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European Journal of Pharmacology 432 (2001) 121–128

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Interaction between the Gln–Arg 192 variants of the paraoxonase gene and oleic acid intake as a determinant of high-density lipoprotein cholesterol and paraoxonase activity

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Received 10 May 2001; received in revised form 15 October 2001; accepted 19 October 2001

Abstract

Olive oil, rich in oleic acid, could play a particular beneficial role in the anti-atherogenic effects attributed to the Mediterranean diet. Paraoxonase (PON1) has emerged as the component of high-density lipoproteins (HDL) most likely to explain its ability to attenuate the oxidation of low-density lipoproteins. We hypothesised that oleic acid intake might be associated with changes in PON1–HDL associated particles, and investigated the impact, if any, on this association of the PON1–192 polymorphism, a common polymorphism that strongly modulates PON1 activity. Six hundred and fifty-four men randomly selected from the census were studied. Oleic acid intake was calculated from a 72-h recall questionnaire with specific software. Oleic acid intake groups (low vs. high) were created by stratifying the population according the median value as a cut-point. After adjusting for confounding variables, high oleic acid intake was associated with increased HDL cholesterol levels and PON1 activity only in subjects with the QR and the RR genotypes, respectively. Analyses of the variance showed a statistically significant interaction between PON1–192 genotypes and oleic acid intake for log PON1 activity ($P=0.005$) and a marginally significant interaction for HDL cholesterol ($P=0.066$). These results suggest that the beneficial effect of increasing oleic acid intake on HDL and PON1 activity at population level is especially observed in subjects carrying the R allele of the PON1–192 polymorphism.

Keywords: Paraoxonase; Gene–diet interaction; High-density lipoprotein

1. Introduction

The so-called "French paradox" has been described as an apparent coexistence of high-fat diet with low incidence of coronary heart diseases (Artaud-Wild et al., 1993). An extension of this paradox can be found in other southern European countries, such as Spain, where low acute myocardial infarction incidence and mortality rates have been found together with high cardiovascular risk factor prevalence at population level (Masiá et al., 1998; Pérez et al., 1998). The factors in Spain that confer sufficient protection to compensate for the high cardiovascular risk factor prevalence remain to be elucidated. However, it is likely that lifestyle factors, such as diet or physical activity and their interactions with genes, may contribute to neutralising other factors with negative effects.

Diet is one of the major environmental factors playing an important role in the different coronary heart disease incidence rates between northern Europe or the United States and Mediterranean countries. Olive oil, rich in oleic acid, could play a particular beneficial role in coronary heart disease prevention and in the antiatherogenic effects attributed to the Mediterranean diet. Oleic acid may exert beneficial effects on the pathogenesis of vascular disease through a variety of mechanisms, such as reducing smooth muscle cell DNA synthesis and low-density lipoprotein (LDL) levels, and protect LDL from oxidation (Mata et al., 1997), inducing less monocyte chemotaxis and adhesion

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01482-0

when exposure to oxidative stress exists (Tsimikas et al., 1999), and inhibiting endothelial activation (Carluccio et al., 1999). With regard to oxidative modification of lipoproteins, it has also been described that phenolic compounds present in olive oil may contribute to the endogenous antioxidant capacity of LDL (Wiseman et al., 1997) and that an oleic acid-rich diet protects against the oxidative modification of high-density lipoproteins (HDL) (Solà et al., 1997). Concerning lipids and lipoproteins, dietary oleic acid was considered to be "neutral", neither raising nor lowering serum cholesterol levels (Grundy, 1996).

Paraoxonase (PON1) is a calcium-dependent esterase closely associated with HDL-containing apolipoprotein AI that has been reported to confer antioxidant properties to HDL by decreasing the accumulation of lipid peroxidation products (Mackness et al., 1991a). It has been suggested that PON1 is related to coronary heart disease risk (Ruiz et al., 1995; Serrato and Marian, 1995) and that its activity, usually measured using paraoxon as a substrate, is under genetic and environmental regulation and appears to largely vary among individuals and populations. One molecular basis of the variations in PON1 activity is a polymorphism in the PON1 gene located in chromosome 7, which is clustered with at least two other related genes, PON2 and PON3 (Primo-Parmo et al., 1996). PON1 genetic polymorphism comprises PON1 Q, an isoform with low activity towards paraoxon hydrolysis, which has a glutamine at position 192, while the high-activity PON1 R isoform contains an arginine at position 192 (Mackness et al., 1996).

