Familial Aggregation of Blood Lipid Response to Exercise Training in the Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Family Study

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Background—Fasting levels of plasma lipids and lipoproteins are reported to improve with regular exercise training. However, little is known on whether the training responses are influenced by heritable factors.

Methods and Results—The lipid profile was assessed in 115 black (224 individuals) and 99 white families (469 individuals), who participated in the HERITAGE Family Study, while in a sedentary state (baseline visit) and after exercise training for 20 weeks (post visit). Variables included total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), apolipoprotein B (ApoB), high-density lipoprotein cholesterol (HDL-C), apolipoprotein A-I, and HDL-C subfractions 2 (HDL_2-C) and 3 (HDL_3-C). Familial correlations for the training responses (Δ=post−baseline) were significant for most variables, and the percent variance accounted for by familial factors (ie, maximal heritabilities) ranged from 25% to 38%. Exceptions were for higher heritabilities near 60% for ΔApoB in blacks and ΔHDL_2-C in whites and a lower estimate of zero for ΔLDL-C in blacks.

Conclusions—Heritable factors in part determine lipid profile responses to regular exercise. Maximal heritabilities were similar across ethnic groups and variables, except for ΔLDL-C, ΔApoB, and ΔHDL_2-C. Molecular studies to identify the markers and genes associated with these influences are currently underway. (***Circulation***. 2002;105:1904-1908.)

Key Words: genetics ■ heritability ■ lipids ■ exercise

Plasma levels of lipids and lipoproteins are risk factors for coronary heart disease (CHD).[1] Apolipoprotein B (ApoB), the primary surface component of low-density lipoprotein (LDL), functions by binding to specific receptors in the liver and peripheral tissue surface membrane for cholesterol delivery[2] and is strongly associated with an increased risk for CHD.[1] The anti-atherogenic effects of high-density lipoprotein (HDL)[3] are thought to be associated with cholesterol removal from peripheral tissues (including arteries) directly to the liver by the reverse cholesterol transport processes.[4] HDL subfraction 3 (HDL_3) assimilates lipids and is converted to HDL subfraction 2 (HDL_2),[5] and CHD risk increases with reduced levels of either subfraction.[6] Increases in the levels of the principal surface protein of HDL (apolipoprotein A-I [ApoA-I]) may reflect higher HDL synthesis and conversion of HDL_3 to HDL_2. Exercise training can improve the lipid profile and may reduce CHD risk.[7–11] Moreover, these responses can be affected by several inter-acting factors[12,13] such as age, sex, ethnicity, and steroid hormones, although the strongest predictors are exercise-induced plasma volume changes, dietary habits, and initial fitness levels.

Although it is well documented that baseline lipids and lipoproteins are influenced by heritable factors,[14] less is known of familial components for lipid responses to exercise. That is, are some individuals, because of their genetic make-up, more likely to have an improved lipid profile with exercise training? The HERITAGE Family Study is unique with regard to investigating this question because sedentary families were assessed at baseline and then again after 20 weeks of endurance exercise training. Although there were mean changes only for high-density lipoprotein cholesterol (HDL-C), ApoA-I, and triglyceride (TG),[15] there was a great deal of variability for all lipids. This variability, when contrasted between and within families, constitutes the basis for estimates of heritability. In the current investigation of

these HERITAGE families, we address whether the responses to exercise training cluster in families.

Methods

Sample and Study Design

The HERITAGE Family Study of 99 white families (229 males, 240 females) and 115 black families (83 males, 141 females) included data at baseline and again after 20 weeks of exercise training. Family structures consisted of 2 parents and at least 2 to 3 offspring, although the black family units were often smaller. The criteria for participation have been described elsewhere.[16] Briefly, parents were <66 years of age and offspring were >16 years of age. All were sedentary at baseline, which was defined as no regular strenuous physical activity over the previous 6 months (ie, lasts 30 minutes or more and involves an energy expenditure of $\geq$7 METs [1 MET=3.5 mL O_2 uptake/kg/min] for subjects 50 years or older and $\geq$8 METs for subjects younger than 50 years). With a few exceptions approved by a physician, body mass index was $\leq$40 kg/m². Systolic blood pressure was $\leq$150 mm Hg, and diastolic blood pressure was $\leq$99 mm Hg. Analysis of dietary questionnaires[17] ensured that the subjects maintained the same diet composition throughout the protocol.[15]

