Ethnic differences in hepatic lipase and HDL in Japanese, black, and white Americans: role of central obesity and *LIPC* polymorphisms

Molly C. Carr,[1],* **John D. Brunzell,*** **and Samir S. Deeb[†]**

Division of Metabolism, Endocrinology, and Nutrition* and Division of Medical Genetics,[†] Department of Medicine, University of Washington, Seattle, WA

Abstract Hepatic lipase activity (HLA) is a determinant of HDL levels, and a polymorphism in the hepatic lipase gene (*LIPC*) promoter (C−514T) has been hypothesized to account for higher HDL in blacks and Japanese compared with whites. To determine whether the polymorphism contributes to ethnic differences in HDL, we compared *LIPC* allele frequencies and HLA in Japanese American (JA; n = 84), black American (BA; n = 94), and white American (WA; n = 110) men and women. The *LIPC* polymorphism was associated with HLA in all cohorts (BA, $P = 0.012$; JA, $P = 0.008$; WA, $P = 0.009$). WA men had 49% and 58% higher HLA than BA and JA men, respectively (both $P < 0.05$), yet no differences in HLA were found between the women. The higher HLA in the WA men remained after adjustment for the *LIPC* polymorphism's effect on HLA ($P = 0.037$) but was erased after adjustment for waist-to-hip-ratio ($P = 0.46$). Although the WA men had lower HDL and HDL_3 than the JA and BA men (all $P < 0.05$), there were no differences in HDL_2, implying that variance in HLA may not underlie the ethnic differences in HDL levels. ■ These results suggest that *1*) the *LIPC* promoter polymorphism contributes to variation in HLA and HDL_2 in the three ethnic groups; *2*) WA men had higher HLA than BA and JA men, related to ethnic differences in central adiposity but not *LIPC* allele frequency; and *3*) the higher HLA in WA men did not contribute to the ethnic differences in HDL, as the differences in HDL were made up entirely of differences in HDL_3 and not HDL_2.—Carr, M. C., J. D. Brunzell, and S. S. Deeb. **Ethnic differences in hepatic lipase and HDL in Japanese, black, and white Americans: role of central obesity and *LIPC* polymorphisms.** *J. Lipid Res.* 2004. **45:** 466–473.

Supplementary key words cholesterol • low density lipoprotein • triglyceride • high density lipoproteins 2 and 3 • ethnic • hepatic lipase gene

Hepatic lipase (HL) is a lipolytic protein that catalyzes the hydrolysis of triglyceride (TG) and phospholipid in LDL and HDL particles and may also act as a ligand be-tween these particles and receptors (1–3). HL activity contributes to plasma HDL levels, as it promotes the conversion of large, buoyant HDL_2 to small, dense HDL_3 (4). Increased HL activity is associated with reduced plasma HDL levels and reduced large, buoyant HDL_2 particles, thought to be the more anti-atherogenic subspecies of total HDL (5, 6). Plasma HDL levels are known to be strongly influenced by genetic factors, including a functional polymorphism (C−514T) in the HL gene promoter that is associated with plasma HDL and HDL_2 levels (7–9).

The human hepatic lipase gene (*LIPC*), located on chromosome 15q21, spans more than 120 kb of DNA and encodes a protein of 449 amino acids (10, 11). There are six common genetic polymorphisms in the proximal promoter region, in complete linkage disequilibrium (G−50A, G−250A, C−480T, C−514T, T−710C, and A−763G), that together define two common haplotypes (7, 8). The C→T base pair substitution at the −514 position (T allele) is associated with an ∼30% reduction in promoter activity in vitro (12, 13), reduced postheparin HL activity, and increased plasma HDL, HDL_2, and large, buoyant LDL particles (7, 9, 14). On a population level, the *LIPC* promoter polymorphism is quite common, with allele frequencies ranging from ∼20% in whites to ∼35% in Koreans, Chinese, Czechs, and Hispanics to ∼50% in blacks and Japanese (7–9, 15–19). The higher allele frequency of the *LIPC* promoter polymorphism in Japanese and black populations has been hypothesized to account for the known ethnic differences in plasma HDL levels via lower levels of HL activity (15, 19, 20).

Numerous studies have shown that black men have ∼20% higher plasma HDL levels than similarly aged white men, but the underlying reasons remain unclear (21–24). These ethnic differences in HDL are present from childhood and persist even after adjusting for variables that influence plasma HDL levels, such as age, body weight, to-

---

[1] To whom correspondence should be addressed.
  e-mail: carr@u.washington.edu

This article is available online at http://www.jlr.org

bacco use, alcohol consumption, and TG levels (23, 25, 26). Lower HL activity in blacks compared with whites has been hypothesized to account for the differences in HDL levels (15, 27). Although many fewer studies have compared lipids in Japanese and white cohorts, it does appear that people of Japanese heritage also have higher HDL levels than whites (28).

We sought to investigate the relationships of the HL (C−514T) gene promoter polymorphism and HL activity with plasma HDL in three groups of healthy Japanese American (JA), black American (BA), and white American (WA) men and women. The study was designed to determine if ethnic differences in the allele frequency of the HL gene promoter polymorphism and HL activity accounted for the higher plasma HDL levels in BA and JA compared with WA men and women.

METHODS

Subjects

We studied 110 WA, 94 BA, and 84 JA men and women. The study participants were not taking lipid-lowering medications, β-blockers, or estrogen. They had no lipid disorders or other medical conditions affecting lipid metabolism, including diabetes, liver disease, pregnancy, or tobacco use. The participants (aged 18–70 years) were recruited from the Seattle metropolitan area and identified themselves as non-Hispanic white, Japanese, or black. The JA and BA participants were recruited specifically for the present study and compared with WA men and women who had been recruited to compare gender-related influences on lipid metabolism (29). The participants reported that both of their parents were of the same ethnic background. Participants were excluded from the study with body mass index (BMI) > 40 kg/m², TG or LDL cholesterol levels greater than the 95th percentile for age (30), or tobacco use. The Human Subjects Review Committee of the University of Washington approved the study protocol. Informed consent was obtained from all participants.

