Effect of Physical Activity on Lipid Levels in a Population-Based Sample of Men With and Without the Arg192 Variant of the Human Paraoxonase Gene

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The prevalence of cardiovascular risk factors in Gerona, Spain, is high for the low myocardial infarction incidence and mortality rates in the province. Physical activity is a protective factor against coronary heart disease. We investigated whether the genetic variants Q and R of the paraoxonase Gln-Arg 192 polymorphism were involved in different responses of lipids to physical activity. Serum triglycerides, HDL-cholesterol concentrations, and the paraoxonase Gln-Arg 192 polymorphism were determined in 262 men randomly selected from a representative population sample in a cross-sectional study conducted in Gerona, Spain. The Minnesota Leisure Time Physical Activity Questionnaire was used to assess energy expenditure in leisure time physical activity. No differences were found in lipid levels among tertiles of physical activity distribution in subjects with the QQ genotype. However, R carriers showed a significant decreasing trend in triglyceride levels and in log-triglyceride-to-HDL-cholesterol ratio and a significant increasing trend in HDL-cholesterol concentration with the amount of physical activity. R carriers included in the low tertile of physical activity distribution had HDL-cholesterol levels significantly lower than those of QQ homozygous men in the same physical activity category (1.04 mmol/L vs. 1.22 mmol/L, \( P = 0.024 \)). R carriers of the higher tertile of physical activity distribution showed
PON1 Genetic Polymorphism and Physical Activity

the most favorable lipid profile in this genetic group. A statistically-significant interaction between paraoxonase genotypes and physical activity was observed for log triglycerides ($P = 0.018$), HDL-cholesterol concentration ($P = 0.017$), and log triglyceride-to-HDL-cholesterol ratio ($P = 0.008$). The beneficial association of the amount of physical activity and lipid traits found in men with the R allele suggests that this population subgroup needs to be physically active to achieve a favorable lipoprotein phenotype similar to that observed in QQ homozygous men. Genet. Epidemiol. 18:276–286, 2000. © 2000 Wiley-Liss, Inc.

Key words: lipids; genetics; paraoxonase; physical activity

INTRODUCTION

In terms of cardiovascular disease, the so-called “French paradox” has been described as an apparent coexistence of high-fat diet with low incidence of coronary heart diseases (CHD) [Artaud-Wild et al., 1993]. An extension of this paradox can be found in other South European countries such as Spain where, although ischemic heart disease is the leading cause of death, 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]. Interaction of life-style factors, including physical activity, with genetic factors may account for differences in CHD among different geographic areas.

Physical activity and physical fitness have been identified as protective factors against the occurrence and progression of coronary heart disease [Powell et al., 1987; Lakka et al., 1994; Erikssen et al., 1998] and premature mortality [Pekkanen et al., 1987]. Such protection, among other factors, has been related to improvement in the lipid profile, particularly lowered triglyceride and increased high-density lipoprotein (HDL) cholesterol concentrations [Marrugat et al., 1996]. The average daily amount of physical activity was 16% higher in Gerona, Spain, compared with Minnesota [Masiá et al., 1998; McGovern et al., 1996]. Since the incidence rate of acute myocardial infarction in Minnesota men was three times higher than in Gerona, [McGovern et al., 1996; Pérez et al., 1998], the wider practice of physical activity in Spain may conceivably account for part of the differences in this incidence.

Cumulated evidence involves serum paraoxonase (PON), an HDL-linked enzyme, in the oxidation hypothesis of atherosclerosis, since it has been described that this enzyme exerts its effect by removing lipid-peroxidation products [Mackness et al., 1991]. Serum levels of PON activity, based on the capacity of the enzyme to hydrolyze paraoxon, appear to vary among individuals and populations. The molecular basis of these variations 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, that has a glutamine at position 192, while the high-activity PON1 R isoform contains an arginine at position 192 [Mackness et al., 1996].

The report by Ruiz et al. [Ruiz et al., 1995] was the first in a series of studies on PON1-192 gene polymorphism and CHD. An overall view of these studies reveals different results, even in studies conducted in the same ethnic population.
In one study carried out in the United States [Serrato and Marian, 1995], the R allele was clearly associated with CHD. In northern Europe, two studies failed to show any association between PON1-192 and CHD [Antikainen et al., 1996; Rice et al., 1997]; neither did a study in a French population [Herrmann et al., 1996] or another in Italians [Ombres et al., 1998]. This great variability in results suggests that an unknown third factor or gene-environment interactions might be present in the proposed relationship between PON1 polymorphism and CHD.