Each individual was trained on a cycle ergometer 3 times per week for 20 weeks. The training intensity and/or duration was adjusted every 2 weeks to ensure that the participant was working at a heart rate associated with 75% of baseline VO_2max for 50 minutes during the last 6 weeks. The power output was adjusted automatically to the heart rate response by a computer program that controlled the cycle ergometer during all training sessions. Sessions were supervised on site, and adherence to the protocol was strictly monitored.[18]

Measures

Blood samples were obtained from an antecubital vein into Vacutainer tubes that contained EDTA and were taken in the morning after a 12-hour fast with participants in a semirecumbent position, twice at baseline, and again at 24- and 72-hours after the last training session. For eumenorrheic women, all samples were obtained in the early follicular phase of the menstrual cycle when blood plasma cholesterol alterations are minimal.[19] Plasma samples for lipid assays were packed with ice for shipping to the Core Laboratory at the Lipid Research Center of Laval University.

Total cholesterol (TC) and TG levels were determined in plasma and in lipoproteins by enzymatic methods using a Technicon RA-500 Analyzer (Bayer Corporation Inc, Tarrytown, NY). Plasma VLDLs were isolated by ultracentrifugation.[20] The HDL-C fraction was obtained after precipitation of LDL in the infranatant by the heparin manganese chloride method.[21] Selective precipitation was used to isolate the HDL_2-C and HDL_3-C subfractions using dextran sulfate.[22] ApoA-I was measured in the infranatant and ApoB was measured in the plasma and infranatant fraction by the rocket immunoelectrophoretic method.[23] The Apo measurements were calibrated with reference standards from the Centers for Disease Control and Prevention (Atlanta, Ga). Extensive quality control procedures[24] verified the reproducibility of the plasma measurements.[25] Daily variation was characterized by high intraclass correlations (from 0.79 for HDL_3-C to 0.95 for ApoB and TC), and the coefficient of variation was between 4.2% (ApoA-I) and 21.8% (TG). The laboratory measurement error was low, with repeated-measure and split-sample intraclass correlations that range from 0.93 (HDL_3-C) to 0.99 (TC, TG, HDL-C, LDL-C, ApoB) and coefficient of variations that range from 0.7% to 10%.

To adjust for plasma volume changes associated with exercise, plasma total proteins were assayed by the biuret method at baseline and after training. The lipid levels after training were then corrected on the basis of the correlation of baseline to post-training protein levels. The 2 baseline lipid values were averaged to obtain the baseline variable, and the average of the 2 protein-corrected post-training lipid values constituted the post-training variable. TG was nonnormally distributed, and a log transformation reduced the skewness. Training responses were computed (Δ=post-training−baseline).

Covariates

The effects of variables suspected to influence the lipid profile, especially in response to exercise training, were explored with a regression procedure. Dietary habits (2 scales that indexed a high- and a low-fat diet) were derived from the Minnesota Eating Pattern Assessment Tool (EPAT).[26,17] Habitual activity levels (work, sports, and leisure indexes) were derived from the Atherosclerosis Risk in Communities study (ARIC)/Baecke Physical Activity Questionnaire.[27,28] A menstrual history assessed hormone usage (scored as Yes/No), and a health screening questionnaire assessed use of antineoplastic drugs or cardioprotective agents such as beta-blockers or diuretics. Diabetics and people on beta-blockers, other antihypertensive drugs, and lipid-lowering agents were excluded from the study. Fitness was assessed with the baseline VO_2max exercise test.[29] In addition, baseline fasting insulin level was used as a general index of glucose homeostasis. Blood samples were collected under EDTA, and the tubes were centrifuged at 1000g at a temperature of 4°C for 10 minutes. Plasma was kept frozen at −20°C until the time of assay. Plasma insulin was measured by radioimmunoassay with polyethylene glycol separation.[30]

Each Δ (response) was adjusted for the effects of baseline level and of covariates using a stepwise multiple regression procedure separately for each sex by generation by race group. Only terms that were significant at the 5% level were retained.

