Paraoxonase1-192 polymorphism modulates the effects of regular and acute exercise on paraoxonase1 activity

Marta Tomás,[1,*] Roberto Elosua,[*] Mariano Sentí,[*,†] Luis Molina,[§] Joan Vila,[*] Roger Anglada,[*,†] Montserrat Fitó,[*] Maria Isabel Covas,[*,] and Jaume Marrugat[*]**

Lipids and Cardiovascular Epidemiology Unit,* Institut Municipal d'Investigació Mèdica (IMIM); Departament de Ciències Experimentals i de la Salut,† Universitat Pompeu Fabra; Servei de Cardiologia,§ Hospital del Mar; and Laboratori de Referència,** Barcelona, Spain

Abstract **Regular exercise practise is a protective factor against coronary heart disease and enhances antioxidant systems, whereas acute exercise appears to be a major source of increased oxidative stress. Paraoxonase1 (PON1) is an antioxidant HDL-linked enzyme, whose activity toward paraoxon (PON1 activity) is strongly modulated by the PON1-192 polymorphism, comprising Q and R alleles for low and high PON1 activity, respectively. Another polymorphism at the PON1 locus, the PON1-55, modulates PON1 protein and activity levels. PON1 activity, lipid levels, and oxidized LDL concentration were determined in 17 healthy young volunteers before and after a 16-weeks aerobic exercise training period. Furthermore, PON1 activity was analyzed after a bout of exercise in both situations. We found that regular exercise was associated with a decrease in oxidized LDL levels, and an increase in PON1 activity in QQ subjects and with a decrease in PON1 activity in R carriers. A bout of exercise produced an increase in PON1 activity just after the bout of exercise, followed by a decrease in its activity. A recovery of the basal PON1 activity levels at 24 h was found in QQ subjects regardless of their training status and in trained R carriers, but not in untrained R carriers. These results suggest that the effects of regular and acute exercise on PON1 activity levels are modulated by PON1-192 polymorphism. Changes were less evident for the PON1-55 polymorphism.**—Tomás, M., R. Elosua, M. Sentí, L. Molina, J. Vila, R. Anglada, M. Fitó, M. I. Covas, J. Marrugat. **PON1-192 polymorphism modulates the effects of regular and acute exercise on paraoxonase1 activity. *J. Lipid Res.* 2002.** 43: **713–720.**

Supplementary key words bout of exercise • exercise training • PON1 genotypes • oxidative stress

Regular physical activity (PA) has been identified as a protective factor against the occurrence and progression of coronary heart disease (CHD) (1, 2). Regular PA improves lipid profile (3), and reduces blood pressure (4) and non-insulin-dependent diabetes incidence (5). Nevertheless, these effects explain only a part of the protection against CHD related to PA (6). The beneficial effect of regular PA is also supported by the fact that the relative risk for physical inactivity of CHD is similar in magnitude to that of hypertension, hypercholesterolemia, and smoking (1).

Oxidative stress has been found to be linked to the development of atherosclerosis (7). In this respect, the balance between free radical generation and antioxidant activity seems to play a key role in the pathogenesis of CHD. Paraoxonase1 (PON1) is an antioxidant enzyme linked to the HDL-containing apolipoprotein (apo)A-I (8, 9) and apoJ (9). Several emerging lines of evidence suggest that PON1 is responsible for the antioxidant properties of HDL on LDL particle (10, 11). PON1 enzyme activity for paraoxon as a substrate is modulated at the PON1 locus (12), by the PON1-192 and PON1-55 polymorphisms, among others. The former comprises Q and R alleles (13), whose protein allozymes have been suggested to differ both in the PON1 activity and in their ability to confer protection against oxidative conditions (14–17) (Q allele for lower PON1 activity and higher protection than R allele). The PON1-55 polymorphism, which comprises L and M alleles, seems to modulate PON1 mRNA (18) and protein levels (L allele corresponds to higher concentration than M allele) (19), probably due to a certain degree of linkage disequilibrium between PON1-55L and the -108C allele of the functional polymorphism at the promoter region (20), and to the different stability of each allozyme (21).

In contrast to regular PA, isolated bouts of exercise are considered to be a major source of free radical generation, oxidative stress, and lipid peroxidation (22). LDL ox-

Abbreviations: APO, aerobic power output; BMI, body mass index; CHD, coronary heart disease; HDL-C, serum HDL-cholesterol; LDL-C, serum LDL-cholesterol; MANOVA, multivariate analysis of variance; MPO, maximum power output; PA, physical activity; PON1, paraoxonase1; PON1 activity, Paraoxonase1 activity towards paraoxon; RER, respiratory-exchange ratio; TBARS, thiobarbituric acid reactive substances method; TC, serum total cholesterol; TG, serum triglycerides; VO_2, oxygen uptake; VO_{2max}, maximal oxygen uptake; VT, ventilatory anaerobic threshold.

[1] To whom correspondence should be addressed at Lipids and Cardiovascular Epidemiology Unit, Institut Municipal d'Investigació Mèdica (IMIM), Dr Aiguader 80, E-08003 Barcelona, Spain.
e-mail: mtomas@imim.es

idation may inactivate PON1 through interactions between a free sulphydryl group of PON1 and the oxidized LDL lipids (16).

