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Denis Blache
University of Bourgogne

Suzanne Lussier-Cacan
Clinical Research Institute of Montreal

Jacques Gagnon
University of Moncton, Shippagan

Arthur S. Leon
University of Minnesota - Twin Cities

D.C. Rao
Washington University School of Medicine in St. Louis

See next page for additional authors

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Effect of Exercise Training on *In Vitro* LDL Oxidation and Free Radical–Induced Hemolysis: The HERITAGE Family Study

DENIS BLACHE,[1] SUZANNE LUSSIER–CACAN,[2] JACQUES GAGNON,[3] ARTHUR S. LEON,[4] D.C. RAO,[5] JAMES S. SKINNER,[6] JACK H. WILMORE,[7] TUOMO RANKINEN,[8] CLAUDE BOUCHARD,[8] and JEAN DAVIGNON[2]

ABSTRACT

Oxidant stress and overproduction of reactive oxygen species (ROS) contribute to the development of cardiovascular disease. Oxidative modifications of low-density lipoproteins (LDL) are thought to play an early and critical role in atherogenesis. LDL oxidation can be reproduced *in vitro*, but results usually show a large interindividual variation not entirely explained by the environment. Free radical-induced hemolysis is also proposed to reveal the overall antioxidant capacity. The roles of genetic factors and exercise on the variability of both measures were investigated. The study was conducted in 146 healthy individuals from 28 families participating in a 20-week exercise-training program. In addition to important biological and environmental influences on variation, significant familial aggregation was detected in all oxidation measures. Exercise did not significantly modify the LDL oxidation parameters, but significantly increased resistance was observed in the free radical-induced hemolysis, especially in women, this effect was not observed in smokers. In total, the findings suggest the presence of familial effects in the response to *ex vivo* oxidation. Further, smoking negates the beneficial effect of exercise training on erythrocyte resistance to free radical-induced hemolysis. These observations emphasize the importance of context in the evaluation of exercise and oxidant stress. *Antioxid. Redox Signal.* 8, 123–130.

INTRODUCTION

Experimental and clinical studies have documented the role of reactive oxygen species (ROS) in various pathologies, including cancer and cardiovascular disease (24). In particular, the oxidative modification of low-density lipoprotein (LDL) has received much attention because of its suggested participation in the early events of atherogenesis (2, 52). Recent documentation of the involvement of oxidized forms of LDL in inflammatory processes adds another perspective to the importance of oxidized LDL in atherogenesis (34). Furthermore, there is evidence in humans that circulating oxidized LDL correlates with severity of atherosclerosis and contributes to plaque instability (18, 36).

Although the precise mechanisms for *in vivo* LDL oxidation are unknown, several attempts have been made to determine the degree and significance of this phenomenon. One method, the susceptibility of LDL to copper-initiated oxidation (7, 21), has been abundantly used as a marker for enhanced sensitivity to atherosclerosis, although its relevance to

[1]INSERM U 498, Biochimie des Lipoprotéines et Interactions Vasculaires, and Faculté de Médecine, Université de Bourgogne, Dijon, France.
[2]Hyperlipidemia and Atherosclerosis Research Group, Clinical Research Institute of Montreal, Montreal, Quebec, Canada.
[3]Coastal Zones Research Institute, University of Moncton, Shippagan, New Brunswick, Canada.
[4]School of Kinesiology, University of Minnesota, Minneapolis, Minnesota.
[5]Division of Biostatistics, and Departments of Genetics and Psychiatry, Washington University School of Medicine, St Louis, Missouri.
[6]Department of Kinesiology, Indiana University, Bloomington, Indiana.
[7]Department of Kinesiology and Health Education, The University of Texas at Austin, Austin, Texas.
[8]Human Genomics Laboratory, Pennington Biomedical Research Center, Louisiana State University System, Baton Rouge, Louisiana.

in vivo atherogenesis has not been clearly established (13, 26, 44). In addition to LDL *in vitro* oxidation, another measure of overall antioxidant defense based on the susceptibility of erythrocytes to free radical-induced hemolysis has been set up and used under various clinical and experimental conditions (5, 22, 32).

In addition to the impact of pathologies, one of the difficulties associated with *in vitro* oxidation methodology is the large interindividual variation in response that is observed and that can be explained only partially by environmental factors, such as diet or the endogenous antioxidant concentrations (20). Further, the role of genetic factors in the determination of this variability is largely unknown (27, 51). Concurrently, although the influence of acute aerobic exercise on markers of oxidant stress and lipid peroxidation is believed to depend on its intensity and duration (28, 42, 50), the impact of exercise training on the association between the usual determinants of response and the oxidation markers is unknown. The objective of the current study was to search, in a first step, for indications of a familial component to the interindividual variability of the susceptibility or resistance of LDL and erythrocytes to *in vitro* oxidation in healthy women and men and, second, to evaluate the influence of an exercise-training program on these measures.

METHODS

Sample

This satellite study includes members of 28 two-generation nuclear families (both biological parents and at least three biological children), all Caucasians and of French–Canadian descent, recruited in Quebec City as part of the HERITAGE Family Study. The overall objective of this multicenter project was to investigate the effects of regular exercise on several cardiovascular disease and diabetes risk factors and to determine the role of the genotype in cardiovascular, metabolic, and hormonal responses to endurance exercise training. The specific aims, design, recruitment criteria, and measurement protocols of this study have been reported previously (8, 38). The Quebec City subjects were healthy, sedentary, and capable of undertaking and completing an exercise-training program. The participants were required to be between ages 16–65 years (16–40 years for children, and ≤65 years for parents). They were submitted to extensive physical and biological evaluation both before and after the physical training period. Consequently, volunteers acted as their own controls.