Blood collection

Blood was collected in 0.1% EDTA after a 12–16 h fast for DNA isolation and lipoprotein measurements. A heparin bolus of 60 U/kg was given, and blood was collected after 10 min in lithium heparin tubes for the measurement of lipase activity. All assays were performed in the same laboratories.

Lipid determinations

Plasma total cholesterol, HDL, and TG were quantitated by published techniques (31) using an Abbott Spectrum Bichromatic Analyzer (Irving, TX) at the Northwest Lipid Research Laboratory. LDL cholesterol was calculated by Friedewald's formula (32). HDL and HDL_3 cholesterol were determined after plasma precipitation with dextran sulfate and magnesium chloride (33).

Postheparin lipase activity

The total lipolytic activity was measured in plasma after heparin bolus as previously described (34). Glycerol tri-[1-¹⁴C]oleate (Amersham, Arlington Heights, IL) and lecithin were incubated with postheparin plasma for 60 min at 37°C, with LPL activity calculated as the lipolytic activity removed from the plasma by incubation with the specific 5D2 monoclonal antibody against LPL and HL activity determined as the activity remaining after incubation with the LPL antibody. The intra-assay coefficient of varia-

tion (CV) of HL activity was 2.7%, and the interassay CV was 10.4%.

Density gradient ultracentrifugation

A discontinuous salt density gradient was created using a modification (35) of a previous method (36). Samples were centrifuged at 65,000 rpm for 70 min (total angular velocity = 1.95 × 10¹¹) at 10°C in a Beckman VTi 65.1 (Palo Alto, CA) vertical rotor. The relative flotation rate (Rf), the LDL peak buoyancy, was obtained by dividing the fraction containing the LDL cholesterol peak by the total number of fractions collected with a CV of 3.6%.

DNA isolation and analysis

DNA was extracted from leukocytes of 10 ml of freshly drawn blood by the method of Miller, Dykes, and Polesky (37). The C−514T HL gene promoter polymorphism was determined by PCR amplification as described previously (38). The N193S and L334F HL gene polymorphisms, in exons 5 and 7, respectively, were determined in the BA men and women using previously reported oligonucleotide primers (39).

Statistical methods

Statistical analyses were performed using SigmaStat version 3.0 and SigmaPlot version 8.0.2 (SPSS, Chicago, IL). Comparisons between ethnic groups and genotypes were performed using ANOVA and ANOVA on ranks with all pairwise multiple comparisons. HL activity was adjusted linearly for the effect of waist-to-hip ratio (WHR). The relationship of genotype and HL activity was assessed by linear regression. The frequencies of the −514 C and T alleles were compared with the expected allele frequencies (Hardy-Weinberg equilibrium) using Chi-square analysis. The significance level was set at α = 0.05.

RESULTS

Comparison of lipid and anthropomorphic measures

The ethnic differences in lipid and anthropomorphic characteristics between the three groups of men and women are provided in **Tables 1 and 2**. The WA men had significantly lower total HDL than the JA and BA men (both $P < 0.05$). There was no significant difference in plasma LDL between the three groups of men, but the JA men had significantly higher TG levels than the BA men. LDL particle density (LDL-Rf) was significantly lower (more buoyant) in the BA men compared with the WA and JA men (both $P < 0.05$). The JA men had significantly lower LPL activity than both the WA and BA men (both $P < 0.05$), and there was no correlation between LPL activity and total HDL in the men ($R = 0.009$, $P = 0.93$). The WA men had significantly higher WHR than the JA and BA men (both $P < 0.05$) and higher BMI than the JA men. The BA men were significantly younger than the WA and JA men (both $P < 0.05$). The WA men had lower HDL_3 than the JA and BA men (all $P < 0.05$), but there was no significant difference in HDL_2 between the three ethnic groups (**Table 3**).

The BA women did not have higher HDL levels than the WA women, as seen in the men (**Tables 2** and **4**). The JA women had significantly higher HDL than the BA women ($P < 0.05$). BA women had significantly higher

TABLE 1. Comparison of lipids by ethnic group in men

Lipid	WA (n = 43)	JA (n = 38)	BA (n = 32)	P
Total cholesterol (mg/dl)	177 ± 32 (177)	194 ± 32 (197)	179 ± 40 (171)	0.066
LDL (mg/dl)	117 ± 29 (121)	122 ± 30 (127)	118 ± 44 (106)	0.47
TG (mg/dl)	102 ± 55 (92)	113 ± 37 (119)	80 ± 38^a (65.5)	0.004
Total HDL (mg/dl)	41 ± 11 (38)	49 ± 11^b (48)	49 ± 11^b (49)	<0.001
LDL-Rf	0.29 ± 0.04 (0.289)	0.31 ± 0.03 (0.324)	0.34 ± 0.03^{a,b} (0.338)	<0.001
LPL activity (nmol/ml/min)	288 ± 80 (266)	215 ± 93^b (202)	289 ± 100^a (258)	<0.001
BMI (kg/m^2)	29.0 ± 4.7 (29.0)	25.9 ± 3.7^b (24.7)	27.5 ± 5.0 (26.7)	0.011
WHR	0.93 ± 0.1 (0.96)	0.85 ± 0.06^b (0.85)	0.83 ± 0.09^b (0.82)	<0.001
Age (years)	51 ± 15 (49)	50 ± 15 (49)	37 ± 11^{a,b} (35)	<0.001

Data are reported as means ± SD (median) with one-way ANOVA and ANOVA on ranks. Ethnic groups are as follows: BA, black American; JA, Japanese American; WA, white American; BMI, body mass index; LDL-Rf, LDL particle density (relative flotation rate); LPL, lipoprotein lipase; TG, triglyceride; WHR, waist-to-hip ratio.
^{a} $P < 0.5$ for BA versus JA.
^{b} $P < 0.05$ for WA versus JA and WA versus BA.

BMI and younger age than the WA and JA women (both $P < 0.05$). As in the men, the LDL-Rf was significantly higher (more buoyant) and the TG was lower in the BA women compared with the JA and WA women (all $P < 0.05$).

Effects of ethnicity and gender on HL activity

The WA men had 58% and 49% higher HL activity than the BA and JA men, respectively (both $P < 0.05$) (**Fig. 1**). The percentage distribution of HL activity in the three ethnic groups (**Fig. 2**) revealed a shift toward higher HL activity in WA men compared with BA and JA men.