Whereas some authors have failed to find associations between the variation in PON1 gene and changes in lipoprotein concentrations (Antikainen et al., 1996; Sanghera et al., 1997), others have found significant associations of PON1–192 genetic variants with changes in HDL-cholesterol levels and in triglyceride concentrations in a relatively genetically isolated population (Hegele et al., 1995; Boright et al., 1998).

Since an olive oil-rich diet has been recognised to have antioxidant properties, we hypothesised that oleic acid intake might be associated with changes in PON1–HDL associated particles. On the other hand, we investigated the impact of the PON1–192 polymorphism, if any, on the relationship between oleic acid intake and PON1 activity and lipoproteins in a random sample population.

2. Subjects and methods

2.1. Subjects

Six hundred and fifty-four men, aged 25–74, were randomly selected from a representative population sample in a cross-sectional study (the REGICOR study) designed to establish the prevalence of main cardiovascular risk factors in the province of Gerona, Spain where the incidence of myocardial infarction was found to be low (Masiá et al., 1998; McGovern et al., 1996; Marrugat and Sentí, 2000). All subjects completed a smoking questionnaire consisting of eight questions regarding current and past cigarette consumption. Since all ex-smokers of the studied population had stopped smoking at least 6 months before examination, nonsmokers were classified as either those who had never smoked or ex-smokers. The Minnesota Leisure Time Physical Activity Questionnaire was used to assess energy expenditure in leisure time physical activity (EEPA) during the previous year (Taylor et al., 1978). The EEPA questionnaire has been validated for use among Spanish men (Elosua et al., 1994) and was administered by a trained interviewer. All subjects gave their written informed consent to participate. The protocol was approved by an ethics committee. Thirty-seven men taking anti-hypertensives or lipid lowering drugs were excluded from the analyses that included comparisons of parameters by genotype and oleic acid intake groups.

2.2. Dietary assessment

Dietary information was obtained using a 72-h recall questionnaire, which was administered by a trained interviewer and validated for use among Spanish people (Schröder et al., 2001). The questionnaire contained a food list. Participants were requested to precisely describe their food and beverage intakes during the previous 3 days. Each of the foods listed was characterised by a full description of the usual serving size. Ninety different foods typical of the eating habits in northeastern Spain (e.g. local bread with olive oil and tomatoes, or the amount and type of oil used for salad and vegetable dressing) were selected to fit typical population alimentary habits. Furthermore, some generic foods had open questions for the participants to specify details of their type and serving sizes. In this case, the interviewer requested the exact food type (e.g. type of cheese or salad ingredients). The precise preparation of foods was taken into account to include all the ingredients (e.g. oil or butter for frying). Oleic acid intake was calculated from the 72-h recalls with the software *Diet Analysis Nutritionist IV* (N Squared Computing, San Bruno, CA). The database of this software includes 9879 food items complemented with food items from Spanish food composition tables (Hurren et al., 1987; Jiménez Cruz et al., 1994; Moreiras et al., 1992).

2.3. Biochemical analyses

2.3.1. Analyses of lipids and lipoproteins

Blood samples were collected after an overnight fast. Serum cholesterol (Roche Diagnostica, Basel, Switzerland, Ref: 0736635) and triglyceride levels (Roche Diagnostica, Ref: 0736791) were determined enzymatically. LDL cholesterol was calculated by the Friedewald formula (Friedewald et al., 1972). HDL cholesterol was measured as

cholesterol after precipitation of apolipoprotein B-containing lipoproteins with phosphotungstic-Mg^{2+} (Boehringer Mannheim, Mannheim, Germany, Ref: 543004) (Lopes-Virella et al., 1977). Inter-assay coefficients of variation were 2.5%, 4.5% and 3.2% for total cholesterol, HDL cholesterol, and triglycerides, respectively.