Exercise training program

The training protocol is thoroughly outlined in Bouchard *et al*. (8). Briefly, each individual trained on a cycle ergometer in the laboratory under supervision three times a week for 20 weeks. The intensity and duration of exercise were adjusted for each individual every 2 weeks so that each participant was working at a heart rate associated with 75% of their maximum baseline oxygen uptake for 50 min at the last 6 weeks of training. The power output was adjusted automatically in the cycle ergometers to meet the designed heart rate response to exercise training. All training sessions were supervised on site, and adherence to the protocol was strictly monitored (1).

Blood sampling and measures

Fasting blood samples were drawn at the Laval University Medical Center in Quebec City and prepared according to a standard protocol. For women, samples were obtained during the early follicular phase. Plasma lipid, lipoprotein, apolipoprotein B concentrations, and LDL particle size were measured in the lipid core laboratory at the Lipid Research Center by standard procedures as described (38, 45). Routine biochemistry was performed at the Medical Center Central Laboratory. For the lipid oxidation study, plasma and blood cells were separated by centrifugation at 4°C, cells were washed with saline (NaCl 0.15 *M*) and the samples were shipped within 2 h of drawing to the Hyperlipidemia and Atherosclerosis Research Laboratory of the Clinical Research Institute of Montreal. Immediately upon reception of the samples, the plasma and the blood cells were processed as described below.

Lipid oxidation study

LDL was isolated by sequential ultracentrifugation of plasma between densities 1.019 and 1.063 g/mL, dialyzed, and kept under nitrogen at 4°C in the dark until analysis (39). After protein measurement, with bovine albumin as the standard (29), LDL was filtered through a disposable 14-cm desalting column (Econo-Pac 10 DG, Bio-Rad, Mississauga, Ontario, Canada) to remove the ethylenediamine tetraacetate (EDTA) just before the oxidation experiments (within 3 days of ultracentrifugation). EDTA-free LDL (100 μg protein/mL PBS) was oxidized with 2 μmol/L $CuSO_4$. Monitoring of the production of conjugated dienes and determination of the lag time and propagation rate were done essentially as described (39). The intra-assay coefficients of variation (CVs) were determined from triplicate analyses of five samples performed on five different days and found to be 5.3 ± 2.9% for lag time and 6.0 ± 3.9% for propagation rate. The between-assay CVs for lag time and propagation rate were 5.2% and 9.1%, respectively.

The *in vitro* resistance of intact red blood cells to oxidation was evaluated with 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH, Spiral, Dijon, France), a free radical generator. Isolated blood cells were prepared as described previously (32) and incubated with increasing AAPH concentrations (40–260 mmol/L) at 37°C for 150 min. After centrifugation, the absorbance of the supernatant fluid (index of hemolysis) was measured at 405 nm with a microplate reader (Bio-Tek Instruments, Inc., Burlington, VT). The AAPH concentration corresponding to 50% hemolysis (C_{50}-AAPH in mmol/L) was evaluated with Prism software (GraphPad Inc., San Diego, CA) and is interpreted as the erythrocyte resistance to free radical attack. The intra- and between-assay CVs are 2.2% (n = 6) and 5.4% (n = 7), respectively. Total plasma thiobarbituric acid-reactive substances (TBARS) were measured with a spectrophotometric assay (32); results are expressed as nanomoles per liter of malondialdehyde (MDA) equivalents per milliliter of plasma

after use of the molar extinction coefficient for MDA of $\epsilon_{234\ nm} = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Fatty acid analysis

After extraction with chloroform/methanol (2:1, vol/vol), the thin-layer-chromatography-isolated plasma phospholipids (PL) were transmethylated with 14% boron trifluoride in methanol at 100°C for 90 min under a N_2 atmosphere in accordance with the protocol of Morrison and Smith (35). The fatty acid-methyl esters were injected into a gas chromatograph (HP-6890; Hewlett–Packard, Palo Alto, CA) equipped with a 0.25 mm × 30 m Innowax capillary column (Hewlett–Packard, Mississauga, Ont.) and a flame ionization detector, with helium as the carrier gas. Calibration and peak identification were performed using reference fatty acids (Nu-Chek) and results are expressed as percentages of the sum of all identified peaks.

Antioxidants and aminothiols

Plasma α-tocopherol, β-carotene, and retinol concentrations were determined by HPLC (3, 46). Concentrations of plasma total homocysteine (tHcy), cysteine, cysteinyl glycine, and glutathione (oxidized and reduced forms: GSSG + GSH) were also measured by HPLC as described (17). Erythrocyte glutathione peroxidase (GSH-Px) activity was determined by a spectrophotometric assay, with the use of a commercial kit (Ransel, Randox Laboratories, Mississauga, Ontario, Canada); results are expressed as Units per gram of hemoglobin.