To our knowledge, the role of PA on PON1 activity, particularly the effects of regular and a bout of PA, has not been yet established. The objectives of the present study were, firstly, to assess the effect of aerobic exercise training on PON1 activity in young healthy subjects and to determine whether this effect is dependent on the PON1-192 and PON1-55 polymorphisms, and secondly, to assess the effect of a bout of exercise on PON1 activity according to the training status and the PON1-192 and PON1-55 polymorphisms.

MATERIALS AND METHODS

Subjects

Seventeen sedentary healthy young volunteers (seven men and 10 women) participated in this study. They were selected among medical students. Subjects with a previous history of cardiovascular disease, diabetes mellitus, dyslipemia, physical disability, or chronic respiratory disease were excluded, as well as those with a body mass index (BMI) over 30, alcohol consumption greater than 40 g/day, illicit drug use, or long-term medication use including mineral or vitamin supplements. Another exclusion criterion was to have performed regular exercise for more than 2 h per week during the 3 months prior to inclusion in the study. The local ethical committee approved the protocol and all participants signed an informed consent.

Intervention

A general scheme of the designed intervention is presented in **Fig. 1**. An initial individual physical fitness assessment was performed. A week later, 30 min of acute aerobic exercise were performed at adequate individual intensity. Thereafter, a 16 week-training period was initiated. Another physical fitness assessment was carried out 1 week before ending the training period, and another acute aerobic exercise was performed at the end of the period.

Physical fitness assessment. A continuous, incremental maximum cycling test on a electromagnetically braked ergocycle (Marquette Hellige Medical Systems, EC 1200, Milwaukee) was performed by all the participants. The test was initiated with a warm-up at 25 W (women) or 50 W (men) for 5 min. After this, the power output was increased by 25 W every 2 min until exhaustion. During the test, oxygen uptake (VO_2), minute ventilation, and respiratory-exchange ratio (RER) were continuously measured using an Oxicon Alpha (Jaeger, Wuerzburg, Germany). Maximal VO_2 (VO_{2max}) was calculated as the average of the three highest VO_2 registered. The ventilatory anaerobic threshold (VT) was defined as the point where three consecutive RER equal or greater than one were observed. The maximal power output (MPO) was defined as the highest achieved power completed for at least 1 min. The aerobic power output (APO) was defined as the power achieved at VT level.

Bout of aerobic exercise. All the participants performed a bout of aerobic exercise 1 week after the physical fitness assessment, both before and after the training period. Participants cycled an ergometer for 30 min at the level corresponding to their APO. They warmed-up for 5 min at 25 or 50 watts (in the case of women or men, respectively), and achieved the APO, maintaining this power during the remaining 25 min.

This exercise was performed 3 hours after lunch. Each participant was requested to adhere to the same diet 3 days prior to both bouts of exercise.

Training period. Participants were enrolled in an aerobic exercise training program, designed and monitored by a specialist in physical education, during a 16-week period. Initially the frequency of training sessions was four per week and the duration was 30 min per session. The frequency and duration of the sessions were progressively increased up to 5 days per week and 50 min per day during the first 8 weeks, and continued so for another 8 weeks. Only aerobic physical activities (running, swimming, aerobic games) were undertaken, and the intensity of training was adapted to the basal physical fitness of each participant. The training was performed in the installations of the Servei d'Activitat Física of the Universitat Autònoma de Barcelona.

Blood sampling

A blood sample was drawn prior to each bout of exercise (basal). After the bout of exercise, blood samples were collected immediately after exercising (0 h), and at 0.5 h, 1 h, 2 h, and 24 h thereafter.

Laboratory methods

PON1 activity toward paraoxon. PON1 activity was measured after 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 an autoanalyzer (Cobas-Mira Plus, Roche Diagnostica, Switzerland). 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 were used as an internal control; these were thawed just before the beginning of the assay. At least one aliquot of serum pool

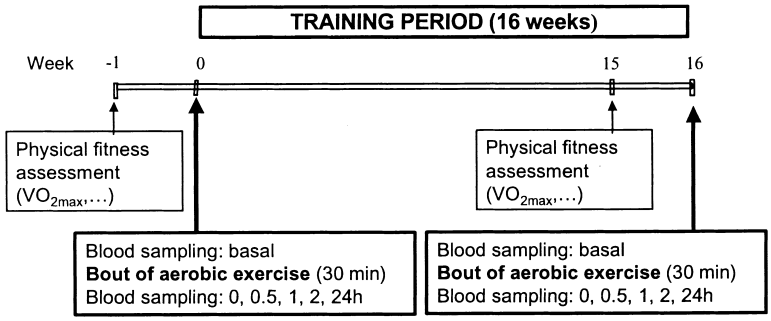

Fig. 1. Temporal diagram of exercise training period, bouts of exercise, physical fitness assessment, and blood sampling. VO_{2max}, maximal oxygen uptake.

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 liter of serum. The molar extinction coefficient of p-nitrophenol is 18,053 l mol^{-1} cm^{-1} at pH 8.5. The intra- and inter-assays coefficients of variation were 0.70% and 0.76%, respectively.