2.3.2. Analysis of PON1 activity

For PON1 activity analysis, samples frozen at −70 °C, which were thawed just before the beginning of each assay, were used. PON1 activity towards paraoxon was measured following the reaction of paraoxon hydrolysis into *p*-nitrophenol and diethylphosphate catalysed by the enzyme. PON1 activity was determined from initial velocity of *p*-nitrophenol production (subtracting the spontaneous paraoxon hydrolysis) at 37 °C and recorded at 405 nm by a Cobas-Mira Plus autoanalyzer (Roche Diagnostica). Forty microliters of serum was added to a basal assay mixture to reach final concentrations of 5 mM paraoxon, 1.9 mM $CaCl_2$, 90 mM Tris–HCl (at pH 8.5) and 3.6 mM NaCl. Two strategies were followed to avoid spontaneous hydrolysis of diluted paraoxon solutions. First, a blank determination of basal assay mixture without serum was made. Second, 5 mM paraoxon basal assay mixture aliquots frozen at −40 °C were used and thawed just before the beginning of each assay. Frozen aliquots of a serum pool, used as an internal control, were thawed just before the beginning of assay. One aliquot of serum pool was measured in triplicate every 24 samples. The serum pool was used to correct for inter-assay variations. A PON1 activity of 1 U/l was defined as 1 μmol of *p*-nitrophenol formed per min per serum litre. The molar extinction coefficient of *p*-nitrophenol is 18,053 M^{-1} cm^{-1} at pH 8.5. Analytical within-run imprecision was determined by 20 replicate measurements of three different serum samples with different PON1 activity. Analytical between-run imprecision was determined from 20 day-to-day measurements of the same three samples. The intra- and inter-assay coefficients of variation were under 1.7%.

2.4. PON1–192 genotype determination

Genomic DNA was isolated from white cells by the salting out method (Miller et al., 1989). Polymerase chain reactions (PCR) were performed using primer sequences derived from published data (Humbert et al., 1993). We used the following primers for amplification of the 99 base pairs sequence encompassing codon 192: 5′ TAT TGT TGC TGT GGG ACC TGA G 3′ and 5′ CAC GCT AAA CCC AAA TAC ATC TC 3′ . The amplification cycle was performed on a Perkin Elmer Cetus 2400 Thermal Cycler with initial denaturation for 4 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 1 min at 61 °C and 1 min at 72 °C, and finally by 7 min of extension at 72 °C. PCR products were digested with *Alw*I for 4 h at 37 °C and the samples electrophoresed in 3% agarose gels for 75 min at 60 V.

2.5. Statistical analysis

The chi square test was used to analyse the association between categorical variables. PON1 activity was log transformed to fit a normal distribution. For comparisons of

Table 1
Clinical characteristics, lipid and lipoprotein concentrations, log PON1 activity and PON1–192 genotypes of men stratified by the categorised oleic acid intake[a]

