG ($p < 0.01$) and γ-

294 tocopherol levels ($p < 0.05$) levels. In PON-1 55LM+MM carriers, WM consumption

295 increased CAT ($p < 0.01$), SOD ($p < 0.01$), PON-1 ($p < 0.05$) and PON-1/HDL

296   cholesterol (p<0.05) activities and GSH (p < 0.001), GSSG (p < 0.001) and γ-

297   tocopherol (p < 0.05) levels. In PON-1 55LL, the CM intake increased GSH levels (p <

298   0.05) but decreased PON-1 activity (p < 0.05). In PON-1 55 LM+MM, the CM intake

299   increased GSSG levels (p < 0.05) and decreased the PON-1 activity (p < 0.05) (Tables 4

300   and 5).

301   When the net nutritional effects on the antioxidant status in volunteers

302   presenting concurrence of both PON-1 192 and 55 polymorphisms were tested, increase

303   in CAT (p = 0.004), SOD (p = 0.002), GSH (p = 0.020), GSSG (p = 0.004), and PON-1

304   (p = 0.012) were observed in (LM+MM)QQ carriers while only significant increase was

305   observed in PON-1 activity in LL(QR+RR) counterparts (data not shown). CM

306   significantly increased concentrations of GSH (p < 0.05) in LL (QR+RR) and those of

307   GSSG (p < 0.05) in (LM+MM, QQ) carriers. WM did not increase any antioxidant

308   parameters except GSSG levels (p < 0.01) in LL(QR+RR) individuals, but it increased

309   CAT (p < 0.01), SOD (p<0.01), GSH (p < 0.01), GSSG (p = 0.001), and PON-1 activity

310   (p < 0.05) and decreased the redox index (p < 0.05) in (LM+MM),QQ carriers (data not

311   shown).

312

313   **4. Discussion**

314   This is the first study to date that evaluates the effects of two potential functional

315   meat products, WM and CM, on several antioxidant status markers in volunteers at high

316   risk of developing CHD carrying different PON-1 polymorphisms. Despite the

317   relatively low number of individuals studied for each polymorphism, the present paper

318   provides new data regarding differences in response of PON-1 192QQ *vs*. 192QR+RR

319   and PON-1 55LL *vs*. LM+MM carriers and in volunteers carrying PON-1 192 and

320   PON-1 55 polymorphisms.

321   No significant differences were observed in energy consumed by volunteers during

322   the WM and CM diets period; although the former diet was somewhat richer in energy

323   than the latter, as it contributed a greater amount of fat and polyunsaturated fatty acids.

324   In addition to their high ω-6 and ω-3 polyunsaturated fatty acid content [3-5, 34],

325   walnuts also contain compounds such as arginine, γ-tocopherol and polyphenols [4,34]

326   that improve antioxidant status and may be associated with decreased prevalence of

327   CHD [1,2,13] and MS risk factors [1].

328   No significant differences in energy and macronutrient energy intakes were found

329   between PON-1 192QQ *vs*. QR+RR and between PON-1 55LL *vs*. LM+MM allele

330 carriers through the study; thus, results have to be related to differences in response to
331 diet of volunteers due to their respective PON-1 192 and/or PON-1 55 polymorphisms.
332 Basal data on lipids and blood pressures suggest, in general terms, that PON-1 192QQ
333 and PON-1 55LM+MM showed lower CHD risk than their QR+RR and LL respective
334 counterparts. These results agree with the available information on PON-1
335 polymorphisms and CHD risk [13,18]. The differences in PON-1 basal activities found
336 by Mackness et al. [35] between QQ and QR/RR carriers were not observed in the
337 present paper. Although no clear explanation is available, the tendency to consume
338 higher PUFA and tocopherol amounts by QQ carriers, would explain, at least partially,
339 such values, as PUFA consumption has been related to PON-1 activity [13]. Oliveira et
340 al. [36] found that the M allele was more prevalent among control subjects than in CHD
341 patients; however, in a meta-analysis study, Wheeler et al. [37] reported no significant
342 association between the PON-1 55 polymorphisms and CHD. The effect of WM and
343 CM diets on blood pressure were noticeable during the whole trial. The dietary increase
344 of nuts and/or the reduction of saturated fat is known to positively affect blood pressure
345 [1,34,38]. Only QR+RR and LL significantly decreased both systolic and diastolic
346 blood pressures during the WM period. In addition, only QQ decreased significantly
347 diastolic blood pressure during the CM period, suggesting clear differences in response
348 to those meat derivates in PON-1 polymorphism carriers.