On the other hand, 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 these changes are also characteristic of physical activity practice and because it is particularly important to study gene-environmental interactions that may modulate phenotypic expression of PON1 in different populations, we attempted to ascertain whether an interaction that modifies serum lipoproteins exists between PON1-192 genetic polymorphism and physical activity. The present study is part of a larger study designed to establish protective interactions against CHD between genetic characteristics and environmental factors in our area [Masiá et al., 1998]. The candidate variables were physical activity, dietary factors, smoking, and genetic polymorphisms in the human paraoxonase gene and genes involved in lipid metabolism.

SUBJECTS AND METHODS

Subjects

Two hundred and sixty-two men, aged 25 to 74, were randomly selected from a representative population sample of 1,750 subjects (50% men) in a cross-sectional 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 [McGovern et al., 1996; Masiá et al., 1998]. The reference population was composed of six regions of the province of Gerona comprising 189 towns and 509,618 inhabitants according to the 1991 census. Thirty-three towns were randomly selected in the first phase of the study and selected people were notified in their own towns by a letter informing them of the aims of the study. All subjects completed a smoking questionnaire consisting of eight questions regarding current and past cigarette consumption. Smoking was defined as consumption of at least 1 cigarette per day, or having been a smoker until less than 1 month before examination. 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]. EEPA data was available for 256 participants. This questionnaire was validated for use among Spanish men [Elosua et al., 1994] and was administered by a trained interviewer. Data on alcohol consumption in g/week was also obtained by a questionnaire. None of the men included in the present study were on lipid-lowering drug therapy. All subjects gave their written informed consent to participate. The protocol was approved by an ethics committee.
Laboratory Analyses

Blood samples were collected after an overnight fast. Serum triglyceride levels were determined enzymatically (Roche Diagnostica, Basel, Switzerland). HDL cholesterol was measured as cholesterol after precipitation of apolipoprotein B-containing lipoproteins with phosphotungstic-Mg++ (Boehringer Mannheim, Mannheim, Germany). Analyses were performed in a Cobas Mira Plus (Roche Diagnostica, Basel, Switzerland).

Genomic DNA was isolated from white cells by the salting-out method [Miller et al., 1989]. Polymerase chain reaction was performed using primer sequences derived from published data [Humbert et al., 1993]. 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 sec at 94°C, 1 min at 61°C and 1 min at 72°C, and finally by 7 min of extension at 72°C. After amplification, polymerase chain reaction products were digested with AlwI for 4 hr at 37°C and the samples electrophoresed in 3% agarose gels for 75 min at 60 V.

Statistical Analysis

The chi-square test was used to test the association between categorical variables and the deviation of genotype frequencies from those predicted by the Hardy-Weinberg equilibrium. Allele frequency for the polymorphism was estimated by the gene counting method. Effects of physical activity on lipid traits were evaluated by comparing lipid values in the tertiles of physical activity distribution by one-way analysis of variance, and differences between pairs tested by Tukey’s test. Linear trends for continuous variables were assessed by Spearman correlation coefficient. Two-way interaction between genotype and physical activity in determination of lipid traits was estimated using multiple analysis of variance. A multiple regression analysis was conducted to analyze the magnitude of the association between physical activity and lipid traits in each genotype. Triglyceride values were log-transformed prior ANOVA and regression analysis. Based on previous data showing that triglyceride content of very-low-density lipoproteins (VLDL) divided by HDL cholesterol reflects triglyceride metabolism [Havel 1990; Sentí et al., 1992], we defined a lipid parameter consisting of the ratio of triglycerides (which strongly correlate with VLDL triglycerides) and HDL-cholesterol levels. Analyses were performed using the SPSS package.

RESULTS

Relative allele frequencies were 0.697 and 0.303 for Q and R alleles, respectively. Genotype frequencies did not deviate from those predicted by the Hardy-Weinberg equilibrium. Genotype groups had similar age, BMI, EEPA, alcohol consumption, and percentage of smokers (Table I). No significant differences were found in lipid traits between PON1 genotypes. (Table I).