PON1-192 and PON1-55 genotypes determination. Genomic DNA was isolated from white cells by the salting-out method (23). PCR for PON1-192 and PON1-55 polymorphisms were performed using primer sequences derived from published data (13). 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. For PON1-192 polymorphism, 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. For PON1-55 polymorphism, PCR products were digested with *Hsp*92II and electrophoresed in the same conditions as described above.

Lipids and lipoproteins. Serum total cholesterol (TC) and TG were measured by enzymatic methods. HDL cholesterol (HDL-C) was measured as cholesterol after precipitation of apoB-containing lipoproteins with phosphotungstic-Mg^{2+} (Boehringer Mannheim, Mannheim, Germany). LDL-C was calculated by the Friedewald's formula (24).

Oxidized LDL. Oxidized LDL was determined in serum by an enzyme-linked immunosorbent assay using two antibodies against the antigenic determinants of oxidized apoB molecule (ox-LDL, Mercodia AB, Uppsala, Sweden). Values were expressed in U/l. The intra- and inter-assays coefficients of variation were 2.8% and 10.7%, respectively.

PON1 activity, lipid, lipoprotein, and oxidized LDL levels were adjusted for plasma volume changes, as described elsewhere (25).

Other measurements

Weight and height were measured in underwear using a calibrated scale. BMI was calculated as the weight (in kg) divided by the squared height (in meters). Smokers were defined as those smoking more than one cigarette per day during the previous 3 months.

Statistical analysis

χ-Square test was used to test differences in categorical variables. Subjects were grouped by PON1-192 or -55 genotype, as QQ and R carriers, or LL and M carriers, respectively. Unpaired and paired Student's *t*-test were used to compare the mean of continuous variables according to PON1-192 or -55 genotype, and to training status, respectively. A multivariate ANOVA (MANOVA) for repeated measures was used to test for interactions on PON1 activity levels. To assess changes on PON1 activity after the bout of exercise with respect to basal values, paired Student's *t*-test was also used with the Bonferroni correction for multiple comparisons. $P < 0.05$ was considered as statistically significant. PON1 activity is represented in the figures as the percentage of change with respect to basal point, in order to visually compare the activity of different genotypes. However, for statistical purposes, comparisons were performed using the absolute values.

RESULTS

The clinical characteristics, lipid profile, PON1 activity, and oxidized LDL concentration of the overall study participants and those of subjects stratified by PON1-192 and PON1-55 genotypes, before the training period are shown in **Table 1**. PON1 activity and oxidized LDL concentration were normally distributed. In subjects carrying the R allele, serum TC was significantly higher than in QQ subjects, but this trait was not significantly different between PON1-55 genotypes. As expected, PON1 activity was significantly higher in R carriers and in LL homozygotes. There were no differences in sex, age, smoking, BMI, HDL-C, LDL-C, TG, or oxidized LDL concentrations when subjects were stratified by PON1-192 and PON1-55 genotypes. There were no statistically significant differences in PON1 activity or oxidized LDL concentrations according to sex or smoking (data not shown). Although the proportion of smokers in the R carrier group was higher than in the QQ genotype group, the difference was not statistically significant. Moreover, there was no association between smoking and PON1 activity.

Effects of exercise training

Changes after training are presented in **Table 2**. Statistically significant increases in VO_{2max}, VT, and APO were observed together with a marginally significant increase in MPO.

There were no statistically significant differences in BMI or the percentage of smokers between before and after training. In fact, the number of cigarettes smoked per day by a particular subject did not change during the intervention period. Non-significant decreases in TC, LDL-C, and TG, and a non-significant increase in HDL-C were observed as well.

No significant change in PON1 activity due to training either in the overall study group or in subjects stratified by PON1-55 genotypes was observed at the basal point. However, an opposite effect of training on PON1 activity was found when subjects were classified according to PON1-192 genotypes. QQ subjects showed significantly increased PON1 activity levels after training, whereas a significant decrease was found in R carriers.

Oxidized LDL levels decreased after training, and this reduction was statistically significant in the overall and in the LL subject groups.

Effects of a bout of exercise

The effect of a bout of exercise on PON1 activity and oxidized LDL concentration was assessed comparing the levels at 0h, 0.5 h, 1 h, 2 h, and 24 h to the basal value. In the overall group, the PON1 activity time response pattern was similar before and after training (**Fig. 2**).

An initial increase in PON1 activity just after the bout of exercise was followed by a decrease during the 0.5–2 h period and a trend to recover the basal levels at 24 h. By analyzing the overall group, no statistical differences in PON1 activity between before and after training were found at any considered time point. However, before training PON1 activity at 24 h was significantly lower than the basal activity, a circumstance that was not observed after training. Conversely, PON1 activity at 0 h was significantly higher than basal activity only after training.

It is noteworthy that the PON1 activity time response pattern was different according to PON1-192 genotype (**Figs. 3A, B**), but not to PON1-55 genotype (Fig. 3C, D).