	All (n=654)	Low oleic acid intake (n=327)	High oleic acid intake (n=327)	P
Age (years)	50.3 (13.9)	48.6 (14.1)	52.0 (13.5)	0.002
Kilocalories per day	2306.2 (522.9)	2338.0 (527.8)	2274.3 (516.8)	0.120
Oleic acid intake (g×100 kcal)	1.41 (0.31)	1.17 (0.17)	1.65 (0.22)	<0.001
BMI (kg/m^2)	26.5 (3.8)	26.2 (3.8)	26.9 (3.8)	0.034
EEPA (kcal/day)	247.0 (125.0–452.0)	237.0 (102.2–422.7)	254.0 (138.0–494.0)	0.156
Alcohol (g/week)	249.0 (36.0–504.0)	182.0 (0.0–476.0)	252.0 (71.2–504.0)	0.042
Smokers (n (%))	196 (30.0)	105 (32.1)	91 (27.8)	0.232
Total cholesterol (mg/dl)	223.3 (43.8)	221.4 (43.6)	225.1 (44.1)	0.281
Triglycerides (mg/dl)	99.0 (72.0–139.7)	99.0 (74.0–140.0)	98.0 (71.0–138.0)	0.669
LDL cholesterol (mg/dl)	151.4 (40.2)	150.5 (38.2)	152.2 (42.2)	0.602
HDL cholesterol (mg/dl)	47.8 (13.8)	46.7 (12.9)	49.0 (14.5)	0.036
Log PON1 activity	5.45 (0.49)	5.42 (0.46)	5.48 (0.51)	0.277
PON1–192 genotypes				
QQ (n (%))	308 (47.1)	161 (49.2)	147 (45.0)	
QR (n (%))	281 (43.0)	139 (42.5)	142 (43.4)	0.282
RR (n (%))	65 (9.9)	27 (8.3)	38 (11.6)	

Continuous variables are expressed as mean (S.D.) or median (interquartile range).

[a] Men were stratified in two groups according to the median value of oleic acid intake×100 kcal. BMI, body mass index; EEPA, daily energy expenditure in leisure-time physical activity.

continuous variables between groups, Student's *t*-test or Mann–Whitney *U*-test were performed. Pearson correlation coefficients were used to test the strength of the association between oleic acid intake and serum parameters. Oleic acid intake groups (low vs. high) were created by stratifying the population into two groups using the median oleic acid intake (in g/100 kcal of daily caloric intake) as a cut-point. Analyses of the variance were performed to test for gene–diet interaction on log PON1 activity and HDL cholesterol in models that included age, body mass index [calculated as weight (kg)/height^2 (m^2)], smoking, alcohol consumption and daily EEPA.

3. Results

Subjects in the high oleic acid intake group were older, showed higher mean values of body mass index, HDL

Table 2
Clinical characteristics, lipid and lipoprotein concentrations, and log PON1 activity of men stratified by PON1–192 genotypes and the categorised oleic acid intake