Participants were classified as sedentary, active, and very active according to the tertiles of daily EEPA distribution. The relationship between physical activity and lipid traits for the total group of subjects was examined (Table II). Serum triglycerides and mean log triglyceride-to-HDL-cholesterol ratio were significantly lower
in subjects of the higher tertile of physical activity distribution compared with those of the lower tertile. Although differences did not reach statistical significance, mean HDL-cholesterol concentration tended to be higher in very active men. Furthermore, there was a clear dose-dependent relationship between daily EEPA and serum triglycerides, HDL cholesterol, and log triglyceride-to-HDL-cholesterol ratio.

The hypothesis that PON1-192 polymorphism might modify the relationship between physical activity and lipid traits was explored in an analysis stratified by PON1 genotypes. (Table III). No differences were found between lipid values of tertiles of physical activity distribution in the QQ genotype. However, R carriers showed a significant decreasing trend in triglyceride levels and in log triglyceride-to-HDL-cholesterol ratio and a significant increasing trend in HDL-cholesterol concentrations with the amount of daily EEPA. Similar trends were found when the R homozygotes were removed from the R-carrier group (results not shown). Sedentary subjects with the R allele had an HDL-cholesterol mean concentration significantly lower than sedentary QQ homozygotes. However, R carriers included in the second tertile of physical activity distribution achieved similar HDL-cholesterol levels and

<table>
<thead>
<tr>
<th>TABLE II. Serum Triglycerides, HDL-Cholesterol, Log Triglyceride-to-HDL-Cholesterol Ratio, and Other Characteristics in Men Stratified by Tertiles of Daily Physical Activity Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kcal/day n = 86</td>
</tr>
<tr>
<td>Age (years) 53.6 (10.8)</td>
</tr>
<tr>
<td>BMI 27.6 (4.2)</td>
</tr>
<tr>
<td>Alcohol consumption 361.4 (342.4)</td>
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<tr>
<td>Triglycerides 1.57 (1.39–1.75)</td>
</tr>
<tr>
<td>HDL-cholesterol 1.14 (0.48)</td>
</tr>
<tr>
<td>Log triglyceride-to-HDL-cholesterol ratio 0.164 (0.236)</td>
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</tbody>
</table>

*Significantly different from sedentary subjects, P < 0.05.
**Test for linear trend, P < 0.005.
TABLE III. Serum Triglycerides, HDL-Cholesterol and Log Triglyceride-to-HDL-Cholesterol Ratio in Men Stratified by Tertiles of Daily Physical Activity Distribution and PON1 Genotypes

<table>
<thead>
<tr>
<th></th>
<th>Sedentary (Kcal/day)</th>
<th>Active (184–3470 Kcal/day)</th>
<th>Very active (&gt;370 Kcal/day)</th>
<th>( P^* )</th>
<th>( P^{**} )</th>
<th>( r^{**} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>QQ homozygotes</td>
<td></td>
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</tr>
<tr>
<td>n = 48</td>
<td>n = 47</td>
<td></td>
<td>n = 33</td>
<td></td>
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</tr>
<tr>
<td>Triglycerides</td>
<td>1.55 (1.29–1.79)</td>
<td>1.69 (1.42–1.96)</td>
<td>1.54 (1.11–1.94)</td>
<td>0.687</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.22 (0.56)</td>
<td>1.19 (0.32)</td>
<td>1.21 (0.29)</td>
<td>0.985</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Log triglyceride-to-HDL-cholesterol ratio</td>
<td>0.136 (0.226)</td>
<td>0.176 (0.199)</td>
<td>0.155 (0.298)</td>
<td>0.734</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>R carriers</td>
<td></td>
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<tr>
<td>n = 38</td>
<td>n = 37</td>
<td></td>
<td>n = 53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.60 (1.32–1.88)</td>
<td>1.34 (1.12–1.55)</td>
<td>1.23 (1.02–1.44)</td>
<td>0.005</td>
<td>0.003</td>
<td>–0.25</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.04 (0.35)</td>
<td>1.18 (0.40)</td>
<td>1.24 (0.32)</td>
<td>0.007</td>
<td>0.001</td>
<td>0.28</td>
</tr>
<tr>
<td>Log triglyceride-to-HDL-cholesterol ratio</td>
<td>0.198 (0.272)</td>
<td>0.094 (0.196)</td>
<td>0.048 (0.197)</td>
<td>0.007</td>
<td>0.001</td>
<td>–0.28</td>
</tr>
</tbody>
</table>

\( P \), significance of differences between tertile 3 (very actives) and tertile 1 (sedentary subjects).