TABLE 1. Sample description before the training period, overall and with subjects stratified by PON1-192 and PON1-55 genotypes

	Overall (n = 17)	QQ (n = 10)	R Carriers[a] (n = 7)	*P*	LL (n = 6)	M Carriers[b] (n = 11)	*P*
Sex (men)	7	3	4	0.263	3	4	0.585
Smokers	5	2	3	0.309	1	4	0.394
Age (years)	19.5 (1.9)	19.5 (1.4)	19.4 (0.8)	0.907	19.0 (0.0)	19.7 (1.4)	0.120
BMI (kg/m2)	23.5 (2.1)	22.9 (2.2)	24.6 (1.4)	0.080	23.2 (1.4)	23.8 (2.4)	0.547
TC (mmol/l)	4.41 (0.73)	4.10 (0.76)	4.85 (0.40)	0.032	4.68 (0.92)	4.25 (0.60)	0.258
HDL-C (mmol/l)	1.25 (0.36)	1.19 (0.39)	1.34 (0.32)	0.425	1.08 (0.35)	1.35 (0.34)	0.144
LDL-C (mmol/l)	2.54 (0.67)	2.36 (0.70)	2.80 (0.58)	0.200	2.77 (0.91)	2.42 (0.51)	0.419
TG (mmol/l)	1.39 (0.97)	1.19 (0.41)	1.55 (1.47)	0.461	1.84 (1.53)	1.07 (0.32)	0.275
PON1 activity (U/l)	258 (135)	169 (27)	385 (125)	0.004	333 (184)	217 (85)	0.194
Oxidized LDL (U/l)	48.8 (16.2)	45.2 (17.3)	53.9 (14.2)	0.289	55.9 (13.6)	44.9 (16.7)	0.189

Values are mean (SD), or number of subjects. BMI, body mass index; TC, serum total cholesterol; HDL-C, serum HDL-cholesterol; LDL-C, serum LDL-cholesterol; TG, serum triglycerides.

P values are for genotype comparisons.

[a] R carrier group comprises six QR heterozygotes, and one RR homozygote.

[b] M carriers group comprises ten LM heterozygotes, and one MM homozygote.

First, PON1 activity was significantly higher after than before training at all six points considered in QQ subjects, but conversely it was significantly lower in R carriers. This effect was not observed when stratifying by PON1-55 (except for the activity at 24 h in M carriers). Second, basal PON1 activity level recovery 24 h after the bout of exercise was different depending on the PON1-192 genotype. QQ subjects recovered the basal level 24 h after the bout of exercise, regardless of the training status, whereas R carriers reached it only after, but not before, training. To test for interactions among the three factors (training, acute exercise, and PON1-192 genotype) a MANOVA for repeated measures was carried out (**Table 3**). As expected, PON1-192 genotype had a significant effect on PON1 activity. A clear effect of a bout of exercise on PON1 activity was also observed; this effect differed according to the training level and the genotype. The effect of training was also different according to the genotype. The interaction among training, the bout of exercise, and genotype on PON1 activity was also tested and was not statistically significant ($P = 0.091$).

There was no correlation between HDL-C levels and PON1 activity either in the entire study group or in subjects stratified by genotype at any time point.

Mean basal point oxidized LDL concentration was lower after than before training. Training status was associated to an increase in oxidized LDL levels in the 2 h following the bout of exercise (**Fig. 4**). Before training, a non-significant increase of oxidized LDL just after the bout of exercise was observed.

TABLE 2. Physical fitness variables, body mass index, lipid profile, PON1 activity, and oxidized LDL concentration before and after training basal values, and percentage of change

	Before Training	After Training	Mean Change (%)	*P*
Physical fitness				
VO_{2max} (ml/kg)	37.4 (7.7)	46.2 (11.0)	23.6	<0.001
VT (% VO_{2max})	63.4 (13.4)	77.0 (8.5)	56.6	<0.001
APO (Watts)	131.2 (47.0)	159.4 (63.8)	22.7	0.009
MPO (Watts)	206.2 (55.1)	220.3 (72.0)	5.7	0.057
BMI (kg/m^2)	23.51 (2.1)	23.47 (2.5)	−0.3	0.875
Lipids				
TC (mmol/l)	4.41 (0.73)	4.08 (0.84)	−12.7	0.125
HDL-C (mmol/l)	1.25 (0.36)	1.31 (0.34)	2.3	0.390
LDL-C (mmol/l)	2.54 (0.67)	2.22 (0.81)	−12.3	0.177
TG (mmol/l)	1.39 (0.97)	1.11 (0.35)	−13.5	0.971
PON1 activity (U/l)				
Overall	258 (135)	253 (109)	2.1	0.489
QQ	169 (27)	186 (27)	10.2	<0.001
R carriers	385 (125)	348 (111)	−9.5	0.006
LL	333 (184)	315 (150)	−5.4	0.302
M carriers	218 (85)	219 (63)	0.4	0.880
Oxidized LDL (U/l)				
Overall	48.8 (16.2)	41.1 (10.6)	−15.9	0.043
QQ	45.2 (17.3)	38.7 (12.8)	−14.4	0.242
R carriers	53.9 (14.2)	44.5 (5.2)	−17.6	0.086
LL	55.9 (13.6)	41.5 (8.4)	−25.7	0.018
M carriers	44.9 (16.7)	40.8 (11.9)	−9.2	0.403

Values are mean (SD). VO_{2max}, maximal oxygen uptake; VT, ventilatory anaerobic threshold; APO, aerobic power output; MPO, maximum power output; BMI, body mass index; TC, serum total cholesterol; HDL-C, serum HDL-cholesterol; LDL-C, serum LDL-cholesterol; TG, serum triglycerides.

P values are for comparisons between after and before training.