	All	Low oleic acid intake[a]	High oleic acid intake[a]	*P*
QQ	(*n*=308)	(*n*=161)	(*n*=147)	
Age (years)	50.9 (13.6)	49.5 (14.0)	52.4 (13.1)	0.061
Kilocalories per day	2336.3 (561.6)	2347.1 (548.9)	2324.5 (576.8)	0.725
Oleic acid intake (g×100 kcal)	1.40 (0.29)	1.19 (0.16)	1.63 (0.21)	<0.001
BMI (kg/m^2)	26.5 (3.9)	26.3 (3.9)	26.6 (3.8)	0.498
EEPA (kcal/day)	237.0 (136.0–423.0)	229.5 (126.5–412.0)	247.0 (145.0–494.0)	0.334
Alcohol (g/week)	252.0 (52.0–504.0)	252.0 (52.0–470.0)	252.0 (45.0–504.0)	0.530
Smokers (*n* (%))	91 (29.5)	51 (31.7)	40 (27.2)	0.391
Total cholesterol (mg/dl)	226.5 (42.2)	227.4 (43.2)	225.5 (41.1)	0.702
Triglycerides (mg/dl)	101.5 (73.7–142.5)	104.0 (74.0–145.0)	100.0 (73.0–142.0)	0.784
LDL cholesterol (mg/dl)	153.5 (38.3)	154.4 (37.9)	152.5 (38.8)	0.679
HDL (mg/dl)	48.6 (13.6)	48.4 (13.6)	48.7 (13.7)	0.879
Log PON1 activity	5.06 (0.24)	5.07 (0.26)	5.05 (0.22)	0.733
QR	(*n*=281)	(*n*=139)	(*n*=142)	
Age (years)	49.8 (14.1)	47.7 (13.7)	51.9 (14.2)	0.011
Kilocalories per day	2274.9 (484.1)	2326.6 (511.4)	2224.3 (451.8)	0.076
Oleic acid intake (g×100 kcal)	1.42 (0.33)	1.17 (0.17)	1.67 (0.24)	<0.001
BMI (kg/m^2)	26.7 (3.8)	26.4 (3.7)	27.0 (3.8)	0.179
EEPA (kcal/day)	261.0 (126.0–498.0)	260.0 (98.0–493.0)	267.5 (145.5–509.2)	0.414
Alcohol (g/week)	216.5 (27.5–504.0)	139.0 (0.0–504.0)	252.0 (88.0–495.5)	0.057
Smokers (*n* (%))	85 (30.2)	44 (31.7)	41 (28.9)	0.612
Total cholesterol (mg/dl)	221.4 (44.7)	218.0 (43.9)	224.6 (45.4)	0.223
Triglycerides (mg/dl)	96.0 (71.0–138.0)	98.0 (76.5–143.0)	92.5 (70.0–134.2)	0.213
LDL cholesterol (mg/dl)	150.2 (41.8)	148.3 (39.0)	152.1 (44.4)	0.457
HDL (mg/dl)	47.4 (14.2)	45.0 (12.2)[b]	49.6 (15.6)	0.007
Log PON1 activity	5.81 (0.25)[c]	5.82 (0.26)[c]	5.80 (0.24)[c]	0.578
RR	(*n*=65)	(*n*=27)	(*n*=38)	
Age (years)	49.4 (14.6)	47.7 (16.7)	50.7 (13.0)	0.433
Kilocalories per day	2298.5 (493.3)	2342.6 (499.6)	2267.1 (493.1)	0.547
Oleic acid intake (g×100 kcal)	1.41 (0.32)	1.12 (0.19)	1.62 (0.20)	<0.001
BMI (kg/m^2)	26.2 (3.5)	24.7 (2.9)[d]	27.2 (3.6)	0.005
EEPA (kcal/day)	218.0 (92.0–396.0)	210.0 (25.0–369.0)	219.0 (103.5–437.0)	0.394
Alcohol (g/week)	126.0 (0.0–380.0)	42.0 (0.0–364.0)	159.5 (12.0–467.2)	0.118
Smokers (*n* (%))	20 (30.8)	10 (37.0)	10 (26.3)	0.356
Total cholesterol (mg/dl)	216.7 (47.2)	204.2 (39.4)	225.6 (50.7)	0.071
Triglycerides (mg/dl)	102.0 (69.0–134.0)	96.0 (59.0–121.0)	117.0 (74.0–153.2)	0.055
LDL cholesterol (mg/dl)	146.8 (42.0)	140.1 (33.1)	151.5 (47.1)	0.284
HDL (mg/dl)	46.3 (12.8)	44.5 (11.4)	47.6 (13.7)	0.338
Log PON1 activity	6.16 (0.27)[c]	5.94 (0.25)[c]	6.28 (0.19)[c]	0.001

Continuous variables are expressed as mean (S.D.) or median (interquartile range).

[a] Men were stratified in two groups according to the median value of oleic acid intake×100 kcal. BMI, body mass index; EEPA, daily energy expenditure in leisure-time physical activity.

[b] Significantly different from QQ homozygotes, $P=0.028$.

[c] Significantly different from QQ homozygotes, $P<0.001$.

[d] Significantly different from QQ homozygotes, $P=0.049$.

cholesterol concentration, and alcohol consumption than those in the low oleic acid intake group (Table 1). Log PON1 activity tended to be higher in subjects in the high oleic acid intake category than in the low (involving a mean increase in PON1 activity of around 14 U/l), although differences did not reach statistical significance. No differences between the two groups with regard to PON1–192 genotype distribution were found.

Serum lipid and lipoprotein concentrations of the R carrier groups in the overall study group were similar to those of QQ homozygotes (Table 2). As expected, log PON1 activity was significantly lower in the subset of the low-activity PON1 QQ genotype subjects than in those who were QR or RR ($P<0.001$).