In contrast to the men, there were no significant differences in HL activity between the three groups of women (Fig. 1). A shift toward higher HL activity in the distribution of HL activity in WA women was not seen in the comparison of the women (data not shown). The men had significantly higher HL activity than the women in all ethnic groups (Fig. 1). The BA, JA, and WA men had 66, 51, and 128% higher HL activity than the women within the same ethnic group (all $P < 0.001$).

Allele frequencies

The WA men had a significantly lower frequency of the −514 HL gene promoter T allele than the JA and BA men ($P < 0.001$ and $P = 0.002$, respectively), with allele frequencies consistent with previously published reports (8, 40). The allele frequencies of the *LIPC* T allele (BA = 0.44, JA = 0.50, WA = 0.27) were consistent with previ-

ously published reports (7–9, 15–19), and the populations were in Hardy-Weinberg equilibrium (BA, $P = 0.72$; JA, $P = 0.26$; WA, $P = 0.59$).

Association of the *LIPC* promoter polymorphism (C−514T) with HL activity

There was a significant dose-dependent association of the *LIPC* promoter T allele with HL activity in all three ethnic groups (**Fig. 3**). There also was a significant relationship of this polymorphism with HL activity, as assessed by linear regression analyses, in JA, BA, and WA men and women, with the *LIPC* promoter polymorphism accounting for 11, 5, and 9% of the variation in HL activity, respectively (JA, $P = 0.003$, $R = 0.33$; BA, $P = 0.04$, $R = 0.23$; WA, $P = 0.002$, $R = 0.30$).

After adjusting for the effect of the HL gene promoter polymorphism on HL activity, by comparing men with the same genotype, the WA men with the CC genotype continued to have significantly higher HL activity than both the JA and BA men with the CC genotypes ($P = 0.037$) (**Fig. 4**). The WA men with the CT or TT genotype ($P = 0.22$ or $P = 0.53$, respectively) did not have significantly higher HL activity than the JA or BA men. The higher HL activity in the WA men with the CC genotype was erased after adjustment for the ethnic differences in WHR ($P = 0.46$). WHR was significantly correlated with age in both men ($R = 0.29$, $P = 0.008$) and women ($R = 0.18$, $P = 0.048$)

TABLE 2. Comparison of lipids by ethnic group in women

Lipid	WA (n = 61)	JA (n = 44)	BA (n = 46)	P
Total cholesterol (mg/dl)	194 ± 35 (189)	189 ± 34 (187)	178 ± 28^a (179)	0.07
LDL (mg/dl)	118 ± 33 (114)	105 ± 29 (103)	107 ± 25 (105)	0.06
TG (mg/dl)	92 ± 48 (79)	98 ± 54 (83)	67 ± 29^{a,b} (57)	0.001
Total HDL (mg/dl)	57 ± 14 (54)	63 ± 16 (62)	56 ± 12^b (53)	0.04
LDL-Rf	0.33 ± 0.03 (0.333)	0.33 ± 0.03 (0.324)	0.35 ± 0.03^{a,b} (0.351)	0.003
LPL activity (nmol/ml/min)	288 ± 98 (281)	279 ± 80 (281)	295 ± 113 (265)	0.96
BMI (kg/m^2)	26 ± 4 (25.4)	24 ± 4 (23.3)	30 ± 6^{a,b} (30.0)	<0.001
WHR	0.77 ± 0.06 (0.75)	0.78 ± 0.06 (0.76)	0.78 ± 0.06 (0.78)	0.44
Age (years)	51 ± 2 (50.5)	50 ± 16 (49.0)	40 ± 11^{a,b} (40.0)	<0.001

Data are reported as means ± SD (median) with one-way ANOVA and ANOVA on ranks.
^{a} $P < 0.05$ for WA versus JA and WA versus BA.
^{b} $P < 0.5$ for BA versus JA.

TABLE 3. Comparison of HDL and HDL subspecies by ethnic group in men

Variable	WA (n = 43)	JA (n = 38)	BA (n = 32)	P
HDL (mg/dl)	41 ± 11	49 ± 11[a]	49 ± 11[a]	<0.001
HDL$_2$ (mg/dl)	8 ± 4	8 ± 4	9 ± 4	0.29
HDL$_3$ (mg/dl)	33 ± 8	41 ± 8[a]	40 ± 7[a]	<0.001
Hepatic lipase activity	477 ± 207	321 ± 137[a]	301 ± 169[a]	<0.001
T allele frequency	0.24	0.51	0.50	0.002

Data are reported as means ± SD with one-way ANOVA and ANOVA on ranks.

[a] $P < 0.05$ for WA versus JA and WA versus BA.

(ethnic groups combined). The absolute difference in HL activity between the CC and CT genotypes and between the CT and TT genotypes averaged 115 nmol/ml/min in WA subjects, 47 nmol/ml/min in JA subjects, and 51 nmol/ml/min in BA subjects. These differences in the contribution of the T allele to HL activity were reduced but not eliminated when allelic effects on HL activity were expressed as percentage change in HL activity (BA, 20% decrease; JA, 16% decrease; WA, 36% decrease). Further adjustment of HL activity for WHR revealed no ethnic difference in the effect of the T allele on HL activity (BA, 23% decrease; JA, 12% decrease; WA, 27% decrease) between BA and WA subjects. In the JA cohort, it appears that the contribution of the T allele to HL activity is lower in magnitude than that in the BA and WA cohorts.

Associations of other *LIPC* gene polymorphisms with HL activity

There was no significant association of the *LIPC* N193S ($P = 0.16$) and L334F ($P = 0.15$) polymorphisms, in exons 5 and 7, respectively, with HL activity in the BA men and women combined. Examining the BA men and women separately, there was a trend toward lower HL activity with the F allele of the 334 variant in the BA men ($P = 0.065$, $R = 0.33$) but not in the women. The JA and WA cohorts were not genotyped for the *LIPC* N193S or L334F polymorphisms, as these gene variants had very low allele frequencies (our unpublished observations).