DISCUSSION

This study assessed the effects of a single bout of regular exercise on PON1 activity for the first time in humans. The different effects of the two types of exercise on PON1 activity according to genotypes have also been addressed here.

The present experimental study showed that training was not associated with increased basal PON1 activity levels in the overall study subjects. A previous report of a cross-sectional study showed significantly increased PON1 activity values in well-trained rugby players compared with sedentary controls (26). However, the different design and participant characteristics make it difficult to compare the two studies.

In our study, an increase in PON1 activity immediately after the bout of exercise was followed by a decrease in the following 2 h and a recovery at 24 h. One study performed

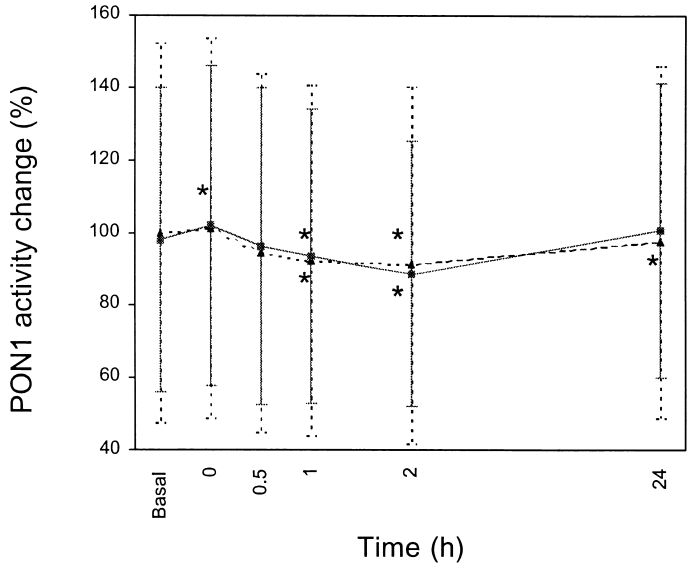

Fig. 2. Percentage of change in paraoxonase1 (PON1) activity before (dashed line) and after (solid line) training of the study subjects with respect to basal mean value before training. Bars indicate standard deviation. $*P < 0.05$ with respect to basal value.

in rats showed a similar effect: a single bout of PA inhibited serum PON1 activity in rats (27). An increase in oxidized LDL just after and during the 2 h following the bout of exercise was also found. These data support the idea that acute exercise induces oxidative stress by increasing lipid peroxidation which, in turn, decreases PON1 activity (22, 28).

The trained status was associated with a significant increase in PON1 activity just after the bout of exercise and a recovery of basal PON1 activity at 24 h. This finding suggests that training attenuates the effect of a bout of exercise on PON1 activity. It remains unclear whether the effect of exercise training on PON1 activity response to a bout of PA goes through direct or indirect mechanisms. A bout of exercise produced an increase in certain antioxidant gene transcription and a reduction in lipoperoxidation in trained rats (29, 30), but these effects were not observed in untrained specimens (31). In humans, exercise training appears to enhance antioxidant systems (22, 32, 33) and reduce lipid peroxidation (22), improving plasma oxidant status (34). The decrease in oxidized LDL levels after training observed in the present study is in accordance with that notion. Oxidized LDL levels were somewhat higher in the trained status after the bout of exercise than in the basal point. This supports the idea that the protection given by exercise training may not be sufficient

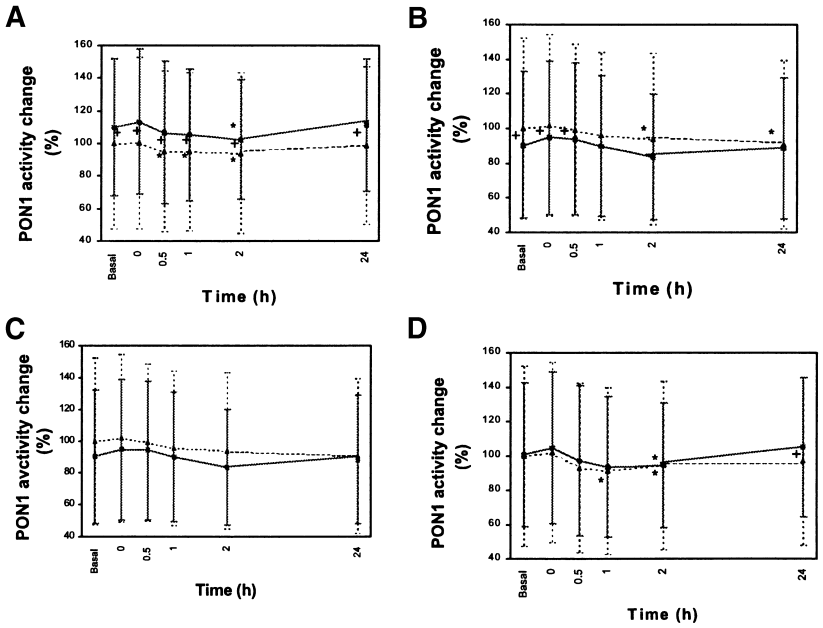

Fig. 3. Percentage of change in PON1 activity before (dashed line) and after (solid line) training of the (A) QQ subjects, (B) R carriers, (C) LL subjects, and (D) M carriers with respect to basal mean value before training. Bars indicate standard deviation. $^{+}P < 0.05$ after versus before training. $*P < 0.05$ with respect to basal value.