Statistical analyses

Descriptive analyses were carried out, either separately in the four subgroups (fathers, mothers, sons, and daughters), or by generation or gender, using the SAS software (SAS Institute Inc, Cary, NC). The homogeneity of each variable mean among subgroups was tested by analysis of variance (ANOVA) (43). The contribution of each trait (independent variables) to the prediction of variability of measures of oxidation (dependent variables) was evaluated by simple regression analysis: kinetics of LDL oxidation (lag time and propagation rate), erythrocyte resistance to hemolysis following free radical attack, and plasma TBARS. The predictors of measures of oxidation were first identified by considering each independent trait separately to establish whether it made a moderately significant ($p \leq 0.10$) contribution to variability in at least two of the four subgroups. In a second step, a backward stepwise regression analysis was carried out for each measure of oxidation on the pooled sample, to select the combination of the independent traits identified in step 1 that predicted significant amounts of variation. The threshold criterion for exclusion from the regression model was taken to be $p > 0.10$. Next, the contribution of familial aggregation to the variation in each measure of oxidation was estimated using ANOVA and variance components-based methods. The ANOVA model was used to derive an F-ratio, which compares the between-family with the within-family variances and thereby provides an estimate of the familial resemblance. A variance components-based method as implemented in the QTDT software package was used to estimate the phenotypic variance due to polygenic and nonshared environmental factors (Center for Statistical Genetics, http://www.sph.umich.edu/esg/abecasis/QTDT/). The polygenic variance reflects both genetic and shared environmental sources of variance. Maximal heritability of the trait was expressed as the proportion of total variance explained by the polygenic variance. Paired *t* tests were used to assess differences between variables measured before and after the exercise-training program. Correlations are reported as Pearson's *r* coefficients.

RESULTS

The baseline biological characteristics of the participants are presented by family membership in Table 1. The ages varied from 43 to 62 and from 17 to 36 years in parents and children, respectively. Parents had significantly higher values than their children for biological measurements ($p < 0.001$), except for serum albumin, which was higher in children ($p < 0.05$) and serum uric acid and high density lipoprotein (HDL) cholesterol,

TABLE 1. BASELINE BIOLOGICAL CHARACTERISTICS OF PARTICIPANTS BY FAMILY MEMBERSHIP

*Variables**	*Fathers*	*Mothers*	*Sons*	*Daughters*	*p*†
Number of subjects	28	25	40	53	
Age (years)	51.2 ± 4.1	49.8 ± 3.2	21.7 ± 4.0	22.5 ± 4.9	0.0001
BMI (kg/m²)	26.9 ± 3.6	26.2 ± 3.4	24.1 ± 4.1	22.2 ± 3.7	0.0001
Serum glucose (mmol/L)	4.99 ± 0.58	4.83 ± 0.48	4.67 ± 0.40	4.53 ± 0.36	0.0001
Serum albumin (g/L)	43.9 ± 2.9	41.0 ± 3.3	45.7 ± 3.6	42.5 ± 3.5	0.0001
Serum uric acid (μmol/L)	358 ± 65	255 ± 48	344 ± 55	246 ± 49	0.0001
Serum creatinine (μmol/L)	89 ± 10	69 ± 7	84 ± 9	72 ± 8	0.0001
LDL					
Cholesterol (mmol/L)	3.70 ± 1.01	3.28 ± 0.77	2.57 ± 0.61	2.67 ± 0.77	0.0001
apoB (mg/dL)	97 ± 24	84 ± 22	68 ± 16	69 ± 21	0.0001
Particle size (nm)	25.84 ± 0.88	25.97 ± 0.76	26.05 ± 0.72	26.01 ± 0.82	NS
HDL⁻cholesterol	1.02 ± 0.19	1.27 ± 0.26	1.01 ± 0.18	1.18 ± 0.26	0.0001

*Values are means ± S.D.

†Significance of the differences among subgroups by ANOVA.

which did not differ significantly between generations. Male subjects (fathers and sons together) had higher values ($p < 0.05$) than females (mothers and daughters) for every biological variable except HDL cholesterol ($p < 0.001$). No significant differences in LDL particle size were noted among subgroups.

Table 2 shows the lipid oxidation parameters, as well as plasma aminothiols and antioxidants in the four subgroups at baseline. No significant differences among groups were found for the measures of LDL or erythrocyte oxidation and plasma TBARS concentrations. However, there was a gender difference for plasma tHcy, men having higher values than women ($p < 0.001$), whereas plasma glutathione was higher in children than in parents ($p < 0.05$). Plasma α-tocopherol, β-carotene ($p < 0.001$), and retinol levels ($p < 0.005$) were found to be higher in parents than in children, as was the activity of erythrocyte GSH-Px ($p < 0.05$). This enzyme was higher in women than in men ($p < 0.005$). The plasma PL fatty acid profile was determined in subjects for whom LDL oxidation data were available for use in regression analyses (data not shown).

Among the 41 independent variables (physical and biochemical measures) included in the simple regression analysis, the significant predictors were entered into a backward stepwise regression analysis of the pooled sample. The significant positive and negative influences were LDL cholesterol (-), plasma α-tocopherol (+), serum uric acid (+) and plasma PL oleic acid (+) and they contributed 31.6% (adjusted $R^2 \times 100$; $p < 0.001$) of the variance in LDL oxidation *lag time*. Plasma glutathione (+) and PL eicosapentaenoic acid (EPA) (−) (7.8%; $p < 0.01$) were retained in the model for *propagation rate*. Plasma glutathione (+) and plasma α-tocopherol (+) were respectively significant predictors for *hemolysis* (6.8%; $p < 0.01$) and for *TBARS* (2.1%; $p < 0.05$). As shown in Table 3, all four *in vitro* oxidation parameters were characterized by significant familial aggregation. The baseline traits showed 2–3 times more variance between families than within families, and maximal heritability estimates ranged from 30.8% to 42.5%.