Familial Aggregation

A sex-specific family correlation model was used to assess heritability. Four types of individuals (fathers=F, mothers=M, sons=S, and daughters=D), lead to 8 correlations within 3 groups: 1 spouse (FM), 4 parent-offspring (FS, FD, MS, and MD), and 3 sibling (SS, DD, and SD). Correlations were estimated using maximum likelihood methods, as outlined in more detail elsewhere (see Data Supplement). Maximal heritability (h²), defined as the percent of variance due to additive familial effects, was adjusted for degree of spouse resemblance and indexed both genetic and familial environmental sources of variance.

Results

Table 1 gives means and standard deviations for baseline measures. For covariate adjustments (see Data Supplement), baseline level was the most consistent predictor and accounted for 10% to 40% of the variance. Other significant covariates (eg, physical activity and fitness, dietary habits, and hormonal and medication usage) accounted for <10% of the variance.

A summary of the most parsimonious familial correlations is in Table 2. A detailed description of all of the tests that lead to these models is found elsewhere (see Data Supplement). There was significant familial resemblance for most variables, with ≈30% of the phenotypic variance accounted for by familial factors (maximal heritabilities). The exceptions were for no heritability for ΔLDL-C in blacks (0%) and higher estimates of ≈60% for ΔApoB in blacks and ΔHDL_2-C in whites. These traditional heritabilities are expressed as a percentage of the residual variance (ie, after removing effects caused by covariates). Heritability may be expressed alternatively as a percent of total variance (Data Supplement), ie, $h^{2*}=h^2\times(1-R^2)$, in which h^2 is the heritability in Table 2 and R^2 is the variance due to covariates.

Discussion

Previous studies that investigate lipid and lipoprotein responses to exercise training distinguish between acute and

TABLE 1. Sample Statistics for Baseline Variables and Covariates

Variables	Black Sample				White Sample			
	Fathers (n=20)	Mothers (n=43)	Sons (n=63)	Daughters (n=98)	Fathers (n=91)	Mothers (n=88)	Sons (n=138)	Daughters (n=158)
Age, years	50.1±7.2	46.6±6.6	27.0±7.2	27.7±7.5	53.5±5.3	52.0±5.1	25.4±6.1	25.4±6.3
BMI, kg/m^2	27.5±5.2	29.3±5.2	27.4±5.7	27.9±7.0	28.4±4.4	27.6±5.0	25.6±4.9	23.7±4.4
VO$_2$max, mL/min	2251.5±301.2	1558.4±290.7	2921.9±424.6	1826.8±373.9	2616.0±460.4	1636.2±255.5	3287.6±496.8	2062.0±298.1
Insulin, pmol/L	67.0±34.5	86.2±78.5	72.9±46.1	79.9±62.9	80.1±66.1	63.2±30.5	66.8±41.2	58.6±26.5
Leisure index	1.9±0.53	2.0±0.55	1.9±0.49	2.0±0.55	2.2±0.50	2.2±0.45	2.2±0.48	2.3±0.47
Sports index	1.6±0.83	1.6±0.79	2.0±0.97	1.5±0.77	1.9±0.96	1.7±0.94	2.1±1.01	1.8±0.86
Work index	2.7±0.76	2.3±0.70	2.4±1.00	2.0±0.94	2.4±0.74	2.3±0.92	2.2±1.14	2.0±1.00
High fat index	23.9±6.1	24.9±5.2	28.3±5.3	26.1±5.2	26.8±4.6	25.4±5.2	29.4±4.4	25.9±4.8
Low fat index	25.6±3.4	25.5±3.0	25.3±3.1	25.3±3.2	27.5±3.1	28.5±3.3	26.8±3.8	27.1±3.0
CPA drugs,* %	15	10	0	1	2	1	0	0
NEO drugs,* %	0	0	0	0	0	1	0	0
HORMSTAT,* %	0	17	0	23	0	37	0	40
TC, mg/dL	186.9±37.2	177.2±34.4	162.3±34.5	154.4±28.4	197.9±32.8	190.7±29.9	161.9±36.2	160.7±31.4
TG, mg/dL	4.7±0.5	4.4±0.5	4.5±0.5	4.2±0.4	5.1±0.5	4.7±0.5	4.6±0.5	4.5±0.4
LDL-C, mg/dL	126.7±30.9	120.3±30.1	109.4±31.7	102.9±25.8	135.0±30.3	127.4±26.7	108.1±30.4	105.0±28.1
ApoB, mg/dL	94.0±27.5	87.6±23.2	79.8±23.4	73.9±17.7	105.0±21.1	91.9±21.7	81.2±22.9	76.1±20.8
HDL-C, mg/dL	39.2±20.2	45.4±12.2	37.9±8.5	42.8±10.1	35.2±8.2	46.0±10.8	36.3±7.3	43.2±9.5
ApoA-I, mg/dL	114.7±18.0	122.6±18.6	112.0±14.4	112.9±15.1	116.4±17.8	127.4±16.2	112.6±15.2	118.1±16.7
HDL$_2$-C, mg/dL	12.4±13.4	15.4±8.9	10.4±6.1	15.6±7.7	10.1±4.7	17.4±8.0	10.3±4.5	16.0±7.1
HDL$_3$-C, mg/dL	26.8±7.3	30.0±6.1	27.5±4.7	27.2±5.3	25.1±5.0	28.6±5.8	26.0±4.5	27.2±4.7