**Test for linear trend, Spearman correlation coefficients between physical activity, and lipid characteristics as continuous variables, when ANOVA result was statistically significant \( (P < 0.05) \).

***Significantly different from QQ homozygous subjects, \( P < 0.05 \).
log triglyceride-to-HDL-cholesterol ratios than QQ homozygotes, with their triglyceride levels being significantly lower. In both PON1-192 genotypes, no significant differences of mean BMI values and alcohol intake were observed between physical activity groups (results not shown).

To further explore whether an interaction existed between genotype and physical activity, a multiple analysis of variance was made in which physical activity, categorized according to the first tertile of daily EEPA distribution, and PON1 genotypes were entered as factors, and age, BMI, smoking, and alcohol consumption as covariates. A statistically-significant interaction between genotype and physical activity was observed for all lipid traits (Table IV). No evidence of interaction for the lipid traits was found between PON1 genotype and categorized BMI or alcohol consumption (results not shown).

Multiple linear regression analysis was conducted separately in men with the QQ genotype and in R carriers to analyze the magnitude of the association between physical activity and lipid traits, adjusting for age, BMI, alcohol, and cigarette consumption. No evidence of colinearity was found among the variables for the prediction of lipid traits. In fact, the tolerance of colinearity for all the variables ranged from 0.852 to 0.970 in the 3 regression models. In the subset of QQ homozygotes, physical activity was not significantly associated with any lipid trait. In the subset of R carriers, the amount of daily EEPA was positively associated with HDL-cholesterol concentrations (partial $r^2 = 0.043, P = 0.023$) and inversely with log triglycerides (partial $r^2 = 0.040, P = 0.036$) and log triglyceride-to-HDL-cholesterol ratio (partial $r^2 = 0.060, P = 0.006$). In R-carrier men, age, BMI, alcohol consumption, smoking, and daily EEPA accounted for 13 to 23% of the variability in these specific traits.

**DISCUSSION**

**Physical activity and Lipids**

The prevalence of cardiovascular risk factors in Gerona, Spain, is high for the low myocardial infarction incidence in the province [Masiá et al., 1998]. Which factors confer sufficient protection to compensate for this high prevalence remain to be determined. However, it is likely that lifestyle factors such as diet, physical activity,

| TABLE IV. Effect of Interaction of PON1 Genotype With Physical Activity on Levels of Log Triglycerides, HDL-Cholesterol and on Log Triglyceride-to-HDL-Cholesterol Ratio |
|-----------------|----------------|-----------------|-----------------|
|                 | F value | P value | F value | P value | F value | P value |
| Age             | 1.21    | 0.273   | 2.77    | 0.097   | 4.12    | 0.044   |
| Body mass index | 12.24   | 0.001   | 10.05   | 0.002   | 8.11    | 0.005   |
| Alcohol consumption | 0.13 | 0.721   | 19.17   | <0.001  | 8.75    | 0.003   |
| Physical activity$^a$ | 0.01   | 0.961   | 3.68    | 0.056   | 2.97    | 0.086   |
| Smoking         | 4.68    | 0.032   | 1.79    | 0.182   | 3.60    | 0.059   |
| Genotype        | 0.03    | 0.861   | 2.42    | 0.121   | 3.89    | 0.050   |
| Genotype by physical activity | 5.66 | 0.018   | 5.78    | 0.017   | 7.20    | 0.008   |

$^a$Coded as sedentary and active or very active according to the first tertile value (183 Kcal/day) of daily energy expenditure in leisure time physical activity as a cut-off point.
and their interactions with genes may contribute to neutralizing atherogenic factors. Among the former, physical activity exerts part of its beneficial effect by increasing HDL-cholesterol levels and lowering the atherogenic index [Marrugat et al., 1996]. In the present study, the amount of physical activity was associated with decreasing triglyceride and increasing HDL-cholesterol level trends. Both features resulted in a significant decreasing trend in triglyceride-to-HDL-cholesterol ratio, a lipidic parameter that indirectly reflects triglyceride catabolism [Havel, 1990; Sentí et al., 1992]. These findings concur with a previous report that described an association between physical training and more efficient catabolism of plasma triglycerides and prolonged survival of HDL lipoproteins [Weintraub et al., 1989].