The hypothesis that PON1–192 polymorphism might modify the relationship between the oleic acid intake and serum parameters was explored by stratifying subjects in the three PON1–192 genotypes and oleic acid intake categories (Table 2). No differences were found in clinical characteristics, lipids and lipoproteins, or log PON1 activity between oleic acid intake groups of QQ homozygotes. However, QR heterozygotes with high oleic acid intake showed significantly higher HDL cholesterol concentration than low oleic acid intake subjects. RR subjects included in the high oleic acid intake group showed significantly higher PON1 activity than those of the low intake group. It is noteworthy that HDL cholesterol concentration in QR subjects was significantly lower than QQ homozygotes only in the low oleic acid intake group. PON1 activity was significantly higher in QR subjects and RR homozygotes than in QQ homozygotes, despite the oleic acid intake.

A significant correlation between oleic acid intake and log PON1 activity was found only in RR heterozygotes ($r=0.595$, $P=0.001$). No significant correlation was found between these variables in the overall study group, or between oleic acid intake and HDL cholesterol, or between HDL cholesterol and log PON1 activity in any considered category. To further explore whether an interaction existed between genotype and oleic acid intake, analyses of the variance adjusting by age, body mass index, smoking, alcohol consumption and physical activity covariables were carried out. A statistically significant interaction between PON1–192 genotypes and oleic acid intake for log PON1 activity was observed ($P=0.005$) (Table 3). The interaction between oleic acid intake and PON1–192 genotypes for HDL cholesterol was marginally significant ($P=0.066$).

Table 3
Effect of interaction of oleic acid intake and PON1–192 genotype on log PON1 activity and HDL cholesterol concentration

	Log PON1 activity		HDL cholesterol	
	F value	*P* value	*F* value	*P* value
Oleic acid intake[a]	7.31	0.007	3.17	0.075
PON1–192 genotype	421.54	<0.001	0.58	0.562
Oleic acid intake× PON1–192 genotype	5.46	0.005	2.73	0.066
Age	22.98	<0.001	0.01	0.914
BMI	0.15	0.703	33.52	<0.001
Smoking	1.59	0.208	10.84	0.001
Alcohol consumption	1.90	0.174	42.48	<0.001
EEPA	0.01	0.930	2.05	0.152

[a] Men were stratified in two groups according to the median value of oleic acid intake×100 kcal. BMI, body mass index; EEPA, daily energy expenditure in leisure-time physical activity.

4. Discussion

Human and animal studies strongly support the hypothesis that oxidative modification of LDLs plays a crucial role in the pathogenesis of atherosclerosis (Berliner and Heinecke, 1996). Therefore, mechanisms preventing LDL oxidation appear to be antiatherogenic. In this respect, HDL-associated PON1 may be a major defence barrier against lipid peroxides from oxidised LDLs (Mackness et al., 1993). Conversely, PON1 activities (activities towards paraoxon and towards oxidised lipids) can be inhibited by oxidative stress, and the degree of inhibition depends on the PON1–192 genotype (Aviram et al., 1999). R allozyme is supposed to give worse protection against oxidation and be more easily impaired than the Q allozyme (Mackness et al., 1997, 1998; Aviram et al., 1998, 2000).

The Mediterranean diet has been claimed to be one of the reasons for the low incidence of coronary heart disease, despite the high prevalence of cardiovascular risk factors (Masiá et al., 1998; Marrugat and Sentí, 2000). The Mediterranean diet is rich in monounsaturated fatty acids, particularly oleic acid, which confers antiatherogenic benefits through a variety of mechanisms.

Since an olive oil-rich diet has been recognised to have antioxidant properties, and since PON1–192 polymorphism has been shown to be related to a variation in HDL cholesterol levels in some population based studies, the hypothesis under consideration was that this genetic marker may influence the possible association of oleic acid intake with changes in PON1 HDL associated particles. Our results showed that the interaction between PON1–192 genotype and the oleic acid intake resulted in differences, particularly in PON1 activity levels. High oleic acid intake was associated with increased PON1 activity levels only in men who were homozygotes for the R allele. HDL cholesterol concentration was significantly higher in subjects with a high oleic acid intake than in those with a low oleic acid intake only in the QR genotype. HDL cholesterol was also found increased in RR subjects with a high oleic acid intake, but differences did not reach statistical significance, probably owing to the small number of RR subjects. On the other hand, QR subjects in the lower oleic acid intake category had a HDL cholesterol concentration mean significantly