349 In the totally of volunteers the WM *vs.* CM consumption in all the volunteers
350 improved the antioxidant status. Interestingly, PON-1 activity during the WM period
351 increased in all volunteers, except in PON-1 192QR+RR carriers, while it decrease in
352 all of the volunteers during the CM period. The consumption of ω-3 polyunsaturated
353 fatty acids has been found to increase PON-1 activity [39]. In the CM period, decrease
354 in the PON-1 seemed to be parallel with the changes of HDL cholesterol. The reduction
355 of dietary fat has been related to decrease in HDL [40] that may have resulted in the
356 decrease of PON-1 enzyme activity associate to HDL particles. However, other
357 compounds present in food, walnut and/or WM, e.g. polyphenols, could affect PON-1
358 results [41,42]. Rantala et al. [43] reported decrease in PON-1 activity in women after
359 the consumption of vegetable rich diet for 5 weeks. Strunz et al. [44] found that Brazil
360 nuts affect PON-1 while Nus et al. [25] observed increase in the arylesterase activity
361 after walnut enriched-meat consumption. As volunteers maintained their food habits
362 throughout the whole study, an average reduction of about 8.5 g/day in the fat
363 consumed and an increase in the intake of about 4 g polyunsaturated fatty acids could be

364 calculated with respect to their regular diet after the CM and WM, respectively, which

365 might explain, at least partially, the change in PON-1 activity.

366   The PON-1 192 polymorphism has been associated with diminished PON-1

367 activities and an increased risk for CHD in RR-allele subjects [24]. No significant

368 baseline differences between QQ or R or between LL and M allele carriers in PON-1

369 and other antioxidant enzymes and substrates were observed in either of the two dietary

370 periods. Differences in the study design and type of volunteers tested would explain, at

371 least partially, these discrepancies. QQ *vs*. QR+RR or LL *vs*. LM+MM carriers did not

372 show significant differences in the PON-1 activity standardized for HDL cholesterol;

373 however, QQ and LM+MM carriers tended to present the highest values for that ratio

374 while QR+RR and LL carriers the lowest. Changes in the PON-1 activities during the

375 WM period cannot be attributed to HDL cholesterol changes as the PON-1/HDL

376 cholesterol ratio tended to increase both in PON-1 192 and 55 polymorphisms.

377   Tocopherols are known to efficiently protect erythrocyte from lipid peroxidation

378 [45]. According to present LPO and GSSG results, R carriers responded to WM *vs*. CM

379 more efficiently than QQ carriers. These findings are important considering the higher

380 CHD risk of R carriers [13,18]. Moreover, dietary γ-tocopherol in 192QR+RR increased

381 significantly serum γ-tocopherol but not in QQ counterparts or in PON-1 55 carriers

382 after the trial (WM *vs*. CM) explaining, at least in part, the decrease in erythrocyte LPO

383 observed in R carriers. As far as we know this is the first report suggesting that the

384 influence of diet on γ-tocopherol levels might be dependent of PON-1 allele; however,

385 this conclusion has to be carefully interpreted due to the low number of volunteers

386 tested in the study. Differences in tocopherol absorption and metabolization in the

387 various PON-1 polymorphisms cannot be ruled out. Bub et al [24] found reduced lipid

388 peroxidation and improved antioxidant status in R-allele-carriers but not in QQ

389 homozygous elderly subjects on a tomato-rich diet. Davies et al. [46] suggested that

390 paraoxon PON-1 activity was greatest in R allele carriers but Akçay et al. [47] found

391 that R isoform carriers are less able to prevent accumulation of lipid peroxides in HDL

392 and to protect LDL cholesterol from oxidation than Q allele carriers.

393   Sözmen et al. [48] explain the increase in CAT and SOD activities observed in their

394 study as a compensatory induction of these enzymes to oxidative stress. In fact, certain

395 substrates are thought to promote the so-called "hormesis" property, by which a

396 relatively low degree of peroxidation induces a strong antioxidant defense response

397   [49]. Barbosa et al. [50] justified a positive relation between ox-LDL and GPx activity

398   by compensatory induction of the activity of this antioxidant enzyme. However, it has to

399   be pointed out that ox-LDL were measured by Barbosa et al. [49] in plasma while in the

400   present paper LPO were tested in erythrocytes.

401   In the presently studied polymorphisms, as a consequence of LPO and/or GSSG

402   changes, various modifications in the antioxidant enzyme activity and in GSH were

403   observed. A detailed study of data shows that the GSSG levels were significantly

404   increased in PON-1 192QQ and in PON-1 55LM+MM volunteers suggesting, at least in

405   part, that the significant increase observed in PON-1, SOD, and GSH in those carriers

406   were related to an efficient "hormesis" property. In QR+RR volunteers less oxidation

407   was obtained, thus increases in CAT were not found. On the other hand, in PON-1

408   55LL carriers, no other change than in PON-1 activity due to the WM *vs*. CM

409   consumption was observed suggesting that the "hormesis" effect was not totally

410   required as the LPO and glutathione system were not significantly affected. WM and

411   CM consumption affected LM+MM,QQ carriers and LL(QR+RR) individuals to similar

412   degree as previously discussed in QQ and LM+MM individuals, suggesting that the

413   results were not clearly influenced by the concurrence of PON-1 55/192

414   polymorphisms.