TABLE 3. MANOVA for repeated measures for PON1 activity

Factors and Interactions	*F*	*P*
Training	0.643	0.440
Bout	16.060	0.001
PON1-192 genotype	17.771	0.001
Training*PON1-192 genotype	27.304	<0.001
Bout*PON1-192 genotype	8.157	0.008
Training*Bout	5.538	0.022

P values are for *F* value significance.

to completely protect highly fit individuals against oxidative stress resulting from a bout of exercise (33). The global antioxidant activity, enhanced by exercise training, might indirectly mitigate the inhibition of PON1 caused by acute exercise-induced oxidative stress, and, as a result, PON1 activity may recover basal levels faster in trained status. Besides these effects, exercise training may also exert a direct action on the PON1 protein or PON1 carrier lipoprotein. PON1 enzyme is mainly linked to the HDL particle. However, no correlation was found between HDL-C levels and PON1 activity either in all subjects or in genotype groups at any time point. Therefore, it is unlikely that HDL-C changes explain changes in PON1 activity.

A major finding of this study is that the effects of a bout of exercise and of exercise training on PON1 activity differed depending on the PON1-192 genotype. There was an opposite trend between QQ and R carriers genotype in PON1 activity changes after training, not only at basal status, but at almost all time points considered after the bout of exercise. These changes were not statistically significant when analyzing by PON1-55 genotype, except for M carriers 24 h after the bout of exercise. Furthermore, before training only QQ subjects recovered basal PON1 activity at 24 h, whereas after training all subjects did, regardless of the PON1-55 genotype. Subjects of both PON1-55 genotypes reached basal PON1 activity levels 24 h after the bout whatever the training status was.

All these findings suggest that the response of PON1 activity to a bout of exercise was modulated by the previous training status and also by the PON1-192 genotype. In agreement with the notion that the PON1-192 polymorphism may influence the effects of PA, we previously found that PA was associated with a better lipid profile only in R carriers, whereas no association was observed in QQ subjects (35). However, at present it is difficult to explain the opposite effects of training on PON1 activity depending on PON1-192 genotype. The extent to which PON1 is inhibited by oxidized LDL lipids (28) seems to differ between the PON1-192 allozymes. Most of the studies (15) suggest that Q allozyme provides the best protection against oxidation in vitro, and its PON1 activity is less reduced in highly oxidative conditions. Therefore, exercise training may not provide enough protection to R carrier subjects against the oxidative stress resulting from a bout of exercise. On the other hand, smoking has been associated with decreased serum PON1 activity (36) and increased lipid peroxide generation. We previously described an interaction between smoking and the QQ genotype for the risk on myocardial infarction (37). However, in the present study smoking was not a confounding factor, since it was not associated with PON1 activity or with genotype.

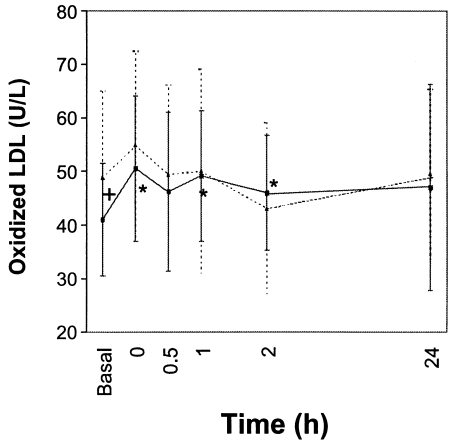

Fig. 4. Oxidized LDL before (dashed line) and after training (solid line). Bars indicate standard deviation. $^{+}P < 0.05$ after versus before training. $^{*}P < 0.05$ with respect to basal value.

Two hypotheses to explain the effect of differing genotype may be put forward. First, regular exercise produces repeated increases of free radicals after each exercise session which may act as a transcription inductor of endogenous antioxidant genes (30, 38) (PON1 or other antioxidant enzymes), but the greater susceptibility of the R allozyme to be inhibited would result in a decrease in this particular allozyme PON1 activity. And second, a linkage disequilibrium between the PON1-192 polymorphism and a polymorphism in the PON1 gene promoter or other region, which may modulate PON1 synthesis or activity and be more responsive to oxidative stress when linked to Q allele, cannot be ruled out. For instance, the A-162G polymorphism, located within a PON1 promoter region, which shows high homology to an interleukin-6 (IL-6)-responsive element, has recently been described to be in linkage disequilibrium with the PON1-192 (20). Acute exercise practice increases oxidized phospholipids, which in turn induce the secretion of the proinflammatory cytokine IL-6 leading to a decrease in PON1 activity and concentration in mice and HepG2 human cell (39). In this respect, the effect of IL-6 on PON1 expression might depend to a large extent on the polymorphism within IL-6-responsive element linked to PON1-192 polymorphism.

Little data about possible chronobiological and hormonal variations in PON1 activity is available at the moment. In this study, the two bouts of exercise performed by participants were carried out at the very same hour of the day. Hormone-replacement therapy increased serum arylesterase (40) and PON1 activity (Sentí M, unpublished data) in diabetic and non-diabetic postmenopausal women, respectively. In the same direction, estrogens have been shown to prevent vascular free radical production (41). In our study, no differences in PON1 activity or oxidized LDL according to sex were observed.