PON1 Polymorphism, Physical Activity, and Lipids

Since the PON1-192 polymorphism has been shown to be related to a variation in the lipoprotein profile in some population-based studies [Hegele et al., 1995; Boright et al., 1998], the hypothesis under consideration was that this genetic marker may influence lipid level response to physical activity. Overall, our results suggest no influence of PON1-192 genetic variation on lipids and lipoproteins. However, daily EEPA was associated with increased HDL-cholesterol levels and decreased triglyceride levels, which determined a decreased log triglyceride-to-HDL-cholesterol ratio only in men carrying the R allele. R carriers included in the lower physical activity tertile had an HDL-cholesterol mean concentration significantly lower than QQ homozygous men in the same physical activity category. In contrast, R carriers of the second tertile achieved HDL-cholesterol levels similar to those of QQ subjects and, interestingly, better serum triglycerides. Very active R carriers in the third tertile of daily EEPA showed the most favorable lipid profile in this genotype group. Therefore, the existence of a single R allele seems to strongly impair the lipid profile, particularly HDL-cholesterol concentrations, in men if physical activity is not regularly undertaken, but our results also suggest that with moderate increases in physical activity the beneficial effect on lipids is already obtained in subjects with the R allele. An example of the cut-off point that defines active participants included in the second tertile of physical activity distribution used in the present study (183 Kcal/day) may be equivalent to three 20-min sessions per week of running at 5 miles/hr or 40-min sessions of brisk walking (3 miles/hr), plus 100 Kcal/day expended in other daily physical activities such as walking or climbing stairs.

Metabolic Hypothesis

Most lipid changes following regular physical activity practice have been attributed to elevated levels of post-heparin lipoprotein lipase (LPL) [Weintraub et al., 1989]. It has also been reported in one study [Nevin et al., 1996] that variations in LPL affect paraoxonase through their independent effects on HDL levels and that paraoxonase and LPL activities seem to correlate in normolipidemic subjects homozygous for the Q allele. However, no correlation of LPL activity with paraoxonase was demonstrated in R carriers [Nevin et al., 1996]. Unfortunately, information on physical activity was not available in that study. If LPL can be assumed to be a physiological determinant of triglyceride-rich lipoproteins and HDL-cholesterol levels [Kuusi et al., 1989], it seems reasonable to postulate that the interaction between the amount of physical activity and PON1-192 genetic polymorphism may modulate
the linear relationship of LPL with paraoxon activity and this may affect lipoprotein concentrations. However, whether physical activity can modulate the expression of the PON gene, and this in turn affect LPL activity, is at this time a matter of conjecture and additional investigations are required to clarify this point.

It is conceivable that the extent of the PON1 polymorphism-lipid profile relationship depends on the presence of specific environmental factors such as lifestyle, particularly physical activity. This could explain some of the discrepancies observed in the literature. For example, in the Hutterites study [Hegele et al., 1995], PON1-192 might be associated with variations in lipoproteins, particularly HDL, due to overall low physical activity rather than a specific genetic background. On the other hand, R allele, which has been related to increased cardiovascular risk in some studies [Ruiz et al., 1995; Serrato and Marian, 1995; Zama et al., 1997], may be deleterious only in sedentary R-carrier subjects, who appear to have a considerably more adverse lipoprotein phenotype than sedentary subjects with the QQ genotype.

Study Characteristics and Clinical Implications

The sample used in the present study is representative of a Mediterranean population in the northeast of Spain. More remarkably, the population prevalence of the QR or RR allele pairs was found to be very high (50%). In consequence, impact of such a genetic characteristic in the population is considerable. Any intervention directed towards increasing the amount of physical activity in R carriers should theoretically reduce the risk of atherosclerosis with considerable specificity. The design of the present study is cross-sectional. Thus, our findings should be confirmed in case-control and cohort studies.

The beneficial association between the amount of physical activity and lipid traits found in PON1-192 R carriers suggests that this population subgroup, which constitutes 50% of the whole, needs to be physically active to achieve favorable lipid levels similar to those observed in PON1 QQ homozygous men.

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