DISCUSSION

These data demonstrate three findings. First, there was a significant association of the HL gene promoter poly-

TABLE 4. Comparison of HDL and HDL subspecies by ethnic group in women

Variable	WA (n = 61)	JA (n = 44)	BA (n = 46)	P
HDL (mg/dl)	57 ± 14	63 ± 16	56 ± 12[a]	0.04
HDL$_2$ (mg/dl)	14 ± 8	14 ± 7	12 ± 4	0.54
HDL$_3$ (mg/dl)	43 ± 8	49 ± 11[b]	44 ± 9[a]	0.009
Hepatic lipase activity	209 ± 80	213 ± 60	181 ± 68	0.061
T allele frequency	0.27	0.49	0.43	0.012

Data are reported as means ± SD with one-way ANOVA and ANOVA on ranks.

[a] $P < 0.5$ for BA vs. JA.

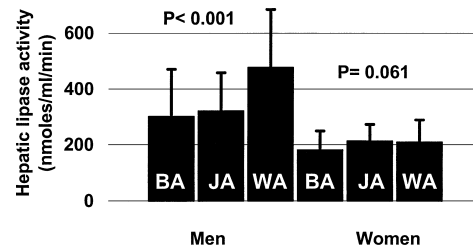[b] $P < 0.05$ for WA vs. JA and WA vs. BA.

Fig. 1. Comparison of hepatic lipase (HL) activity by ethnicity and gender. Shown are means ± SD of HL activity in the three ethnic groups. The white American (WA) men had significantly higher HL activity ($P < 0.001$), whereas the women showed no differences ($P = 0.06$). BA, black American; JA, Japanese American.

morphism T allele with lower HL activity in all three ethnic groups. Second, the *LIPC* promoter polymorphism did not account for the higher HL activity in the WA men compared with the JA and BA men. Third, the higher HL activity in the WA men did not account for the ethnic differences in HDL, as the higher plasma HDL levels in the BA and JA men were composed entirely of differences in HDL$_3$ and not HDL$_2$ cholesterol.

Similar effects of *LIPC* polymorphism (C−514T) and gender on HL activity in ethnic groups

The *LIPC* promoter polymorphism contributed to the variance in HL activity in all three ethnic groups. As seen in previously studied ethnic groups (7, 14, 15, 19, 40, 41), the *LIPC* promoter polymorphism T allele was associated with significantly lower HL activity in the current groups of JA, BA, and WA men and women. This is the first time that the influence of the −514 promoter polymorphism on HL activity has been shown in people of Japanese heritage, accounting for ∼11% of the variance in HL activity in JA subjects.

We have confirmed that the strong effect of gender on HL activity was evident in JA and BA cohorts, as the JA and BA men both had higher HL activity than the JA women. The gender dimorphism in HL activity is well established (42, 43) and thought to be related to gender differences in both intra-abdominal fat and sex-steroid hormones
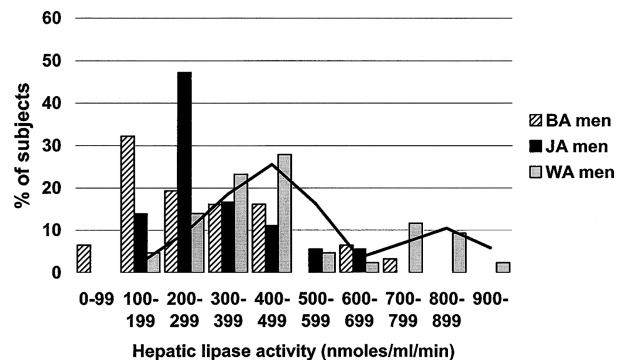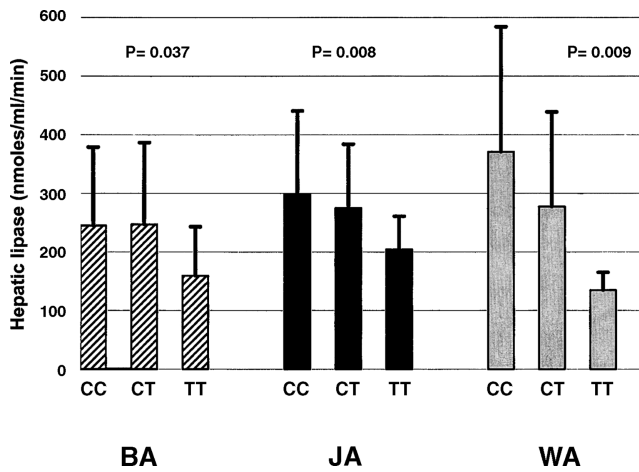

Fig. 2. The distribution of HL activity in men of the three ethnic groups. Shown is the percentage distribution of HL activity, revealing a shift toward higher HL activity in the WA men compared with BA and JA men, as indicated by the line plot for WA men.
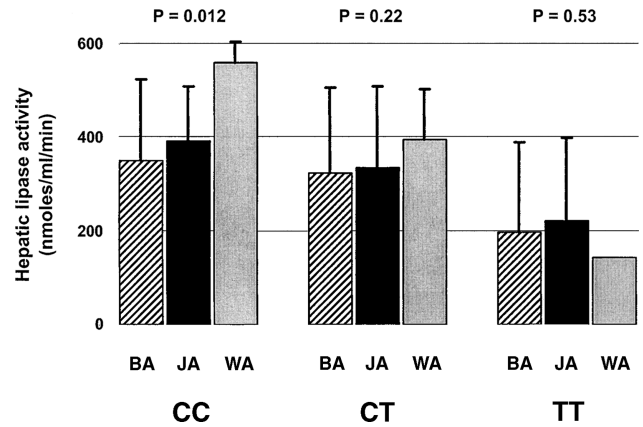
Fig. 3. Contribution of the hepatic lipase gene (*LIPC*) polymorphism to HL activity in men and women. HL activity is plotted as a function of *LIPC* genotype. There was a significant (by ANOVA) dose-dependent association of the *LIPC* promoter T allele with HL activity in all three ethnic groups.

Fig. 4. Ethnic differences in HL activity in men of the same *LIPC* genotype. The WA men with the CC genotype continued to have significantly higher HL activity than both the JA and BA men with the CC genotype (*P* = 0.037). The higher HL activity in the WA men with the CC genotype was erased after adjustment for the ethnic differences in waist-to-hip ratio (*P* = 0.46) (see Results).