Values are mean±SD or percent. TG is log transformed. Categorical indices of activity (leisure, sports, and work) and dietary (high- and low-fat) habits are derived from questionnaire data and have a large number of categories, and the resulting scores are normally distributed.

BMI indicates body mass index.

*For cardioprotective (CPA), antineoplastic (NEO), and hormone (HORMSTAT) variables (scored "yes" or "no"), values represent the percentage of individuals reporting use of the medications.

chronic effects.[31] Acute effects generally occur within 24 to 48 hours after an exercise bout and are transitory, whereas chronic effects are longer-acting adaptations that occur in response to long-term exercise training. Thompson et al[31] suggests that it is often difficult to distinguish between these acute versus chronic effects because of the timing of the post-training measurement and that acute effects may be different in sedentary versus trained individuals. For example, acute changes in LDL-C measured 24 hours after a bout of exercise were significant before training but not after 24 weeks of training.[32] In the HERITAGE study, the lipid and lipoprotein responses are considered to index chronic effects to exercise training in sedentary individuals.[15] Although previous results for chronic effects of training are inconsistent, increases in HDL-C and reductions in TC, LDL-C, and TG are often reported.[13] In the HERITAGE study, similar results were found.[15] HDL-C increased 3.6%, and this was primarily because of an increase in HDL$_2$-C and was associated with an increase in ApoA-I. Although there were no overall mean changes in the remaining responses, there was a great deal of variability in all lipid responses. A recent multivariate regression analysis of these HERITAGE families suggested that at least some of the variability could be accounted for by confounding factors such as fitness levels, body composition, and behavior characteristics.[33] For example, >15% of the variance in ΔHDL-C was attributed to these sources, leaving ≈85% still unaccounted for, which could be due in part to familial/genetic factors.

No previous studies were found reporting heritability estimates for these training responses. In the current study, familial factors accounted for ≈30% of the variation of the adjusted training responses with little evidence for ethnic differences. The exceptions were for no familial influence on ΔLDL-C (h^2=0%) in blacks and higher estimates approaching 60% for ΔApoB in blacks and ΔHDL$_2$-C in whites. These estimates also can be expressed as a percent of the total variation. For example, the total percentage of variance accounted for in ΔLDL-C in blacks is 37%, stratified into that due to the covariates (37%) and heritability (0%). Similarly, in whites, the total variance accounted for is 39%. However, it is stratified differently due to covariates (7%) and familial factors (32%). Thus, for ΔLDL-C, the total accounted for is approximately the same across ethnic groups, although the source is partitioned differently. This discrepancy between blacks and whites may indicate genetic heterogeneity in possible underlying pleiotropic genes (ie, a gene or genes that affect multiple traits) for the LDL-training responses and the covariates. For example, baseline values of LDL-C and VO$_2$max account for a significant percentage of the variance in blacks but not in whites. If the "genes" for initial LDL-C or fitness levels[29] also affect the LDL response (ie, pleiotropic effect), then the adjustment would remove their effects and the heritability would decrease. These results are consistent with Crouse et al[32] who reported that changes in LDL-C vary depending on training level. Our results suggest this depen-

TABLE 2.   Familial Correlations and Maximal Heritabilities for Responses Under the Most Parsimonious Models