In summary, regular exercise was associated with an increase in PON1 activity in QQ subjects and with a de-

crease in R carriers. Increased PON1 activity immediately after a bout of exercise was subsequently followed by a decrease of activity. The recovery of the basal PON1 activity levels at 24 h was found in QQ subjects regardless of their training status and in trained R carriers, but not in untrained R carriers. Altogether, the results of the present study suggest that the effects of regular and acute exercise on PON1 activity levels are modulated by the PON1-192 polymorphism. The role of PON1-55 was much less pronounced.

The authors thank the Servei d'Activitat Física of the Universitat Autònoma de Barcelona and the participants for their help and cooperation. We thank Stephanie Lonsdale and Dave Macfarlane for their revision of the English manuscript. M.T. and M.F. were the recipients of grants from the Fondo de Investigaciones Sanitarias (99/9342 and 98/9562, respectively). This study was partially supported by grant Bayer 1999 of the Spanish Society of Cardiology.

Manuscript received 3 August 2001 and in revised form 21 January 2002.

REFERENCES

1. Powell, K. E., P. D. Thompson, C. J. Caspersen, and J. S. Kendrick. 1987. Physical activity and the incidence of coronary heart disease. *Annu. Rev. Public Health.* **8:** 253–287.
2. Bijnen, F. C., C. J. Caspersen, and W. L. Mosterd. 1994. Physical inactivity as a risk factor for coronary heart disease: a WHO and International Society and Federation of Cardiology position statement. *Bull. World Health Organ.* **72:** 1–4.
3. Marrugat, J., R. Elosua, M. I. Covas, L. Molina, and J. Rubies-Prat. 1996. Amount and intensity of physical activity, physical fitness, and serum lipids in men. The MARATHOM Investigators. *Am. J. Epidemiol.* **143:** 562–569.
4. American College of Sports Medicine. 1993. Physical activity, physical fitness and hypertension. *Med. Sci. Sports. Exerc.* **25:** i–x.
5. Helmrich, S. P., D. R. Ragland, R. W. Leung, and R. S. Paffenbarger. 1991. Physical activity and reduced occurrence of non-insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **325:** 147–152.
6. Ekelund, L. G., W. L. Haskell, J. L. Johnson, F. S. Whaley, M. H. Criqui, and D. S. Sheps. 1988. Physical fitness as a predictor of cardiovascular mortality in asymptomatic North American men. The Lipid Research Clinics Mortality Follow-up Study. *N. Engl. J. Med.* **319:** 1379–1384.
7. Witztum, J. L. 1994. The oxidation hypothesis of atherosclerosis. *Lancet.* **344:** 793–795.
8. Mackness, M. I. 1989. Possible medical significance of human serum paraoxonase. *In* Enzymes Hydrolysing Organophosporus Compounds. E. Reiner, W. N. Aldridge, and F. C. Hoskin, editors. Ellis-Horwood, UK. 202–213.
9. Blatter, M. C., R. W. James, S. Messmer, F. Barja, and D. Pometta. 1993. Identification of a distinct human high-density lipoprotein subspecies defined by a lipoprotein-associated protein, K-45. Identity of K-45 with paraoxonase. *Eur. J. Biochem.* **211:** 871–879.
10. Mackness, M. I., S. Arrol, and P. N. Durrington. 1991. Paraoxonase prevents accumulation of lipoperoxides in low- density lipoprotein. [published erratum appears in FEBS Lett 1991 Nov 4;292(1–2):307]. *FEBS Lett.* **286:** 152–154.
11. Mackness, M. I., S. Arrol, C. Abbott, and P. N. Durrington. 1993. Protection of low-density lipoprotein against oxidative modification by high- density lipoprotein associated paraoxonase. *Atherosclerosis.* **104:** 129–135.
12. Adkins, S., K. N. Gan, M. Mody, and B. N. La Du. 1993. Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 191, for the respective A or B allozymes. *Am. J. Hum. Genet.* **52:** 598–608.
13. Humbert, R., D. A. Adler, C. M. Disteche, C. Hassett, C. J. Omiecinski, and C. E. Furlong. 1993. The molecular basis of the human serum paraoxonase activity polymorphism. *Nat. Genet.* **3:** 73–76.
14. Mackness, B., M. I. Mackness, S. Arrol, W. Turkie, and P. N. Durrington. 1998. Effects of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. *FEBS Lett.* **423:** 57–60.
15. Aviram, M., E. Hardak, J. Vaya, S. Mahmood, S. Milo, A. Hoffman, S. Billicke, D. Draganov, and M. Rosenblat. 2000. Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. *Circulation.* **101:** 2510–2517.
16. Aviram, M., S. Billecke, R. Sorenson, C. Bisgaier, R. Newton, M. Rosenblat, J. Erogul, C. Hsu, C. Dunlop, and B. La Du. 1998. Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities: selective action of human paraoxonase allozymes Q and R. *Arterioscler. Thromb. Vasc. Biol.* **18:** 1617–1624.
17. Mackness, M. I., S. Arrol, B. Mackness, and P. N. Durrington. 1997. Alloenzymes of paraoxonase and effectiveness of high-density lipoproteins in protecting low-density lipoprotein against lipid peroxidation. [letter] *Lancet.* **349:** 851–852.
18. Leviev, I., F. Negro, and R. W. James. 1997. Two alleles of the human paraoxonase gene produce different amounts of mRNA. An explanation for differences in serum concentrations of paraoxonase associated with the (leu-Met54) polymorphism. *Arterioscler. Thromb. Vasc. Biol.* **17:** 2935–2939.
19. Garin, M. C., R. W. James, P. Dussoix, H. Blanche, P. Passa, P. Froguel, and J. Ruiz. 1997. Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. *J. Clin. Invest.* **99:** 62–66.
20. Brophy, V. H., R. L. Jampsa, J. B. Clendenning, L. A. McKinstry, G. P. Jarvik, and C. E. Furlong. 2001. Effects of 5′ Regulatory-Region Polymorphisms on Paraoxonase-Gene (PON1) Expression. *Am. J. Hum. Genet.* **68:** 1428–1436.
21. Leviev, I., S. Deakin, and R. W. James. 2001. Decreased stability of the M54 isoform of paraoxonase as a contributory factor to variations in human serum paraoxonase concentrations. *J. Lipid Res.* **42:** 528–535.
22. Clarkson, P. M. 1995. Antioxidants and physical performance. *Crit. Rev. Food Sci. Nutr.* **35:** 131–141.
23. Miller, S. A., D. D. Dykes, and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16:** 1215.
24. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.
25. Dill, D. B., and D. L. Costill. 1999. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J. Appl. Physiol.* **37:** 247–248.
26. Brites, F., M. Travacio, G. Gambino, J. Verona, S. Llesuy, and R. Wikinski. 2000.Regular exercise improves lipid and antioxidant profile. *Atherosclerosis.* **151:** 261.
27. Pawlowska, D., J. Moniuszko-Jakoniuk, and M. Soltys. 1985. Parathion-methyl effect on the activity of hydrolytic enzymes after single physical exercise in rats. *Pol. J. Pharmacol. Pharm.* **37:** 629–638.
28. Aviram, M., M. Rosenblat, S. Billecke, J. Erogul, R. Sorenson, C. L. Bisgaier, R. S. Newton, and B. La Du. 1999. Human serum paraoxonase (PON 1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. *Free Radic. Biol. Med.* **26**: 892–904.
29. Somani, S. M., and L. P. Rybak. 1996. Comparative effects of exercise training on transcription of antioxidant enzyme and the activity in old rat heart. *Indian J. Physiol. Pharmacol.* **40:** 205–212.
30. Sen, C. K., and L. Packer. 1996. Antioxidant and redox regulation of gene transcription. *FASEB J.* **10:** 709–720.
31. Alessio, H. M., and A. H. Goldfarb. 1988. Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. *J. Appl. Physiol.* **64:** 1333–1336.
32. Alessio, H. M., and E. R. Blasi. 1997. Physical activity as a natural antioxidant booster and its effect on a healthy life span. *Res. Q. Exerc. Sport.* **68:** 292–302.
33. Sen, C. K. 1995. Oxidants and antioxidants in exercise. *J. Appl. Physiol.* **79:** 675–686.
34. Brites, F. D., P. A. Evelson, M. G. Christiansen, M. F. Nicol, M. J. Ba-