The influence of 20 weeks of exercise training on measures of oxidation is shown in Table 4. In both women and men, LDL oxidation changes were characterized by a large interindividual variability and were not significantly different. In this group of 71 subjects, 4 men and 6 women reported cigarette smoking. Their exclusion did not have any impact on the results. Conversely, the rate of resistance to hemolysis increased significantly with training in women but not in men, while plasma TBARS concentrations did not change significantly (Table 4). Whereas exercise training induced several significant changes in biological markers, evaluation of the changes in resistance to hemolysis showed a gender-dependent pattern in the correlates of this parameter (Table 5). In women, the change in plasma α-tocopherol relative to baseline was the only significant correlate of hemolysis change, whereas in men, HDL cholesterol (+) and erythrocyte GSH-

Table 2. Lipid Oxidation Parameters, Plasma Aminothiol Concentrations, and Plasma or Erythrocyte Antioxidants by Family Membership at Baseline

*Variables**	*Fathers*	*Mothers*	*Sons*	*Daughters*	*p*†
LDL oxidation					
Lag time (min)	72 ± 26 (20)‡	64 ± 29 (20)	70 ± 23 (30)	62 ± 24 (36)	NS
Propagation rate (nmol/mg LDL protein/min)	8.5 ± 2.5 (20)	9.4 ± 3.4 (20)	9.0 ± 2.7 (30)	8.7 ± 2.1 (36)	NS
Erythrocyte resistance to hemolysis					
C_{50}-AAPH (mmol/L)	74.8 ± 13.9 (25)	81.6 ± 17.7 (24)	75.0 ± 12.6 (38)	74.7 ± 13.8 (45)	NS
Plasma *TBARS* (nmol MDA/mL)	1.93 ± 0.55 (28)	1.90 ± 0.70 (25)	1.77 ± 0.62 (40)	2.09 ± 0.89 (53)	NS
Plasma aminothiols (μmol/L)					
Total homocysteine (tHcy)	9.4 ± 2.1 (28)	7.8 ± 2.6 (25)	8.9 ± 3.7 (40)	7.7 ± 3.7 (53)	0.1100
Total glutathione (GSSG + GSH)	4.1 ± 1.2 (18)	4.3 ± 1.5 (25)	5.1 ± 1.7 (40)	4.4 ± 1.4 (53)	0.0285
Plasma antioxidant vitamins (μg/L)					
α-Tocopherol ($\times 10^3$)	13.6 ± 4.9 (28)	13.1 ± 5.1 (25)	8.1 ± 2.3 (40)	8.85 ± 2.1 (53)	0.0001
β-Carotene	187 ± 89 (28)	255 ± 138 (25)	128 ± 71 (40)	161 ± 100 (53)	0.0001
Retinol	655 ± 114 (28)	550 ± 110 (25)	552 ± 125 (40)	512 ± 138 (53)	0.0001
Erythrocyte antioxidant enzyme					
Glutathione peroxidase (GSH-Px) (U/g hemoglobin)	59.6 ± 15.9 (24)	61.9 ± 16.3 (24)	50.3 ± 12.8 (39)	58.6 ± 15.3 (45)	0.0107

*Values are means ± SD; †significance of the differences among subgroups by ANOVA; ‡Number of samples tested.

TABLE 3. FAMILIAL AGGREGATION OF THE OXIDATION PARAMETERS IN THE SEDENTARY STATE (BASELINE) AND THEIR RESPONSES TO ENDURANCE TRAINING (RESPONSE)

	ANOVA		
	F	*p value*	h^2
LDL oxidation			
Lag time			
Baseline (106)	3.12	<0.0001	0.380
response (69)	2.97	0.001	0.841
Propagation			
Baseline (106)	2.02	0.009	0.308
Response (70)	2.12	0.0147	0.444
Erythrocyte resistance to hemolysis			
Baseline (132)	3.06	<0.0001	0.360
Response (106)	2.08	0.0081	0.354
Plasma TBARS			
Baseline (142)	3.19	<0.0001	0.425
Response (137)	3.15	<0.0001	0.514
Plasma glutathione			
Baseline (145)	2.56	0.0003	0.444
Response (142)	3.20	<0.0001	0.460
RBC GSH-Px			
Baseline (131)	6.09	<0.0001	1.000
Response (109)	1.04	0.424	0.002

F-value is the ratio of between-family and within-family variances from the ANOVA model. Maximal heritability estimate (h^2) is derived from variance components model and reflects the proportion of total variance due to polygenic variance.

Px (-) differences showed the strongest associations with predominance of the glutathione system.

Further scrutiny of results uncovered the influence of smoking in the response to exercise (Fig. 1). While erythrocyte resistance to hemolysis increased in women nonsmokers, a decrease was observed in those who smoked. A similar pattern was observed in men, although changes in nonsmokers were at the limit of statistical significance.