415   In summary, according to the present results, the consumption of WM should be

416   preferred to that of CM. Although the intake of WM *vs*. CM increased PON-1 and other

417   antioxidant enzymes in volunteers at high CHD risk, this response varied depending on

418   each individual's PON-1 polymorphism. According to LPO and γ-tocopherol, and

419   antioxidant enzyme changes, subjects carrying the PON-1 192QR+RR appear to be

420   target for WM *vs*. CM consumption. Future studies are required to better understand the

421   influence of PON-1 polymorphisms on the antioxidant effect of WM consumption.

Acknowledgements

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Table 1. Proximate composition and energy content of control meat and walnut paste-enriched meat.

Characteristics	Control meat[1]	Walnut paste-enriched meat[1]
Moisture (g/100g meat)	74.7	61.1
Energy (kJ/100g meat)	403.4	873.6
Protein (g/100g meat)	20.6	19.5
Fat (g/100g meat)	1.6	14.5
Ash (g/100g meat)	3.1	3.2
SFA (g/100g total fatty acids)	42.1	11.2
MUFA (g/100g total fatty acids)	38.0	13.7
PUFA (g/100g total fatty acids)	19.8	74.9
n-6/n-3 ratio	10.2	3.8
Lysine/arginine ratio	1.3	1.1
α-tocopherol (mg/100g meat)	0.1	0.2
γ-tocopherol (mg/100g meat)	0.01	4.1
δ-tocopherol (mg/100g meat)	nd	0.8
Magnesium (mg/100g meat)	20.2	41.4

[1]corresponds to restructured steaks and sausages. SFA saturated fatty acids; MUFA monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; nd, not detected. Adapted from Serrano et al. [10].

Table 2. Daily energy intake, macronutrients and fatty acid energy contribution during walnut paste-enriched meat (WM), low-fat meat (CM) periods and the whole trial of participants according to their PON-1 192QQ and QR+RR and PON-1 55LL and LM+MM polymorphism variants.

		WM	CM	Net change (%) Mean (95%CI)	Net change (%) (p)	Gen effect (p)
Energy (kJ)	QQ (11)	8104 ± 1138	7654 ± 1371	8.9 (-6.5 to 24.5)	0.230	R[1] 0.068
	QR+RR (11)	7261 ± 1264	7136 ± 956	2.3 (-8.9 to 13.6)	0.653	
	LL (8)	7265 ± 1540	7289± 816	-0.4 (-14.5 to 13.6)	0.948	M[1] 0.238
	LM+MM (14)	7981 ± 1011	7943 ± 1400	9.6 (-3.4 to 22.6)	0.135	
Carbohydrates (% En)	QQ (11)	32.1 ± 7.6	30.5 ± 4.8	10.5 (-8.4 to 29.4)	0.247	R[1] 0.584
	QR+RR (11)	27.7 ± 8.1	36.1 ± 4.7	-23.5 (-37.6 to -9.5)	0.004	
	LL (8)	29.5 ± 7.5	33.9 ± 8.4	-8.6 (-37.7 to 20.4)	0506	M[1] 0.804
	LM+MM (14)	30.5 ± 8.4	32.8 ± 3.6	-2.9 (-19.8 to 13.8)	0.716	
Protein (% En)	QQ (11)	17.6 ± 2.3	18.6 ± 3.5	-1.6 (-17.6 to 14.5)	0.834	R[1] 0.513
	QR+RR (11)	19.4 ± 3.8	19.8 ± 3.1	-0.1 (-16.2 to 15.9)	0.984	
	LL (8)	18.8 ± 3.8	19.4 ± 3.9	-0.9 (-18.5 to 16.7)	0.908	M[1] 0.572
	LM+MM (14)	18.2 ± 2.8	18.9 ± 3.0	-0.9 (-15.7 to 13.8)	0.893	
Fat (% En)	QQ (11)	46.2 ± 1.8	44.2 ± 6.9	6.0 (0.9 to 5.1)*	0.135	R[1] 0.352
	QR+RR (11)	48.6 ± 9.1	40.9 ± 7.1	23.5 (14.1 to 32.4)***	0.008	
	LL (8)	48.3 ± 8.8	44.1 ± 10.3	9.5 (1.0 to 17.5)*	0.062	M[1] 0.791
	LM+MM (14)	46.7 ± 5.8	41.5 ± 5.9	12.9 (6.4 to 20.4)**	0.027	
SFA (% En)	QQ (11)	11.8 ± 2.6	14.0 ± 3.3	-15.7 (-21.9 to -9.5)*	0.340	R[1] 0.120
	QR+RR (11)	13.7 ± 2.8	15.8 ± 5.4	-13.3 (-20.9 to -5.7)*	0.022	
	LL (8)	13.2 ± 2.8	14.7 ± 7.4	-10.4 (-11.1 to 1.1)	0.029	M[1] 0.370
	LM+MM (14)	12.3 ± 2.9	14.5 ± 3.3	-15.2 (-24.3 to -6.1)*	0.270	
MUFA (% En)	QQ (11)	18.8 ± 3.7	20.7 ± 4.4	-6.5 (-22.1 to 9.0)	0.377	R[1] 0.785
	QR+RR (11)	19.6± 6.1	19.0 ± 7.8	15.2 (-16.3 to 46.7)	0.303	
	LL (8)	20.8 ± 7.2	21.6 ± 8.7	6.8 (-26.8 to 40.6)	0.644	M[1] 0.133
	LM+MM (14)	18.2± 2.8	19.0 ± 4.0	1.3 (-18.3 to 21.0)	0.830	

