

Body mass index and hepatic lipase gene (*LIPC*) polymorphism jointly influence postheparin plasma hepatic lipase activity

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Abstract The -514 polymorphism in the hepatic lipase gene (*LIPC*) is associated with decreased hepatic lipase activity. In the present study, the interaction between body mass index (BMI), the -514 polymorphism, and hepatic lipase activity was examined in 118 white men and in 51 African American men. BMI was significantly positively correlated with hepatic lipase activity in both populations. BMI was similar in men with genetic differences in hepatic lipase activity, indicating that high hepatic lipase activity did not cause increased BMI. The data therefore suggest that high BMI leads to increased hepatic lipase activity. The actions of BMI and the -514 polymorphism on hepatic lipase activity appear to be additive and independent, rather than synergistic. This finding indicates that hepatic lipase activity is a multifactorial trait, determined in part by polymorphism within the *LIPC* gene as well as by factors that influence BMI.—Nie, L., J. Wang, L. T. Clark, A. Tang, G. L. Vega, S. M. Grundy, and J. C. Cohen. **Body mass index and hepatic lipase gene (*LIPC*) polymorphism jointly influence postheparin plasma hepatic lipase activity.** *J. Lipid Res.* 1998. 39: 1127–1130.

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Human hepatic lipase is a 476 amino acid glycoprotein (1, 2) that exhibits phospholipase A1 and triglyceride hydrolase activities (3). The role of hepatic lipase in lipoprotein metabolism has been extensively studied (4), but much less is known about the factors that determine the activity of this enzyme in vivo. Several hormones influence hepatic lipase activity (5–9) but the contribution of these hormones to inter-individual variation in hepatic lipase activity is not known. Data from nuclear family (10), and twin (11) studies have suggested that inter-individual variation in hepatic lipase activity is highly heritable. Recently, we identified a common allele of the hepatic lipase gene (*LIPC*) that is associated with decreased hepatic lipase ac-

tivity and increased plasma high density lipoprotein-cholesterol (HDL-C) concentrations in men (12). The allele, designated $-514T$, does not contain mutations in the coding region, or intron/exon boundaries, and is identified by four linked polymorphisms in the 5' flanking region. Postheparin plasma hepatic lipase activity is 30–50% lower in homozygotes for the $-514T$ allele than in homozygotes for the wild type ($-514C$) allele (13). The $-514T$ allele is much more common among African Americans than among white Americans, and contributes to the low hepatic lipase activities observed in African American men (13).

Even among $-514T$ homozygotes, however, hepatic lipase activity is quite variable, indicating that other factors modulate the effects of this allele on hepatic lipase gene expression. One of these factors may be adiposity. Some (14–16) though not all (17–19) studies have suggested a correlation between hepatic lipase activity and adiposity. This inconsistency of results may be related to the relatively small sample sizes used in previous studies. In the present study, we have examined the relation between hepatic lipase activity and adiposity, as reflected by body mass index (BMI), in two large groups of men. In addition, the impact of *LIPC* polymorphism on the relationship between BMI and hepatic lipase activity was assessed.

METHODS

The study was approved by the Institutional Review Boards at the University of Texas Southwestern Medical Center and the State University of New York Health Science Center.

Abbreviations: BMI, body mass index; HDL-C, high density lipoprotein-cholesterol; PCR, polymerase chain reaction.

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Subjects

African American and white men who were apparently healthy and who did not have diabetes or use lipid-lowering medication were recruited through advertisements placed on bulletin boards at the University of Texas Southwestern Medical Center and the State University of New York Health Science Center. Any man aged over 20 years who met these criteria was eligible for the study. Body mass index and hepatic lipase activity were not used as selection criteria.

Assay of postheparin plasma hepatic lipase activity

Hepatic lipase activity was measured in postheparin plasma as described previously (20).

Assay of *LIPC* genotypes

LIPC genotypes were determined by polymerase chain reaction (PCR) amplification and restriction digestion (12).

Statistical analysis

The relationship between hepatic lipase activity, age, and body mass index was examined using Pearson's correlation. The potential influence of outliers was assessed by graphical inspection of the data, and by recalculating the correlation coefficients after exclusion of influential points (see Fig. 1). The mean postheparin plasma hepatic lipase activities of men in the upper and lower BMI tertiles were compared using *t*-tests. A general linear model (implemented in the SAS program) was used to determine whether BMI and the -514 polymorphism had independent effects on hepatic lipase activity (21). The polymorphism and BMI were entered as classification and continuous variables, respectively.

RESULTS

Postheparin plasma hepatic lipase activities and *LIPC* genotypes were determined in 118 white men aged 20 to 68 years, and in 51 African American men aged 20 to 40 years. Data from all individuals were included in the analysis. Hepatic lipase activity was not correlated with age in either group (data not shown).