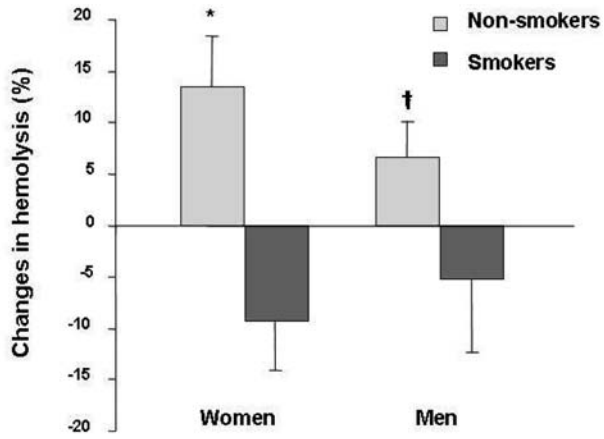

FIG. 1. Influences of smoking on changes in hemolysis rate after 20 weeks of training. Data are expressed as mean differences in percentages (Δ%) ± SEM by gender. Numbers are: 42 women nonsmokers and 12 smokers; 45 men nonsmokers and 7 smokers. *$p = 0.0001$; †$p = 0.0591$

DISCUSSION

One of our goals in this study was to search for indications of genetic contribution to the widely observed interindividual variation in measures of *ex vivo* antioxidant defense, as assessed by LDL oxidation and free radical-induced erythrocyte hemolysis. Our first step to this end was to identify biological and environmental influences and to evaluate their capacity to predict trait variability in the lipid oxidation measures. We were successful in explaining a significant portion of LDL oxidation variability and our findings were compatible with those in other reports and were biologically relevant (12, 16, 47).

Apart from LDL components, several factors (some in the circulation, others in blood cells) are involved in the promotion or inhibition of oxidation *in vivo* (25, 37). Our study provided quantitative data on a large number of biological variables (see Results), including thiols, uric acid, albumin, and erythrocyte glutathione peroxidase activity. The latter was not correlated with the oxidation parameters at baseline; however, plasma glutathione levels made a significant contribution to variability in oxidation for both LDL and erythrocytes. Similarly, plasma retinol and β-carotene levels were also unexpectedly not correlated with oxidation parameters, although a recent study has reported that serum retinol and β-carotene are mostly under genetic control in healthy families (23).

TABLE 4. EFFECT OF 20 WEEKS OF EXERCISE TRAINING ON OXIDATION PARAMETERS*

	LDL oxidation			*Hemolysis*		*TBARS*	
	N	*Lag time (min)*	*Porpagation rate (nmol/mg LDL prot./min)*	*n*	*C^{50}-AAPH†(mmol/L)*	*n*	*nmol MDA/mL‡*
Women	35	3.5 ± 39.5	0.7 ± 4.9	54	4.9 ± 16.7§	78	−0.13 ± 1.0
Men	36	8.8 ± 38.7	−0.2 ± 2.8	52	2.3 ± 15.4	68	0.05 ± 0.8

*Numbers are mean differences from baseline in absolute values ± SD.
†Concentration of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) corresponding to 50% hemolysis.
‡MDA: malondialdehyde equivalents.
§Significance of the difference from baseline: $p < 0.05$.

TABLE 5. CHANGES IN BIOLOGICAL MARKERS AND CORRELATES OF HEMOLYSIS RESISTANCE AFTER 20 WEEKS OF EXERCISE TRAINING

	Women (n = 54)				*Men (n = 52)*			
Variables	Δ% *	*p*	*r* †	*p*	Δ % *	*p*	*r* †	*p*
Uric acid	3.4	0.0944	−0.21	NS	5.5	0.0120	−0.23	0.0937
Albumin	−3.6	0.0005	0.04	NS	−2.4	0.0245	−0.05	NS
Plasma total glutathione	9.4	0.0335	−0.04	NS	18.1	0.0135	0.28	0.0432
Plasma α-tocopherol	8.2	0.0179	0.48	0.0002	4.1	NS	0.03	NS
RBC glutathione peroxidase (GSH-Px)	−0.6	NS	−0.14	NS	−0.3	NS	−0.40	0.0033
HDL cholesterol	5.0	0.0146	0.01	NS	4.1	0.0031	0.50	0.0002
Maximal oxygen uptake	17.6	0.0001	−0.13	NS	13.6	0.0001	−0.04	NS

*Mean percent differences (Δ%) from baseline.
†Pearson correlation coefficients of changes.

Cigarette smoking, which is recognized as an important risk factor for cardiovascular disease and is known to contribute to oxidative stress (4, and reviewed in Ref. 15), did not have a significant impact on LDL oxidation measures in our sample. The generally expected negative effect on the resistance of LDL to oxidative stress has not been consistently observed in healthy individuals (49).

Ours is the first study to have evaluated the interindividual variability in erythrocyte response to free radical attack. We found an association of plasma glutathione with the hemolysis rate, both as a predictor of variability at baseline and as a correlate of change (Table 5). Other elements, not measured in this study, are directly or indirectly involved in hemolytic reactions and may play an important role in the reaction to oxidative stress (25, 33, 37).

All the measures of oxidation showed significant familial aggregation in the sedentary state. This observation could in part be due to shared environmental factors and could also underscore the influence of genetic effects. A Finnish study of 15 families, including parents and male twins 16–18 years of age, has considered the concept of "Familial aggregation" in the response of LDL to oxidation (27). The authors concluded that inherited factors contribute to interindividual variability in the oxidative modification of LDL. In another study, total plasma antioxidant activity (TAS) was determined in 1,337 members of 40 families (Mexican Americans in the San Antonio Family Heart Study) (51). TAS levels were found to be under genetic control: the additive effects of genes explained around 51% of the phenotypic variance in TAS, after adjustment for several biological and environmental factors. However, cellular antioxidants are not taken into consideration in the TAS procedure, which essentially accounts for circulating antioxidants mainly ascribed to albumin (reviewed in Ref. 9). Despite differences in methodology, the above results add weight to our own observations and suggest genetic involvement in the control of the redox system.