PUFA (% En)	QQ (11)	12.7 ± 2.6	7.3 ± 3.2	111 (62.1 to 160)	0.005	R[1] 0.817
	QR+RR (11)	13.4 ± 2.9	7.0 ± 3.9	143 (98.1 to 189)	0.004	
	LL (8)	12.6 ± 2.2	7.8 ± 4.9	136 (82.2 to 188)	0.035	M[1] 0.860
	LM+MM (14)	13.3 ± 3.0	6.8 ± 2.5	120 (87.1 to 154)	<0.001	
ω-6 PUFA/ ω-3 PUFA	QQ (11)	4.2 ± 0.2	9.6 ± 3.8	-45.8 (-63.8 to -27.8)	<0.001	R[1] 0.754
	QR+RR (11)	4.2 ± 0.4	10.9 ± 3.1	-52.7 (-66.4 to -38.9)	<0.001	
	LL (8)	4.4 ± 0.4	8.9 ± 3.3	-40.1 (-62.9 to -17.3)	0.004	M[1] 0.279
	LM+MM (14)	4.2 ± 0.2	10.5 ± 3.5	-54.0 (-66.6 to -41.4)	<0.001	
Alcohol (% En)	QQ (11)	1.7 ± 4.2	1.2 ± 2.1	446 (-589 to 1481)	0.363	R[1] 0.889
	QR+RR (11)	1.6 ± 3.1	1.1 ± 2.8	-13.8 (-37.0 to 9.5)	0.213	
	LL (8)	0.7 ± 2.0	1.1 ± 3.1	-12.5 (-42.0 to 17.1)	0.351	M[1] 0.440
	LM+MM (14)	2.2 ± 4.3	1.1 ± 1.9	380 (-490 to 1251)	0.363	
Cholesterol (mg)	QQ (11)	378.1 ± 165.1	398.1 ± 135.7	11.6 (-34.7 to 58.1)	0.591	R[1] 0.262
	QR+RR (11)	362.9 ± 143.3	313.7 ± 128.1	37.9 (-22.9 to 98)	0.192	
	LL (8)	378.8 ± 110.2	266.6 ± 85.7	61.0 (-9.5 to 132)	0.080	M[1] 0.129
	LM+MM (14)	366.9 ± 175.4	413.0 ± 132.7	2.2 (-37.1 to 41.4)	0.905	
Fiber (g)	QQ (11)	14.6 ± 6.0	11.7 ± 4.6	36.0 (-8.7 to 80.9)	0.104	R[1] 0.746
	QR+RR (11)	13.4± 5.9	14.0 ± 4.7	13.2 (-45.8 to 72.4)	0.624	
	LL (8)	13.9 ± 6.6	13.2 ± 4.1	18.0 (-52.6 to 88.6)	0.506	M[1] 0.874
	LM+MM (14)	14.1 ± 5.6	12.4 ± 5.1	30.1 (-11.8 to 72.0)	0.145	
Total tocopherol (mg)	QQ (11)	15.7 ± 3.7	9.8± 3.8	82.3 (33.6 to 131)	0.003	R[1] 0.269
	QR+RR (11)	15.0 ± 1.3	8.2 ± 3.8	120 (50.7 to 190)	0.004	
	LL (8)	14.8 ± 1.1	8.7 ± 3.6	94.7 (29.2 to 160)	0.011	M[1] 0.527
	LM+MM (14)	15.7 ± 3.5	9.2 ± 4.0	102 (48.1 to 156)	<0.001	

Data are mean values ± standard deviations of volunteers (12 men and 10 women) classified according to their PON-1-55 polymorphism variants. N=number of volunteers. SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids. The net change (%), mean and 95%CI for each variable was calculated taken into account end and baseline of both periods as follow: Net change (%) = (WM-CM)*100/CM. R[1], Gen effect QQ *vs.* QR+RR; M[1], Gen effect LL *vs.* LM+MM.