Parameters	ΔTC		ΔTG		ΔLDL-C		ΔApoB	
	B	W	B	W	B	W	B	W
FM	0.17±0.09	0.15±0.04	0.17±0.09	0	0	0.18±0.05	0	0
FS=FD	0.17	0.15	0.17	0.10±0.07	0	0.18	−0.04±0.17	0.13±0.04
MS=MD	0.17	0.15	0.17	0.21±0.07	0	0.18	0.42±0.13	0.13
SS=DD=SD	0.17	0.15	0.17	0.13±0.06	0	0.18	0.40±0.11	0.13
h^2, %	32±18	29±8	32±18	29±13	0	34±10	59±26	26±8

	ΔHDL-C		ΔApoA-I		ΔHDL$_2$-C		ΔHDL$_3$-C	
	B	W	B	W	B	W	B	W
FM	0.48±0.16	0.37±0.09	−0.48±0.18	0	0.55±0.16	0.39±0.05	0	0.37±0.09
FS	0.25±0.09	0.16±0.05	−0.47±0.26	0	0.36±0.07	0.39	0.25±0.07	0.17±0.05
FD	−0.02±0.14	0.16	−0.64±0.17	0	0.36	0.39	0.25	0.17
MS	−0.02	0.16	−0.06±0.17	0	0.36	0.39	0.25	0.17
MD	0.25	0.16	−0.25±0.13	0	0.36	0.39	0.25	0.17
SS	0.25	0.16	0.40±0.15	0.33±0.07	0	0.39	0	0.17
DD	0.25	0.16	0.51±0.11	0.33	0	0.39	0	0.17
SD	−0.02	0.16	0.24±0.17	0.33	0	0.39	0	0.17
h^2, %	26±22	29±10	38±30	33±7	29±7	64±10	25±7	31±10

B indicates black; W, white.

dency, in part, may have a genetic basis, at least in the black sample.

A related discrepancy specific to the black sample involves LDL-C and ApoB. The heritability for ΔLDL-C is lower ($h^2=0\%$) than that for ΔApoB ($h^2=59\% \pm 26\%$). Although ApoB is the primary surface component of LDL, it is not the only carrier of ApoB; thus, we should not expect the circulating plasma levels and, therefore, the heritabilities for the 2 responses to be the same. The genetic pleiotropic hypothesis outlined above with respect to ΔLDL-C and baseline LDL-C and VO$_2$max is still applicable with respect to the low ΔLDL-C finding. However, we note that the within-individual correlation between the covariate-adjusted ΔLDL-C and ΔApoB responses is significant in the blacks ($r=0.55$), which suggests that some common factors, either genetic or nongenetic, underlie their covariation. If genetic, then presumably they are pleiotropic with respect to baseline LDL-C and fitness measures. These various hypotheses for LDL-C and ApoB responses and baseline LDL-C and VO$_2$max warrant further investigation with multivariate methods to disentangle the possible common genetic causes.

A few studies investigated whether genetic markers are associated with lipid responses to exercise training. For example, a greater training-induced improvement was found in individuals with the E2 or E3 alleles of the apolipoprotein E genotypes.[34] Similarly, the PvuII polymorphism of the lipoprotein lipase and the ID polymorphism of the angiotensin-converting enzyme gene were both associated with training-induced improvements. Other genes that directly or indirectly affect lipid metabolism should also be considered in this context.[35] For example, genes that affect baseline levels of LDL include the LDL receptor, the LDL-receptor binding region of ApoB, cholesterol 7α-hydroxylase, and others mapped to chromosomes 1p (autosomal dominant hypercholesterolemia), 13q (LDL-C–lowering gene), and 15q (precocious hypercholesterolemia). Whether these genes also affect the lipid and lipoprotein responses to exercise training warrant investigation.

In summary, these HERITAGE data represent the first report that indicates chronic responses to exercise training are in part determined by heritable factors. The possible exception is for changes in LDL-C. However, pleiotropic hypotheses among LDL-C, ApoB, and initial fitness measures are needed to thoroughly investigate this issue. It is important to note that the training responses in the current study were adjusted for baseline levels, so that these estimates are specific to the response and are independent of initial levels. In fact, the responses were adjusted for other known confounding factors, including adiposity, physical fitness and activity levels, and hormonal and behavioral characteristics. Although these factors accounted for a significant proportion of the variance in the blood lipid training responses, even more variance was accounted for by familial factors. Thus, some individuals, because of their genetic makeup, will be more likely to have an improved lipid profile with training. Given these results, molecular studies are needed to identify the markers that are linked with these responses.

Acknowledgments

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