(29). An estrogen-responsive AP-1 site in the promoter of the HL gene has recently been described, which may explain the strong suppressive effect of estrogens on HL activity (44).

Higher HL activity in WA men, independent of *LIPC* −514 promoter polymorphism

We have confirmed the previous findings of Vega et al. (15), who compared black and white men and found that white men had 63% higher HL activity than black men. After controlling for the effect of the *LIPC* polymorphism (C−514T), they found that the white men continued to have 59% (CC genotype) and 42% (CT genotype) higher HL activity than the black men. Similarly, we found that WA men had 58% higher HL activity than BA men. After controlling for the effect of the *LIPC* polymorphism in the present cohort, WA men continued to have 60% (CC genotype) higher HL activity than BA men, suggesting that the *LIPC* promoter polymorphism does not account for the ethnic differences in HL activity

The ethnic dissimilarities in HL activity in the men we studied could not be explained by differences in T allele frequency alone, implying that there is another factor(s) contributing to the higher HL activity in the WA men. This other factor appears to be the amount of visceral adiposity, as the current ethnic differences in HL activity were erased after adjustment for WHR (41). The effect of ethnicity on visceral adiposity is well known. Several groups have reported lower amounts of visceral adipose tissue, by computed tomography, in blacks compared with whites matched for BMI and total body fat (45–48). Despres et al. (27) showed that at any level of total body fat, compared with black subjects, whites subjects have more visceral adipose tissue accumulation. These differences in body fat distribution are likely to play a role in ethnic differences in HL activity, as HL activity increases with increasing intra-abdominal fat accumulation (49). The in-

fluence of a novel genetic polymorphism in another region of the HL gene or lifestyle differences (diet, exercise) also may have contributed to the ethnic differences in HL activity.

Shohet et al. (19) recently compared the effect of the *LIPC* promoter variant on HL activity in Turkish, Chinese, black, and white men. They found that a single copy of the −514C allele resulted in a 5 mmol/hr/l increase in HL activity in black men and a 10 mmol/hr/l (nmol/min/ml × 0.06 = mmol/hr/l) increase in white men and suggested that there was a differential effect of the *LIPC* −514 variant on HL activity in the ethnic groups, possibly mediated by another factor that interacts with the *LIPC* gene. We found no differences in the contribution of the T allele to HL activity in the ethnic groups after adjustment for WHR on HL activity. This implies that the lower level central fat in the BA men was the factor that interacted with the *LIPC* gene to produce lower HL activity.

The WA men also had 49% higher HL activity than the JA men. Again, the higher HL activity in the WA men could not be fully accounted for by ethnic differences in the frequency of the *LIPC* promoter polymorphism, as the WA men with the CC genotype continued to have 43% higher HL activity than the JA men with the CC genotype. Although it is believed that Japanese have higher plasma HDL levels than whites, there are few studies comparing lipids and lipoproteins in white and Japanese populations (28, 50–53). Only one study has found higher plasma HDL in Japanese children (aged 8–15 years) compared with Australian white children, but plasma HDL was assayed by different methods and the children were living in different countries (28). The current comparison appears to be the first confirmation that men of Japanese descent have higher plasma HDL than white men.

The ethnic differences in HL activity in the men were not seen in the women, as the WA women had similar HL activity compared with the BA and JA women (Fig. 1). The

association of HL activity with HDL was also not as strong in the women. This may be attributable to the small cohort sizes. The ethnic difference in plasma HDL may also be specific to men, as several large epidemiologic studies have found no significant differences in HDL levels in premenopausal or postmenopausal black and white women (23, 54–56). The strong effects of endogenous estradiol on HL activity may also have obscured the relationship of HL activity with plasma HDL (57).

Ethnic differences in HDL are not related to HL activity

Many epidemiologic studies have shown that black men have higher plasma HDL levels than white men, but the mechanisms remain unclear (23, 24, 26, 54). Vega et al. (15, 27) found lower HL activity and higher plasma HDL levels in BA men compared with WA men. They postulated that higher HL activity may account for the higher total HDL levels in BA men, given that the allele frequency of the HL gene T allele, associated with lower HL activity, is much lower in WA men than in BA men.

Many groups have shown the association of HL activity (29, 43, 58) and the *LIPC* gene promoter variant (9, 59, 60) to be with HDL_2 but not HDL_3 particles. Juo et al. (61) recently showed, in a large (n = 578) cohort of black men, that the HL gene promoter polymorphism was exclusively associated with plasma HDL_2 levels but not HDL_3. Vega et al. (15) also showed higher plasma HDL and higher HL activity in black men compared with white men. They found that the higher plasma HDL in black men was composed entirely of higher HDL_3 cholesterol and found no ethnic differences in HDL_2. The ethnic differences in total HDL in the current cohorts were also not related to differences in HDL_2 but to differences in HDL_3. These data imply that ethnic differences in HL activity and in the frequency of the *LIPC* promoter polymorphism may not account for the ethnic differences in plasma HDL levels.

The current study was limited by significant differences in body weight and age between the ethnic groups, which may have obscured the relationship of HL gene polymorphism with lipids. By adjusting HL activity for WHR, we have attempted to erase these differences in age between the cohorts, as WHR was related to age. The ethnic differences in HL activity were erased after adjustment for WHR. There is clear evidence that whites are more prone than blacks to accumulating fat in a central (intra-abdominal) distribution, which would also contribute to higher HL activity and lower HDL in whites (27, 62). Also, we did not control for environmental factors (diet, exercise, alcohol intake) that could have contributed to plasma HDL levels. Ordovas et al. (60) recently showed a strong gene-nutrient interaction between the *LIPC* promoter variant and dietary fat intake, as the T allele was associated with higher HDL and HDL_2 only in those subjects who consumed a low-fat diet. Gene-nutrient interactions may explain the lower magnitude of the contribution of the *LIPC* T allele to HL activity in the JA cohort. Americans are known to have diverse geographic and ethnic origins, making it difficult to separate the influence of lifestyle from genetic factors.