lower than QQ homozygote men in the same category. HDL cholesterol was also lower in RR subjects with a low acid intake than QQ homozygotes in the same category, but differences did not reach statistical significance due to the small number of RR homozygotes. These differences disappeared when R carrier subjects and QQ homozygotes in the high oleic acid intake group were compared. All these findings suggest that the effects of common polymorphisms on a particular trait depend on the presence of a specific life style and other environmental factors, and may explain discrepancies among association studies. For example, the R allele, which has been related to increased cardiovascular risk in some studies (Ruiz et al., 1995; Serrato and Marian,1995), may be deleterious only in sedentary subjects (Sentí et al., 2000) or in subjects consuming an oleic acid-poor diet, both appear to have considerably more adverse HDL-cholesterol concentrations than subjects with the QQ genotype.

The findings observed in the present study raise some interesting considerations. It has been reported that LDL isolated after diets rich in monounsaturated fatty acids oxidises less readily than LDL obtained from diets rich in polyunsaturated acids, and that this difference depends on the content of oleic acid in LDL particles (Bonanome et al., 1992; Reaven et al., 1993). On the other hand, HDL rich in oleic acid was less easily oxidised, regardless of the content of antioxidants such as vitamins A and E (Solà et al., 1997). We show here that a high oleic acid intake was associated with significantly increased HDL cholesterol concentrations and PON1 activity levels in QR and RR subjects, respectively. We also show that oleic acid intake positively correlated PON1 activity in subjects who were homozygotes for the R allele. Altogether, these findings support the idea that an oleic acid-rich diet gives protection against lipoprotein oxidation, particularly in men carrying one or two R alleles, and introduces the enhanced PON1 activity and its HDL related lipoprotein as a likely mechanism for this effect.

In view of previous findings, in which there were differences in the lipoprotein profile according to the amount of regular physical activity only in R carriers (Sentí et al., 2000), it seems reasonable to postulate that the HDL-R PON1 associated particle is more susceptible to changes in environmental factors, such as physical activity or diet. Although the association of PON1–192 polymorphism with changes in lipid and lipoprotein concentrations remains unclear, PON1 is strongly linked to HDL. It has also been reported that plasma concentrations of PON1 are positively correlated with HDL-cholesterol and apo AI, and negatively with total cholesterol and apo B (Blatter-Garin et al., 1994). PON1 activity seems to be decreased in patients with familial hypercholesterolemia compared with control subjects (Mackness et al., 1991b; Tomás et al., 2000). These observations suggest a relationship between lipoprotein metabolism and PON1, the latter being in turn strongly modulated by the PON1–192 polymorphism. On the other hand, there are cumulating evidence that a number of genes associated with lipid metabolism have their expressions controlled by the intracellular levels of fatty acids (Amri et al., 1996; Cheema and Clandinin, 1996; Sfeir et al., 1997). In this respect, the PON1 gene would be another gene to be added to the list of this kind of genes.

Given the potential benefit of having high HDL cholesterol and PON1 activity levels as a desirable antioxidant status, the results of the present study suggest that any direct intervention towards increasing the amount of oleic acid intake in subjects carrying one or two R alleles should theoretically reduce the risk of atherosclerosis with considerable specificity.

In summary, an oleic acid-rich diet may be beneficial in individuals who carry one or more PON1–192 R alleles, which constitutes 50% of the population, to achieve favourable antioxidant status similar to that observed in PON1 QQ homozygous subjects.

Acknowledgements

This study was supported by grants from the Fondo de Investigaciones Sanitarias 99/0013-01, 99/9342; CICYT 1FD97-0626 and CIRIT 1999SGR00243. We appreciate the English revision made by Ms. Stephanie Lonsdale and Dave Mcfarlane.

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