Table 3. Body mass index (BMI), lipid, glucose and blood pressure (BP) changes during walnut paste-enriched meat (WM) and low-fat meat (CM) periods and whole trial in PON-1 192QQ and QR+RR and PON-1 55LL and LM+MM participants.

	Carrier (nr)	WM period		CM period		Net change (%) Mean (95%CI)	Net change (%) (p)	Gen*treatment Interaction[1] (p)
		Baseline	End	Baseline	End			
BMI (kg/m^2)	QQ (11)	30.0 ± 4.0	29.8 ± 4.1	29.8 ± 4.3	29.6 ± 4.3	0.01 (-0.72 to 0.74)	0.981	R[1] 0.185
	QR+RR (11)	29.0 ± 2.7	28.9 ± 2.6	29.1 ± 2.9	29.0 ± 3.0	-0.11 (-1.9 to 1.6)	0.890	
	LL (8)	29.3 ± 3.4	29.3 ± 3.2	29.7 ± 3.3	29.7 ± 3.4	0.05 (-2.0 to 2.1)	0.958	M[1] 0.057
	LM+MM (14)	29.7 ± 3.7	29.4 ± 3.7	29.4 ± 4.0	29.2 ± 4.1	-0.11 (-0.94 to 0.72)	0.782	
Glucose (mg/dl)	QQ (11)	92.3 ± 9.1	98.3 ± 3.5	94.9 ± 8.5	99.8 ± 8.2	4.2 (-4.2 to 8.4)	0.350	R[1] 0.617
	QR+RR (11)	94.5 ± 6.6	100.3 ± 10.4	98.8 ± 11.4	99.4 ± 6.8	3.0 (-3.4 to 9.4)	0.322	
	LL (8)	98.5 ± 8.3	105.2 ± 8.8	96.3 ± 13.6	100.4 ± 8.6	6.6 (-1.5 to 14.7)	0.095	M[1] 0.223
	LM+MM (14)	91.6 ± 6.9	95.6 ± 4.8	97.4 ± 7.4	98.4 ± 6.5	2.5 (-1.9 to 6.8)	0.241	
Cholesterol (mg/dl)	QQ (11)	194.9± 42.8	197.4 ± 40.0	208.3± 47.3	199.2 ± 45.1	8.7 (-4.0 to 21.5)	0.156	R[1] 0.095
	QR+RR (11)	243.6 ± 41.9	231.1 ± 29.8	241.7 ± 37.5	223.0± 34.5*	-1.3 (-25.0 to 22.5)	0.270	
	LL (8)	240.1 ± 36.6	238.4 ± 33.5	249.0± 44.8	222.3 ± 34.9+	10.6 (-8.8 to 30.1)	0.219	M[1] 0.382
	LM+MM (14)	200.5 ± 49.9	195.8 ± 33.4	209.4 ± 41.4	201.2± 43.7	5.7 (-3.7 to 15.0)	0.205	
HDL (mg/dl)	QQ (11)	42.5 ± 9.8	40.8 ± 8.9	44.2 ± 7.3	39.5 ± 5.2*	8.8 (-4.0 to 21.5)	0.152	R[1] 0.531
	QR+RR (11)	51.3 ± 11.2	46.3 ± 9.1	47.9 ± 10.9	43.7 ± 6.5	-13 (-25.1 to 22.5)	0.903	
	LL (8)	47.4 ± 6.8	45.6 ± 9.7	48.9± 10.2	42.1 ± 5.8*	8.8 (-21.2 to 38.7)	0.487	M[1] 0.795
	LM+MM (14)	45.7 ± 13.4	41.7 ± 8.9*	44.2 ± 8.2	40.9 ± 6.3+	1.5 (-12.0 to 14.9)	0.812	
LDL (mg/dl)	QQ (11)	123.7 ± 31.4	125.0 ± 32.2	137.6 ± 37.0	133.1 ± 37.0	3.3 (-13.3 to 19.9)	0.657	R[1] 0.547
	QR+RR (11)	161.9 ± 31.4	159.0 ± 18.5	169.1 ± 30.8	159.3 ± 23.1	5.2 (-4.9 to 15.2)	0.264	
	LL (8)	155.3 ± 27.2	161.8 ± 19.1	174.8 ± 35.0	164.5 ± 30.6	9.6 (-6.0 to 25.2)	0.176	M[1] 0.704
	LM+MM (14)	131.4 ± 39.1	126.3 ± 30.5	139.7 ± 33.2	134.7 ± 32.5	1.3 (-11.1 to 13.6)	0.826	