In conclusion, we have investigated the relationships of the common HL gene promoter polymorphism with HL activity in three groups of Americans. The relationship of the *LIPC* −514 T allele with HL activity was the same in all three ethnic groups. This is the first time that this association has been shown in people of Japanese heritage. We have confirmed that WA men had higher HL activity than BA men, but we also found that WA men had higher HL activity than JA men. The higher HL activity appears to be related to ethnic differences in central fat accumulation and not to differences in the allele frequency of the *LIPC* gene promoter polymorphism. Although it may appear that differences in HL activity account for the ethnic differences in plasma total HDL levels, our data imply that ethnic differences in total HDL were related to differences in HDL_3, which are not influenced significantly by HL activity.

REFERENCES

1. Santamarina-Fojo, S., C. Haudenschild, and M. Amar. 1998. The role of hepatic lipase in lipoprotein metabolism and atherosclerosis. *Curr. Opin. Lipidol.* **9:** 211–219.
2. Perret, B., L. Mabile, L. Martinez, F. Terce, R. Barbaras, and X. Collet. 2002. Hepatic lipase: structure/function relationship, synthesis, and regulation. *J. Lipid Res.* **43:** 1163–1169.
3. Deeb, S. S., A. Zambon, M. C. Carr, A. F. Ayyobi, and J. D. Brunzell. 2003. Hepatic lipase and dyslipidemia: interactions among genetic variants, obesity, gender, and diet. *J. Lipid Res.* **44:** 1279–1286.
4. Kuusi, T., P. Saarinen, and E. A. Nikkila. 1980. Evidence for the role of hepatic endothelial lipase in the metabolism of plasma high density lipoprotein2 in man. *Atherosclerosis.* **36:** 589–593.
5. Salonen, J. T., R. Salonen, K. Seppanen, R. Rauramaa, and J. Tuomilehto. 1991. HDL, HDL2, and HDL3 subfractions, and the risk of acute myocardial infarction. A prospective population study in eastern Finnish men. *Circulation.* **84:** 129–139.
6. Lamarche, B., S. Moorjani, B. Cantin, G. R. Dagenais, P. J. Lupien, and J. P. Despres. 1997. Associations of HDL2 and HDL3 subfractions with ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1098–1105.
7. Jansen, H., A. J. Verhoeven, L. Weeks, J. J. Kastelein, D. J. Halley, A. van den Ouweland, J. W. Jukema, J. C. Seidell, and J. C. Birkenhager. 1997. Common C-to-T substitution at position −480 of the hepatic lipase promoter associated with a lowered lipase activity in coronary artery disease patients. *Arterioscler. Thromb. Vasc. Biol.* **17:** 2837–2842.
8. Guerra, R., J. Wang, S. M. Grundy, and J. C. Cohen. 1997. A hepatic lipase (LIPC) allele associated with high plasma concentrations of high density lipoprotein cholesterol. *Proc. Natl. Acad. Sci. USA.* **94:** 4532–4537.
9. Zambon, A., S. S. Deeb, J. E. Hokanson, B. G. Brown, and J. D. Brunzell. 1998. Common variants in the promoter of the hepatic lipase gene are associated with lower levels of hepatic lipase ac-

tivity, buoyant LDL, and higher HDL2 cholesterol. *Arterioscler. Thromb. Vasc. Biol.* **18:** 1723–1729.

10. Bensadoun, A., and D. E. Berryman. 1996. Genetics and molecular biology of hepatic lipase. *Curr. Opin. Lipidol.* **7:** 77–81.

11. Cohen, J. C., G. L. Vega, and S. M. Grundy. 1999. Hepatic lipase: new insights from genetic and metabolic studies. *Curr. Opin. Lipidol.* **10:** 259–267.

12. Deeb, S. S., and R. Peng. 2000. The C-514T polymorphism in the human hepatic lipase gene promoter diminishes its activity. *J. Lipid Res.* **41:** 155–158.

13. Botma, G. J., A. J. Verhoeven, and H. Jansen. 2001. Hepatic lipase promoter activity is reduced by the C-480T and G-216A substitutions present in the common LIPC gene variant, and is increased by Upstream Stimulatory Factor. *Atherosclerosis.* **154:** 625–632.

14. Tahvanainen, E., M. Syvanne, M. H. Frick, R-S. Murtomaki, M. Antikainen, Y. A. Kesaniemi, H. Kauma, A. Pasternak, M. R. Taskinen, and C. Ehnholm. 1998. Association of variation in hepatic lipase activity with promoter variation in the hepatic lipase gene. The LOCAT Study Investigators. *J. Clin. Invest.* **101:** 956–960.

15. Vega, G. L., L. T. Clark, A. Tang, S. Marcovina, S. M. Grundy, and J. C. Cohen. 1998. Hepatic lipase activity is lower in African American men than in white American men: effects of 5′ flanking polymorphism in the hepatic lipase gene (LIPC). *J. Lipid Res.* **39:** 228–232.

16. Hong, S. H., J. Song, and J. Q. Kim. 2000. Genetic variations of the hepatic lipase gene in Korean patients with coronary artery disease. *Clin. Biochem.* **33:** 291–296.

17. Talmud, P. J., L. Berglund, E. M. Hawe, D. M. Waterworth, C. R. Isasi, R. E. Deckelbaum, T. Starc, H. N. Ginsberg, S. E. Humphries, and S. Shea. 2001. Age-related effects of genetic variation on lipid levels: the Columbia University BioMarkers Study. *Pediatrics.* **108:** E50–58.

18. Hubacek, J. A., D. M. Waterworth, J. Pitha, S. E. Humphries, P. J. Talmud, and R. Poledne. 2001. Polymorphisms in the lipoprotein lipase and hepatic lipase genes and plasma lipid values in the Czech population. *Physiol. Res.* **50:** 345–351.

19. Shohet, R. V., G. L. Vega, T. P. Bersot, R. W. Mahley, S. M. Grundy, R. Guerra, and J. C. Cohen. 2002. Sources of variability in genetic association studies: insights from the analysis of hepatic lipase (LIPC). *Hum. Mutat.* **19:** 536–542.

20. Inazu, A., Y. Nishimura, Y. Terada, and H. Mabuchi. 2001. Effects of hepatic lipase gene promoter nucleotide variations on serum HDL cholesterol concentration in the general Japanese population. *J. Hum. Genet.* **46:** 172–177.