In our study, we observed no significant exercise training-induced changes in the response of LDL to copper-induced oxidation, in the presence of a persistent, large interindividual variability. The influence of exercise on oxidative stress has been abundantly studied (11, 19, 42, 48). After a 16-week training period, Elosua *et al.* (19) observed a significant decrease in LDL oxidation susceptibility with no change in α-tocopherol, whereas in our conditions, we found significant increases in plasma tocopherol in men and women (Table 5). These divergent results might be explained by different protocols and measures. Conversely, we found that the resistance to hemolysis was improved by regular exercise, only in women, and was associated with markers of positive response in a gender-dependent manner. Increase in HDL cholesterol is a known benefit from endurance training and has been reported elsewhere in the context of the HERITAGE Family Study (14). It is noteworthy that the increase in HDL cholesterol, which was significant in both women and men, was associated with hemolysis resistance only in men. In women, the observed relative increase in vitamin E could be due to mobilization from tissue storage to plasma circulation, as there was no indication of increased intake.

The negative correlation between changes in the resistance to hemolysis and changes in GSH-Px noted in men may appear surprising. It should be emphasized, however, that the mean activity of GSH-Px did not change significantly during the study in either women or men. Further, it is possible that the observed increase in plasma glutathione (total), indicative of an increased physical activity represents an increase in oxidized glutathione (GSSG) more than in the reduced form (GSH). The thiol redox cycle is part of an important antioxidant defense system that includes several other agents, such as vitamins E and C (41). We suggest that the exercise-stimulated interaction of these agents was gender dependent in this study. Such a biological phenomenon has been documented in several situations (30, 31, 40), but has not heretofore been reported in the evaluation of response to exercise and oxidant stress. This aspect warrants further evaluation.

Smoking negatively affected erythrocyte resistance to oxidation after exercise training. While erythrocytes in smokers have been found to be more susceptible to *in vitro* peroxidation, probably because of lower vitamin content compared to nonsmokers (6, 10), the current study demonstrates that smoking counteracts the beneficial effect of exercise in the response to oxidant stress (Fig. 1). The increased resistance to hemolysis observed in nonsmokers was replaced by an in-

creased susceptibility to oxidation in the smokers of both sexes.

In conclusion, this study of healthy adults belonging to 28 unrelated pedigrees has shown that the important interindividual variability associated with the response to *in vitro* oxidative stress is due, not only to endogenous biological factors, but also to shared familial factors that may include genetic effects. Exercise training did not modify the resistance of LDL to *in vitro* oxidation, but there was improvement in the resistance of erythrocytes to free radical attack, probably through an improvement in the antioxidant status. This study illustrates yet another detrimental effect of smoking and emphasizes the importance of context in the evaluation of oxidant stress and exercise.

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ABBREVIATIONS

AAPH, 2,2′-azobis (2-amidinopropane) dihydrochloride; BMI, body massindex; EDTA, ethylenediamine tetraacetate; EPA, eicosapentaenoic acid; GSH-Px, glutathione peroxidase; GSH, reduced form of glutathione; GSSG, oxidized form of glutathione; HDL, high density lipoproteins; LDL, low density lipoproteins; MDA, alondialdehyde; PL, phospholipids; RBC, red blood cells; ROS, reactive oxygen species; TAS, total antioxidant activity (of plasma); TBARS, thiobarbituric acid-reactive substances; tHcy, total homocysteine; TLC, thin layer chromatography.