						Net change (%)	p	Gen effect
Triglycerides (mg/dl)	QQ (11)	143.5 ±.76.3	158.1 ± 63.7	132.8 ±.63.2	155.1 ± 72.1	0.65 (-41.6 to 43.0)	0.972	R^1 0.024
	QR+RR (11)	152.1 ± 80.5	129.5 ± 49.1	142.3 ± 64.5	122.4 ± 45.0	-4.5 (-39.8 to 30.8)	0.773	
	LL (8)	186.8 ± 70.8	155.3 ± 58.4	155.0 ± 63.7	139.3 ± 72.0	-17.4 (-92.2 to 57.4)	0.577	M^1 0.031
	LM+MM (14)	122.2 ± 70.7	139.1 ± 59.5	127.8 ± 62.1	142.0 ± 61.4	6.8 (-13.6 to 27.1)	0.476	
Systolic BP (mmHg)	QQ (11)	138.3 ± 14.1	137.8 ± 15.8	144.2 ± 20.6	138.8 ± 15.9	4.3 (-5.8 to 14.4)	0.364	R^1 0.276
	QR+RR (11)	151.1 ± 28.8	135.3 ± 11.1	147.4 ± 31.8	142.1 ± 26.6	-8.1 (-19.9 to 3.6)	0.152	
	LL (8)	160.7 ± 24.5	139.2 ± 7.5*	158.0 ± 30.7	147.6± 27.6	-7.6 (-25.2 to 10.0)	0.340	M^1 0.033
	LM+MM (14)	134.6 ± 15.3	135.2 ± 16.2	138.6± 20.2	135.9 ± 15.6	1.4 (-6.8 to 9.6)	0.725	
Diastolic BP (mmHg)	QQ (11)	89.2 ± 7.2	87.7 ± 10.7	93.9 ± 11.5	82.7 ± 9.7*	9.5 (2.9 to 16.1)	0.039	R^1 0.149
	QR+RR (11)	107.9 ± 34.1	83.6 ± 6.5*	94.2 ± 18.8	86.6 ± 10.0	-18.3 (-36.0 to -0.49)	0.045	
	LL (8)	114.5 ± 37.4	85.2 ± 10.8*	102.6 ± 14.0	86.0 ± 10.0	-11.5 (-33.7 to 10.6)	0.257	M^1 0.007
	LM+MM (14)	89.5 ± 9.1	85.9 ± 8.1	89.1 ± 14.1	83.9 ± 7.3	-0.3 (-13.4 to 12.8)	0.964	

Data are mean values ± standard deviations of volunteers (12 men and 10 women) classified according to their PON-1-192 and PON-1-55 polymorphism variants. N=number of volunteers. R^1, Gen effect QQ *vs.* QR+RR; M^1, Gen effect LL *vs.* LM+MM. The net change (%), mean and 95%CI for each variable was calculated taken into account end and baseline of both periods as follow: Net change (%) = $(WM_{end}-WM_{baseline})-(CM_{end}-CM_{baseline})*100/CM_{baseline.}$

Table 4. Effects of walnut paste-enriched meat (WM), low-fat meat (CM) and the whole trial on erythrocyte reduced glutathione (GSH), oxidized glutathione (GSSG), redox index and lipid peroxidation (LPO), and serum γ-tocopherol and α-tocopherol changes in PON-1 192QQ and QR+RR and PON-1 55LL and LM+MM participants.