Effect of BMI on hepatic lipase activity

The BMIs of the men in this study ranged from 18 to 43 kg/m². Hepatic lipase activity was positively correlated with BMI both in white men (Fig. 1) and in African American men (Fig. 2). When white men were ranked by BMI, the lower tertile had BMIs below 24 kg/m², while the upper tertile had BMIs greater than 27 kg/m². Mean hepatic lipase activity was significantly higher in the upper tertile than in the middle or lower tertiles (Table 1). Because of the smaller sample size, African Americans were divided into two groups comprising those falling above and those falling below the median BMI. Mean hepatic lipase activity was significantly higher in the group with higher BMIs than in the group with lower BMIs (Table 2).

Effect of *LIPC* genotype on hepatic lipase activity

In both ethnic groups, hepatic lipase activity was highest in homozygotes for the $-514C$ allele, lower in $-514CT$ heterozygotes and lowest in $-514T$ homozygotes (Table 1 and Table 2).

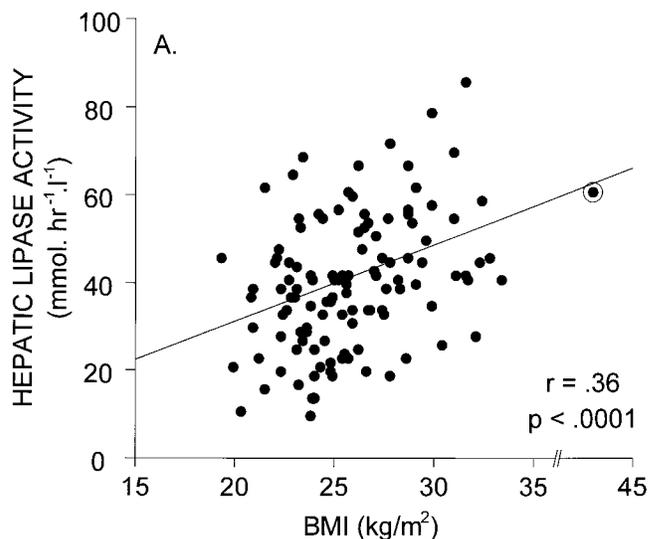


Fig. 1. Scatterplot of body mass index (BMI) and postheparin plasma hepatic lipase activity (PHLA) in 118 white men. The line indicates the least-squares regression line. *r* is the correlation coefficient calculated using all the data. When the circled point was excluded, *r* was 0.34.

Combined effects of *LIPC* genotype and BMI on hepatic lipase activity

To assess the combined effect of *LIPC* genotype and BMI, men were grouped by genotype and then ranked by BMI. Among whites, mean hepatic lipase activity was significantly higher in the upper tertile than in the lower tertile in $-514C$ homozygotes, and in $-514CT$ heterozygotes (Fig. 3, Table 1). Of the seven white men who were homozygous for the $-514T$ allele, hepatic lipase activity was lower in the three who had BMIs within the lower tertile of the whole group (i.e., less than 24 kg/m²), than in the three men who had BMIs in the upper tertile (greater than 27 kg/m²). Among

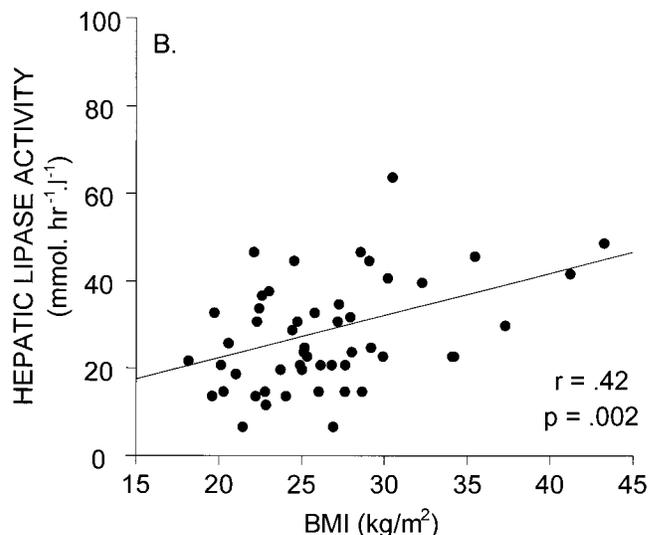


Fig. 2. Scatterplot of body mass index (BMI) and postheparin plasma hepatic lipase activity (PHLA) in 51 African American men. The line indicates the least-squares regression line. *r* is the correlation coefficient.