21. Miller, G. J., N. E. Miller, and M. T. Ashcroft. 1976. Inverse relationship in Jamaica between plasma high-density lipoprotein cholesterol concentration and coronary-disease risk as predicted by multiple risk-factor status. *Clin. Sci. Mol. Med.* **51:** 475–482.

22. Slack, J., N. Noble, T. W. Meade, and W. R. North. 1977. Lipid and lipoprotein concentrations in 1604 men and women in working populations in north-west London. *Br. Med. J.* **2:** 353–357.

23. Tyroler, H. A., C. J. Glueck, B. Christensen, and P. O. Kwiterovich, Jr. 1980. Plasma high-density lipoprotein cholesterol comparisons in black and white populations. The Lipid Research Clinics Program Prevalence Study. *Circulation.* **62:** IV99–IV107.

24. Webber, L. S., S. R. Srinivasan, W. A. Wattigney, and G. S. Berenson. 1991. Tracking of serum lipids and lipoproteins from childhood to adulthood. The Bogalusa Heart Study. *Am. J. Epidemiol.* **133:** 884–899.

25. Srinivasan, S. R., R. R. Frerichs, L. S. Webber, and G. S. Berenson. 1976. Serum lipoprotein profile in children from a biracial community: the Bogalusa Heart Study. *Circulation.* **54:** 309–318.

26. Sprafka, J. M., S. W. Norsted, A. R. Folsom, G. L. Burke, and R. V. Luepker. 1992. Life-style factors do not explain racial differences in high-density lipoprotein cholesterol: the Minnesota Heart Survey. *Epidemiology.* **3:** 156–163.

27. Despres, J. P., C. Couillard, J. Gagnon, J. Bergeron, A. S. Leon, D. C. Rao, J. S. Skinner, J. H. Wilmore, and C. Bouchard. 2000. Race, visceral adipose tissue, plasma lipids, and lipoprotein lipase activity in men and women: the Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) family study. *Arterioscler. Thromb. Vasc. Biol.* **20:** 1932–1938.

28. Dwyer, T., H. Iwane, K. Dean, Y. Odagiri, T. Shimomitsu, L. Blizzard, S. Srinivasan, T. Nicklas, W. Wattigney, M. Riley, and G. Berenson. 1997. Differences in HDL cholesterol concentrations in Japanese, American, and Australian children. *Circulation.* **96:** 2830–2836.

29. Carr, M. C., J. E. Hokanson, A. Zambon, S. S. Deeb, P. H. Barrett, J. Q. Purnell, and J. D. Brunzell. 2001. The contribution of intraabdominal fat to gender differences in hepatic lipase activity and low/high density lipoprotein heterogeneity. *J. Clin. Endocrinol. Metab.* **86:** 2831–2837.

30. Rifkind, B. M., and P. Segal. 1983. Lipid Research Clinics Program reference values for hyperlipidemia and hypolipidemia. *J. Am. Med. Assoc.* **250:** 1869–1872.

31. Warnick, G. R. 1986. Enzymatic methods for quantification of lipoprotein lipids. *Methods Enzymol.* **129:** 101–123.

32. 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.

33. Warnick, G. R., J. Benderson, and J. J. Albers. 1982. Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin. Chem.* **28:** 1379–1388.

34. Iverius, P., and J. Brunzell. 1985. Human adipose tissue lipoprotein lipase: changes with feeding in relation to postheparin plasma enzyme. *Am. J. Physiol.* **249:** 326–334.

35. Hokanson, J. E. 2000. Measurement and clinical significance of low-density lipoprotein subclasses. *In* Handbook of Lipoprotein Testing. N. Rifai, G. Warnick, and M. Dominiczak, editors. American Association for Clinical Chemistry Press, Washington, DC. 329–344.

36. Chung, B. H., T. Wilkinson, J. C. Geer, and J. P. Segrest. 1980. Preparative and quantitative isolation of plasma lipoproteins: rapid, single discontinuous density gradient ultracentrifugation in a vertical rotor. *J. Lipid Res.* **21:** 284–291.

37. 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:** 1215–1222.

38. Fumeron, F., D. Betoulle, G. Luc, I. Behague, S. Ricard, O. Poirier, R. Jemaa, A. Evans, D. Arveiler, P. Marques-Vidal, J. M. Bard, J. C. Fruchart, P. Ducimetiere, M. Apfelbaum, and F. Cambien. 1995. Alcohol intake modulates the effect of a polymorphism of the cholesteryl ester transfer protein gene on plasma high density lipoprotein and the risk of myocardial infarction. *J. Clin. Invest.* **96:** 1664–1671.

39. Nie, L., S. Niu, G. L. Vega, L. T. Clark, A. Tang, S. M. Grundy, and J. C. Cohen. 1998. Three polymorphisms associated with low hepatic lipase activity are common in African Americans. *J. Lipid Res.* **39:** 1900–1903.

40. Nie, L., J. Wang, L. T. Clark, A. Tang, G. L. Vega, S. M. Grundy, and J. C. Cohen. 1998. Body mass index and hepatic lipase gene (LIPC) polymorphism jointly influence postheparin plasma hepatic lipase activity. *J. Lipid Res.* **39:** 1127–1130.

41. Carr, M. C., J. E. Hokanson, S. S. Deeb, J. Q. Purnell, E. S. Mitchell, and J. D. Brunzell. 1999. A hepatic lipase gene promoter polymorphism attenuates the increase in hepatic lipase activity with increasing intra-abdominal fat in women. *Arterioscler. Thromb. Vasc. Biol.* **19:** 2701–2707.

42. Huttunen, J. K., C. Ehnholm, M. Kekki, and E. A. Nikkila. 1976. Post-heparin plasma lipoprotein lipase and hepatic lipase in normal subjects and in patients with hypertriglyceridaemia: correlations to sex, age and various parameters of triglyceride metabolism. *Clin. Sci. Mol. Med.* **50:** 249–260.

43. Applebaum-Bowden, D., S. M. Haffner, P. W. Wahl, J. J. Hoover, G. R. Warnick, J. J. Albers, and W. R. Hazzard. 1985. Postheparin plasma triglyceride lipases. Relationships with very low density lipoprotein triglyceride and high density lipoprotein2 cholesterol. *Arteriosclerosis.* **5:** 273–282.