REFERENCES

1. An P, Rice T, Gagnon J, Borecki IB, Bergeron J, Després JP, Leon AS, Skinner JS, Wilmore JH, Bouchard C, and Rao DC. Segregation analysis of apolipoproteins A-1 and B-100 measured before and after an exercise training program—The HERITAGE Family Study. *Arterioscler Thromb Vasc Biol* 20: 807–814, 2000.
2. Berliner JA and Heinecke JW. The role of oxidized lipoproteins in atherogenesis. *Free Radic Biol Med* 20: 707–727, 1996.
3. Bieri JG, Brown ED, and Smith JC, Jr. Determination of individual carotenoids in human plasma by high performance liquid chromatography. *J Liquid Chromatogr* 8: 473–484, 1985.
4. Blache D. Involvement of hydrogen and lipid peroxides in the acute tobacco smoking-induced platelet hyperactivity. *Am J Physiol* 268: H679–H685, 1995.
5. Blache D, Durand P, Prost M, and Loreau N. (+)-Catechin inhibits platelet hyperactivity induced by an acute iron load in vivo. *Free Radic Biol Med* 33: 1670–1680, 2002.
6. Blache D and Prost M. Free radical attack: biological test for human resistance capability. In: *Proceedings of the IX College Park Colloquium on Chemical Evolution: A Lunar-Based Chemical Analysis Laboratory (LBCAL)*, edited by Ponnamperuma C and Gehrke CW. Washington: 1992, pp 82–98.
7. Blache D, Rodriguez C, and Davignon J. Pro-oxidant effects of 7-hydroperoxycholest-5-en-3b-ol on the copper-initiated oxidation of low density lipoprotein. *FEBS Lett* 357: 135–139, 1995.
8. Bouchard C, Leon AS, Rao DC, Skinner JS, Wilmore JH, and Gagnon J. The Heritage Family Study. Aims, design, and measurement protocol. *Med Sci Sports Exerc* 27: 721–729, 1995.
9. Bourdon E and Blache D. The importance of proteins in defense against oxidation. *Antiox Redox Signal* 3: 293–311, 2001.
10. Brown KM, Morrice PC, and Duthie GG. Erythrocyte vitamin E and plasma ascorbate concentrations in relation to erythrocyte peroxidation in smokers and nonsmokers: Dose response to vitamin E supplementation. *Am J Clin Nutr* 65: 496–502, 1997.
11. Cazzola R, Russo–Volpe S, Cervato G, and Cestaro B. Biochemical assessments of oxidative stress, erythrocyte membrane fluidity and antioxidant status iin professional soccer players and sedentary controls. *Eur J Clin Invest* 33: 924–930, 2003.
12. Chait A, Brazg RL, Tribble DL, and Krauss RM. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. *Am J Med* 94: 350–356, 1993.
13. Chisolm GM and Steinberg D. The oxidative modification hypothesis of atherogenesis: An overview. *Free Radic Biol Med* 28: 1815–1826, 2000.
14. Couillard C, Després JP, Lamarche B, Bergeron J, Gagnon J, Leon AS, Rao DC, Skinner JS, Wilmore JH, and Bouchard C. Effects of endurance exercise training on plasma HDL cholesterol levels depend on levels of triglycerides–Evidence from men of the health, risk factors, exercise training and genetics (HERITAGE) family study. *Arterioscler Thromb Vasc Biol* 21: 1226–1232, 2001.
15. Cross CE and Traber MG. Cigarette smoking and antioxidant vitamins: the smoke screen continues to clear but has a way to go. *Am J Clin Nutr* 65: 562–563, 1997.
16. De Graaf J, Hak–Lemmers HLM, Hectors MPC, Demacker PNM, Hendriks JCM, and Stalenhoef AFH. Enhanced susceptibility to *in vitro* oxidation of the dense low density lipoprotein subfraction in healthy subjects. *Arteriosclerosis* 11: 298–306, 1991.
17. Durand P, Fortin LJ, Lussier–Cacan S, Davignon J, and Blache D. Hyperhomocysteinemia induced by folic acid deficiency and methionine load—applications of a modified HPLC method. *Clin Chim Acta* 252: 83–93, 1996.
18. Ehara S, Ueda M, Naruko T, Haze K, Itoh A, Otsuka M, Komatsu R, Matsuo T, Itabe H, Takano T, Tsukamoto Y, Yoshiyama M, Takeuchi K, Yoshikawa J, and Becker AE. Elevated levels of oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes. *Circulation* 103: 1955–1960, 2001.
19. Elosua R, Molina L, Fito M, Arquer A, Sanchez–Quesada JL, Covas MI, Ordóñez–Llanos J, and Marrugat J. Response of oxidative stress biomarkers to a 16-week aerobic physical activity program, and to acute physical activity, in healthy young men and women. *Atherosclerosis* 167: 327–334, 2003.
20. Esterbauer H and Jürgens G. Mechanistic and genetic aspects of susceptibility of LDL to oxidation. *Curr Opin Lipidol* 4: 114–124, 1993.
21. Esterbauer H, Striegl G, Puhl H, and Rotheneder M. Continuous monitoring of *in vitro* oxidation of human low density lipoprotein. *Free Radic Res Commun* 6: 67–75, 1989.
22. Girodon F, Blache D, Monget AL, Lombart M, Brunet–Lecompte P, Arnaud J, Richard M–J, and Galan P. Effect of a two-year supplementation with low doses of antioxidant vitamins and/or minerals in elderly subjects on levels of nutrients and antioxidant defense parameters. *J Am Coll Nutr* 16: 357–365, 1997.