silico, R. W. Wikinski, and S. F. Llesuy. 1999. Soccer players under regular training show oxidative stress but an improved plasma antioxidant status. *Clin. Sci. (Colch).* **96:** 381–385.
35. Senti, M., C. Aubo, R. Elosua, J. Sala, M. Tomas, and J. Marrugat. 2000. 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. *Genet. Epidemiol.* **18:** 276–286.
36. James, R. W., I. Leviev, and A. Righetti. 2000. Smoking is associated with reduced serum paraoxonase activity and concentration in patients with coronary artery disease. *Circulation.* **101:** 2252–2257.
37. Senti, M., C. Aubo, and M. Tomas. 2000. Differential effects of smoking on myocardial infarction risk according to the Gln/Arg 192 variants of the human paraoxonase gene. *Metabolism.* **49:** 557–559.
38. Sun, Y., and L. W. Oberley. 1996. Redox regulation of transcriptional activators. *Free Radic. Biol. Med.* **21:** 335–348.
39. Van Lenten, B. J., A. C. Wagner, M. Navab, and A. M. Fogelman. 2001. Oxidized phospholipids induce changes in hepatic paraoxonase and ApoJ but not monocyte chemoattractant protein-1 via interleukin-6. *J. Biol. Chem.* **276:** 1923–1929.
40. Sutherland, W. H., P. J. Manning, S. A. de Jong, A. R. Allum, S. D. Jones, and S. M. Williams. 2001. Hormone-replacement therapy increases serum paraoxonase arylesterase activity in diabetic postmenopausal women. *Metabolism.* **50:** 319–324.
41. Wassmann, S., A. T. Baumer, K. Strehlow, M. van Eickels, C. Grohe, K. Ahlbory, R. Rosen, M. Bohm, and G. Nickenig. 2001. Endothelial dysfunction and oxidative stress during estrogen deficiency in spontaneously hypertensive rats. *Circulation.* **103:** 435–441.