	Carrier (nr)	WM period		CM period		Net change (%) Mean (95%CI)	Net change (%) (p)	Gen*treatment Interaction[1] (p)
		Baseline	End	Baseline	End			
GSH (μmol/g Hb)	PON-1 192QQ (11)	3.9 ± 1.7	4.7 ± 1.4**	4.3 ± 1.4	4.6 ± 1.3	13.7 (2.9 to 24.6)	0.046	R[1] 0.830
	PON-1 192QR+RR (11)	3.1 ± 1.2	4.3 ± 1.1*	3.5 ± 0.7	3.8 ± 0.7	11.8 (-23.2 to 46.9)	0.057	
	PON-1 55LL (8)	3.6 ± 1.7	4.3 ± 1.4	3.6 ± 1.3	4.2 ± 1.14*	3.7 (-55.7 to 63.1)	0.869	M[1] 0.047
	PON-1 55LM+MM (14)	3.3 ± 1.5	4.6 ±1.1***	4.0 ± 1.1	4.1 ± 1.0	19.8 (1.7 to 38.0)	0.001	
GSSG (μmol/g Hb)	PON-1 192QQ (11)	0.6 ± 0.3	1.0 ± 0.3***	0.7 ± 0.3	0.8 ± 0.3**	48.5 (33.9 to 63.0)	0.001	R[1] 0.100
	PON-1 192QR+RR (11)	0.6 ± 0.2	0.9 ± 0.3***	0.5 ± 0.2	0.7 ± 0.3	-36.9 (-161.1 to 87.4)	0.554	
	PON-1 55LL (8)	0.7 ±0.2	0.9 ± 0.3**	0.5 ± 0.3	0.7 ± 0.3	-27.6 (-260.7 to 205.5)	0.985	M[1] 0.072
	PON-1 55LM+MM (14)	0.6 ± 0.2	1.0 ± 0.3***	0.6 ± 0.3	0.7 ± 0.3*	49.5 (30.4 to 68.6)	0.001	
Redox index[1]	PON-1 192QQ (11)	7.0 ± 4.2	5.1 ± 2.3*	7.4 ± 3.5	7.2 ± 3.3	-25.7 (-39.3 to -12.1)	0.061	R[1] 0.044
	PON-1 192QR+RR (11)	5.9 ± 2.9	5.6 ± 2.3	8.2 ± 3.5	6.1 ± 2.7	8.3 (-25.8 to 42.4)	0.202	
	PON-1 55LL (8)	5.8 ± 3.1	5.1 ± 2.0	8.2 ± 3.5	6.3 ± 2.5	-1.0 (-59.2 to 37.3)	0.516	M[1] 0.371
	PON-1 55LM+MM (14)	6.7 ± 3.8	5.4 ± 2.5	7.6 ± 3.5	6.8 ± 3.3	-17.6 (-43.7 to 8.6)	0.563	
LPO (μmol/L)	PON-1 192QQ (11)	3.1 ± 2.1	3.1 ± 2.0	2.1 ± 1.3	1.9 ± 1.7	6.9 (-125.3 to 139.1)	0.856	R[1] 0.045
	PON-1 192QR+RR (11)	4.1 ± 1.1	1.4 ± 1.1**	2.2 ± 1.3	2.1 ± 1.9	-147.9 (-400.0 to 103.8)	0.009	
	PON-1 55LL (8)	3.5 ± 1.3	2.1 ± 1.2	1.8 ± 1.0	1.8 ± 2.0	-41.7 (-201.0 to 117.7)	0.208	M[1] 0.827
	PON-1 55LM+MM (14)	3.7 ± 2.1	2.4 ± 2.3	2.4 ± 1.4	2.2 ± 1.3	17.5 (-275.5 to 310.5)	0.368	
γ-tocopherol (μg/dL)	PON-1 192QQ (11)	43.2 ± 12.3	48.0 ± 11.7*	41.4 ± 16.8	40.1 ± 16.3	10.9 (-8.0 to 28.9)	0.103	R[1] 0.086
	PON-1 192QR+RR (11)	41.3 ± 12.1	56.2 ± 27.9*	40.2 ± 13.8	45.1 ± 18.8	29.5 (-6.5 to 63.5)	0.037	
	PON-1 55LL (8)	43.6 ± 12.1	48.9 ± 13.3*	42.1 ± 12.9	42.8 ± 16.2	14.7 (-6.8 to 38.8)	0.092	M[1] 0.641
	PON-1 55LM+MM (14)	39.7 ± 11.9	53.9 ± 28.8*	38.6 ± 19.3	41.8 ± 22.0	26.1 (-4.1 to 56.2)	0.068	
α-tocopherol (μg/dL)	PON-1 192QQ (11)	1324 ± 261	1387 ± 279	1405 ± 330	1359 ± 250	22.0 (-15.1 to 19.5)	0.735	R[1] 0.554
	PON-1 192QR+RR (11)	1197 ± 245	1232 ± 255	1321 ± 395	1329 ± 385	-6.1 (-23.2 to 11.0)	0.606	
	PON-1 55LL (8)	1331 ± 261	1359 ± 287	1356 ± 438	1312 ± 352	-0.3 (-14.3 to 13.8)	0.857	M[1] 0.713
	PON-1 55LM+MM (14)	1138 ± 206	1224 ± 239	1368 ± 322	1362 ± 308	-4.9 (-28.7 to 18.9)	0.340	

Values are mean ± SD of volunteers (12 men and 10 women). Number of volunteers for each polymorphism in parenthesis. [1]Redox index: GSH/GSSG. Values bearing asterisks (*p < 0.05, **p < 0.01, ***p < 0.001) differed significantly from their respective baseline values for the same allele and type of meat consumed. The net change (%), mean and 95%CI for each variable was

calculated taken into account end and baseline of both periods as follow: Net change (%) = $(WM_{end}-WM_{baseline})-(CM_{end}-CM_{baseline})*100/CM_{baseline}$ R[1] or M[1] treatment effect comparison between R and non-R or between M and non-M carriers.