23. Gueguen S, Leroy P, Gueguen R, Siest G, Visvikis S, and Herbeth B. Genetic and environmental contributions to serum retinol and alpha-tocopherol concentrations: the Stanislas Family Study. *Am J Clin Nutr* 81: 1034–1044, 2005.
24. Halliwell B. Antioxidants in human health and disease. *Annu Rev Nutr* 16: 33–50, 1996.
25. Harris ED. Regulation of antioxidant enzymes. *FASEB J* 6: 2675–2683, 1992.
26. Hendrickson A, McKinstry LA, Lewis JK, Lum J, Louie A, Schellenberg GD, Hatsukami TS, Chait A, and Jarvik GP. *Ex vivo* measures of LDL oxidative susceptibility predict carotid artery disease. *Atherosclerosis* 179: 147–153, 2005.
27. Kujala UM, Ahotupa M, Vasankari TJ, Kaprio J, and Tikkanen MJ. Familial aggregation of LDL oxidation. *Scand J Clin Lab Invest* 57: 141–146, 1997.
28. Leaf DA, Kleinman MT, Hamilton M, and Barstow TJ. The effect of exercise intensity on lipid peroxidation. *Med Sci Sports Exerc* 29: 1036–1039, 1997.
29. Lowry OH, Rosebrough NJ, Farr AL, and Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 193: 265–275, 1951.
30. Lussier–Cacan S, Bolduc A, Xhignesse M, Niyonsenga T, Connelly PW, and Sing CF. Impact of age and body size on interindividual variation in measures of lipid metabolism: influence of gender and apolipoprotein E genotype. *Clin Genet* 57: 35–47, 2000.
31. Lussier–Cacan S, Xhignesse M, Piolot A, Selhub J, Davignon J, and Genest J, Jr. Plasma total homocysteine in healthy subjects: Sex-specific relation with biological traits. *Am J Clin Nutr* 64: 587–593, 1996.
32. Mabile L, Piolot A, Boulet L, Fortin LJ, Doyle N, Rodriguez C, Davignon J, Blache D, and Lussier–Cacan S. Moderate intake of n-3 fatty acids is associated with stable erythrocyte resistance to oxidative stress in hypertriglyceridemic subjects. *Am J Clin Nutr* 74: 449–456, 2001.
33. Mackness MI, Mackness B, Durrington PN, Fogelman AM, Berliner J, Lusis AJ, Navab M, Shih D, and Fonarow GC. Paraoxonase and coronary heart disease. *Curr Opin Lipidol* 9: 319–324, 1998.
34. Miller YI, Chang M–K, Binder CJ, Shaw PX, and Witztum JL. Oxidized low density lipoprotein and innate immune receptors. *Curr Opin Lipidol* 14: 445, 2003.
35. Morrison WR and Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *J Lipid Res* 5: 600–608, 1964.
36. Nishi K, Itabe H, Uno M, Kitazato KT, Horiguchi H, Shinno K, and Nagahiro S. Oxidized LDL in carotid plaques and plasma associates with plaque instability. *Arterioscler Thromb Vasc Biol* 22: 1649–1654, 2002.
37. Parthasarathy S and Santanam N. Mechanisms of oxidation, antioxidants, and atherosclerosis. *Curr Opin Lipidol* 5: 371–375, 1994.
38. Pérusse L, Rice T, Després JP, Bergeron J, Province MA, Gagnon J, Leon AS, Rao DC, Skinner JS, Wilmore JH, and Bouchard C. Familial resemblance of plasma lipids, lipoproteins and postheparin lipoprotein and hepatic lipases in the HERITAGE Family study. *Arterioscler Thromb Vasc Biol* 17: 3263–3269, 1997.
39. Piolot A, Blache D, Boulet L, Fortin LJ, Dubreuil D, Marcoux C, Davignon J, and Lussier-Cacan S. Effect of fish oil on LDL oxidation and plasma homocysteine concentrations in health. *J Lab Clin Med* 141: 41–49, 2003.
40. Reilly SL, Ferrell RE, Kottke BA, and Sing CF. The gender-specific apolipoprotein E genotype influence on the distribution of plasma lipids and apolipoproteins in the population of Rochester, Minnesota. II. Regression relationships with concomitants. *Am J Hum Genet* 51: 1311–1324, 1992.
41. Sen CK and Packer L. Thiol homeostasis and supplements in physical exercise. *Am J Clin Nutr* 72: 653S–669S, 2000.
42. Shern–Brewer R, Santanam N, Wetzstein C, White–Welkley J, and Parthasarathy S. Exercise and cardiovascular disease—a new perspective. *Arterioscler Thromb Vasc Biol* 18: 1181–1187, 1998.
43. Sokal RR and Rohlf EJ. In: *Biometry: the Principles and Practice of Statistics in Biological Research*, Freeman, 3rd Ed., New York: 1995.
44. Stocker R and Keaney JF, Jr. Role of oxidative modifications in atherosclerosis**.** *Physiol Rev* 84:1381–1478, 2004.
45. Tchernof A, Moorjani S, Lamarche B, Labrie F, Prud'homme D, Lupien PJ, Nadeau A, and Després JP. The dense LDL phenotype. Association with plasma lipoprotein levels, visceral obesity, and hyperinsulinemia in men. *Diabetes Care* 19: 629–637, 1996.
46. Thurnham DI, Smith E, and Flora PS. Concurrent liquid-chromatographic assay of retinol, alpha-tocopherol, beta-carotene, alpha-carotene, lycopene, and beta cryptoxanthin in plasma with tocopherol acetate as internal standard. *Clin Chem* 34: 377–381, 1988.
47. Tribble DL, Holl LG, Wood PD, and Krauss RM. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. *Atherosclerosis* 93: 189–199, 1992.
48. Urso ML and Clarkson M. Oxidative stress, exercise, and antioxidant supplementation. *Toxicology* 189: 41–54, 2003.
49. Van den Berkmortel FWPJ, Demacker PNM, Wollersheim H, Thien T, and Stalenhoef AFH. Smoking or its cessation does not alter the susceptibility to *in vitro* LDL oxidation. *Eur J Clin Invest* 30: 972–979, 2000.
50. Vasankari TJ, Kujala UM, Vasankari TM, and Ahotupa M. Reduced oxidized LDL levels after a 10-month exercise program. *Med Sci Sports Exerc* 30: 1496–1501, 1998.
51. Wang XL, Rainwater DL, VandeBerg JF, Mitchell BD, and Mahaney MC. Genetic contributions to plasma total antioxidant activity. *Arterioscler Thromb Vasc Biol* 21: 1190–1195, 2001.
52. Witztum JL and Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest* 88: 1785–1792, 1991.

Address reprint requests to:
Dr. Denis Blache
Inserm Unit 498, School of Medicine,
7 boul. Jeanne d'Arc
BP87900 – 21079 Dijon, France

E-mail: dblache@u-bourgogne.fr

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