44. Jones, D. R., R. J. Schmidt, R. T. Pickard, P. S. Foxworthy, and P. I. Eacho. 2002. Estrogen receptor-mediated repression of human hepatic lipase gene transcription. *J. Lipid Res.* **43:** 383–391.

45. Albu, J. B., L. Murphy, D. H. Frager, J. A. Johnson, and F. X. Pi-Sunyer. 1997. Visceral fat and race-dependent health risks in obese nondiabetic premenopausal women. *Diabetes.* **46:** 456–462.

46. Hill, J. O., S. Sidney, C. E. Lewis, K. Tolan, A. L. Scherzinger, and E. R. Stamm. 1999. Racial differences in amounts of visceral adipose tissue in young adults: the CARDIA (Coronary Artery Risk Development in Young Adults) Study. *Am. J. Clin. Nutr.* **69:** 381–387.

47. Lara-Castro, C., R. L. Weinsier, G. R. Hunter, and R. Desmond. 2002. Visceral adipose tissue in women: longitudinal study of the effects of fat gain, time, and race. *Obes. Res.* **10:** 868–874.

48. Bacha, F., R. Saad, N. Gungor, J. Janosky, and S. A. Arslanian. 2003.

Obesity, regional fat distribution, and syndrome X in obese black versus white adolescents: race differential in diabetogenic and atherogenic risk factors. *J. Clin. Endocrinol. Metab.* **88:** 2534–2540.

49. Despres, J. P., M. Ferland, S. Moorjani, A. Nadeau, A. Tremblay, P. J. Lupien, G. Theriault, and C. Bouchard. 1989. Role of hepatic-triglyceride lipase activity in the association between intra-abdominal fat and plasma HDL cholesterol in obese women. *Arteriosclerosis.* **9:** 485–492.

50. Castelli, W. P., G. R. Cooper, J. T. Doyle, M. Garcia-Palmieri, T. Gordon, C. Hames, S. B. Hulley, A. Kagan, M. Kuchmak, D. McGee, and W. J. Vicic. 1977. Distribution of triglyceride and total, LDL and HDL cholesterol in several populations: a cooperative lipoprotein phenotyping study. *J. Chronic Dis.* **30:** 147–169.

51. Castelli, W. P., J. T. Doyle, T. Gordon, C. G. Hames, M. C. Hjortland, S. B. Hulley, A. Kagan, and W. J. Zukel. 1977. HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. *Circulation.* **55:** 767–772.

52. Yano, Y., N. Irie, Y. Homma, M. Tsushima, I. Takeuchi, N. Nakaya, and Y. Goto. 1980. High density lipoprotein cholesterol levels in the Japanese. *Atherosclerosis.* **36:** 173–181.

53. Ueshima, H., M. Iida, T. Shimamoto, M. Konishi, M. Tanigaki, N. Nakanishi, Y. Takayama, H. Ozawa, S. Kojima, and Y. Komachi. 1982. High-density lipoprotein-cholesterol levels in Japan. *J. Am. Med. Assoc.* **247:** 1985–1987.

54. Brown, S. A., R. Hutchinson, J. Morrisett, E. Boerwinkle, C. E. Davis, A. M. Gotto, Jr., and W. Patsch. 1993. Plasma lipid, lipoprotein cholesterol, and apoprotein distributions in selected US communities. The Atherosclerosis Risk in Communities (ARIC) Study. *Arterioscler. Thromb.* **13:** 1139–1158.

55. Proudler, A. J., I. F. Godsland, R. Bruce, M. Seed, and V. Wynn. 1996. Lipid and carbohydrate metabolic risk markers for coronary heart disease and blood pressure in healthy non-obese premeno-

pausal women of different racial origins in the United Kingdom. *Metabolism.* **45:** 328–333.

56. Howard, B. V., M. H. Criqui, J. D. Curb, R. Rodabough, M. M. Safford, N. Santoro, A. C. Wilson, and J. Wylie-Rosett. 2003. Risk factor clustering in the insulin resistance syndrome and its relationship to cardiovascular disease in postmenopausal white, black, Hispanic, and Asian/Pacific Islander women. *Metabolism.* **52:** 362–371.

57. Tikkanen, M. J., T. Kuusi, E. A. Nikkila, and U. H. Stenman. 1986. Variation of postheparin plasma hepatic lipase by menstrual cycle. *Metabolism.* **35:** 99–104.

58. Kuusi, T., C. Ehnholm, J. Viikari, R. Harkonen, E. Vartiainen, P. Puska, and M. R. Taskinen. 1989. Postheparin plasma lipoprotein and hepatic lipase are determinants of hypo- and hyperalphalipoproteinemia. *J. Lipid Res.* **30:** 1117–1126.

59. Couture, P., J. D. Otvos, L. A. Cupples, C. Lahoz, P. W. Wilson, E. J. Schaefer, and J. M. Ordovas. 2000. Association of the C-514T polymorphism in the hepatic lipase gene with variations in lipoprotein subclass profiles: the Framingham Offspring Study. *Arterioscler. Thromb. Vasc. Biol.* **20:** 815–822.

60. Ordovas, J. M., D. Corella, S. Demissie, L. A. Cupples, P. Couture, O. Coltell, P. W. Wilson, E. J. Schaefer, and K. L. Tucker. 2002. Dietary fat intake determines the effect of a common polymorphism in the hepatic lipase gene promoter on high-density lipoprotein metabolism: evidence of a strong dose effect in this gene-nutrient interaction in the Framingham Study. *Circulation.* **106:** 2315–2321.

61. Juo, S. H., Z. Han, J. D. Smith, L. Colangelo, and K. Liu. 2001. Promoter polymorphisms of hepatic lipase gene influence HDL(2) but not HDL(3) in African American men: CARDIA study. *J. Lipid Res.* **42:** 258–264.

62. Lovejoy, J. C., J. A. de la Bretonne, M. Klemperer, and R. Tulley. 1996. Abdominal fat distribution and metabolic risk factors: effects of race. *Metabolism.* **45:** 1119–1124.