Table 5. Effects of walnut-enriched meat (WM), low-fat meat (CM) and the whole trial on catalase, superoxide dismutase (SOD), paraoxonase-1 (PON-1) and PON-1/HDL cholesterol in PON-1 192QQ and QR+RR and PON-1 55LL and LM+MM participants.

	Carrier (nr)	Walnut-enriched meat (WM) diet		Control meat (CM) diet		Net change (%) Mean (95%CI)	Net change (%) (p)	Gen*treatment Interaction[1] (p)
		Baseline	End	Baseline	End			
Catalase	PON-1 192QQ (11)	141.4 ± 59.0	187.5 ± 74.5**	126.7 ± 24.4	131.3 ± 31.0	36.3 (3.0 to 69.8)	0.014	R[1] 0.988
(U/g Hb)	PON-1 192QR+RR (11)	132.9 ± 20.4	165.9 ± 55.0	148.7 ± 36.6	139.7 ± 28.5	22.6 (-14.9 to 60.2)	0.057	
	PON-1 55LL (8)	148.9 ± 60.1	191.1 ± 74.8	147.9 ± 39.8	151.3 ± 25.4	28.7 (-24.3 to 81.7)	0.143	M[1] 0.849
	PON-1 55LM+MM (14)	130.5 ± 30.8	168.5 ± 59.9**	132.6 ± 27.9	126.6 ± 28.2	45.0 (11.0 to -80.2)	0.007	
SOD	PON-1 192QQ (11)	2.4 ± 0.6	2.9 ± 0.6***	2.6 ± 0.6	2.7 ± 0.8	13.1 (-1.7 to 27.9)	0.029	R[1] 0.450
(U/g Hb)	PON-1 192QR+RR (11)	2.3 ± 0.7	2.6 ± 0.6	2.6 ± 0.8	2.3 ± 0.8	16.8 (-6.6 to 40.3)	0.015	
	PON-1 55LL (8)	2.5 ± 0.7	2.9 ± 0.8*	2.8 ± 0.9	2.6 ± 1.1	26.5 (-5.6 to 58.7)	0.082	M[1] 0.948
	PON-1 55LM+MM (14)	2.3 ± 0.6	2.6 ± 0.5**	2.4 ± 0.6	2.2 ± 0.6	18.3 (1.8 to 34.7)	0.003	
PON-1	PON-1 192QQ (11)	55.7 ± 18.0	77.3 ± 25.9**	59.5 ± 20.3	49.5 ± 22.1*	72.0 (38.7 to 105.4)	0.001	R[1] 0.591
(U/L)	PON-1 192QR+RR (11)	48.6 ± 22.5	76.6 ± 55.3	60.8 ± 27.8	46.5 ± 30.1*	60.0 (-10.9 to 131.0)	0.037	
	PON-1 55LL (8)	54.8 ± 22.0	67.7 ± 27.6*	62.0 ± 28.1	48.2 ± 34.9*	48.6 (-3.3 to 100.6)	0.002	M[1] 0.421
	PON-1 55LM+MM (14)	50.8 ± 19.5	82.7 ± 48.1*	58.9 ± 21.7	47.9 ± 19.8*	76.9 (-25.2 to 179.0)	0.009	
PON 1/HDL	PON-1 192QQ (11)	1.3 ± 0.7	1.7 ± 0.3*	1.3 ± 0.5	1.2 ± 0.5	55.8 (-5.9 to 118)	0.050	R[1] 0.632
cholesterol	PON-1 192QR+RR (11)	1.0 ± 0.5	1.7 ± 1.2	1.2 ± 0.6	1.1 ± 0.9	79.4 (-20.6 to 179)	0.045	
(U/mg)	PON-1 55LL (8)	1.1 ± 0.5	1.5 ± 0.7*	1.2 ± 0.6	1.1 ± 1.0	50.0 (-30.6 to 131)	0.160	M[1] 0.594
	PON-1 55LM+MM (14)	1.2 ± 0.7	1.9 ± 0.8*	1.4 ± 0.5	1.2 ± 0.5	77.4 (0.8 to 154)	0.048	

Values are mean $\pm$ SD of volunteers (12 men and 10 women). Number of volunteers for each polymorphism in parenthesis. Values bearing asterisks (*p < 0.05, **p <0.01, ***p <0.001) differed significantly from their respective baseline values for the same allele and type of meat consumed. The net change (%) mean and 95%CI for each variable was calculated taken into account end and baseline of both periods as follow: Net change (%) = (WM$_{end}$-WM$_{baseline}$)-(CM$_{end}$-CM$_{baseline}$)*100/CM$_{baseline}$. R[1] or M[1] treatment effect comparison between R and non-R or between M and non-M carriers.