TABLE 1. Effects of body mass index and *LIPC* genotype on hepatic lipase activity in white men

<i>LIPC</i> Genotype	Hepatic Lipase Activity			
	All	Upper BMI Tertile	Middle BMI Tertile	Lower BMI Tertile
	<i>mmol · h⁻¹ · l⁻¹</i>			
All	41 ± 15 (118)	49 ± 15 ^b (39)	38 ± 14 (40)	37 ± 14 (39)
CC	46 ± 15 ^c (67)	53 ± 16 ^d (22)	45 ± 11 (23)	42 ± 13 (22)
CT	36 ± 14 (44)	44 ± 12 ^e (15)	31 ± 10 (14)	33 ± 15 (15)
TT ^a	26 ± 10 (7)	34 ± 8 (3)		19 ± 7 (3)

Values are means ± standard deviations for hepatic lipase activity. Numbers in each group are given in parentheses. Units are mmol · hr⁻¹ · l⁻¹.

^aThe TT group includes seven men. Three of these men had BMIs greater than 27 and were considered to represent the upper BMI tertile, while another three had BMIs less than 24 and were considered to represent the lower tertile.

^b*P* < 0.002 compared with the middle and lower tertiles.

^c*P* < 0.001 compared with the CT genotype, *P* < 0.05 compared with the TT genotype.

^d*P* < 0.01 compared with the lower tertile.

^e*P* < 0.05 compared with the lower tertile.

African Americans, mean hepatic lipase activity was higher among men with higher BMIs than among men with lower BMIs for each genotype group. These differences in hepatic lipase activity were statistically significant for the -514C and -514T homozygotes, but not for the -514CT heterozygotes.

The effects of BMI and the -514 polymorphism were both highly significant (*P* < 0.001) in the general linear model, indicating that both parameters were significantly and independently related to hepatic lipase activity.

Effect of *LIPC* genotype on BMI

No effect of *LIPC* genotype on BMI was detected in either ethnic group. In white men, mean BMI was 26 ± 4,

TABLE 2. Effects of body mass index and *LIPC* genotype on hepatic lipase activity in African American men

<i>LIPC</i> Genotype	Hepatic Lipase Activity		
	All	High BMI	Low BMI
	<i>mmol · h⁻¹ · l⁻¹</i>		
All	28 ± 12 (51)	32 ± 13 ^a (25)	25 ± 10 (25)
CC	33 ± 9 ^b (13)	37 ± 8 ^a (6)	28 ± 8 (6)
CT	28 ± 12 (24)	30 ± 15 (12)	27 ± 10 (12)
TT	24 ± 12 (14)	30 ± 14 ^a (7)	18 ± 8 (7)

Values are means ± standard deviations for hepatic lipase activity. Numbers in each group are given in parentheses. Units are mmol · hr⁻¹ · l⁻¹. Men were ranked by BMI and divided into two groups. The High BMI groups include men with BMI above the median value, while the low BMI groups include men with BMI below the median value.

^a*P* < 0.05 for High MBI vs. Low BMI.

^b*P* < 0.01 for CC vs. TT.

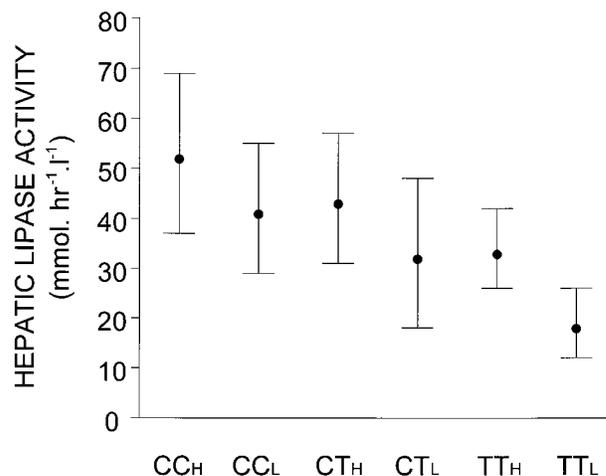


Fig. 3. Postheparin plasma hepatic lipase activity in white men grouped by *LIPC* genotype and body mass index (BMI). The group CC_H includes men who are -514C homozygotes and have a BMI in the upper tertile. CC_L includes men who are -514C homozygotes and have a BMI in the lower tertile. The group CT_H includes men who are -514CT heterozygotes and have a BMI in the upper tertile. CT_L includes men who are -514CT heterozygotes and have a BMI in the lower tertile. The group TT_H includes men who are -514T homozygotes and have a BMI in the upper tertile. TT_L includes men who are -514T homozygotes and have a BMI in the lower tertile.

25 ± 3, and 26 ± 3 for CC, CT, and TT genotypes, respectively (*P* = 0.2). The corresponding mean values in African American men were 26 ± 6, 27 ± 5, and 26 ± 5 for C, CT, and TT genotypes, respectively (*P* = 0.7).

DISCUSSION

A common *LIPC* allele (-514T) is associated with low hepatic lipase activity in white and in African American men (13). In the present study, we investigated the relationship between the -514 *LIPC* polymorphism, BMI, and hepatic lipase activity in these two populations. Two primary observations were established. First, our data provide strong evidence that increased BMI is associated with increased hepatic lipase activity in men. Second, a striking additive effect of BMI and the -514 polymorphism on hepatic lipase activity was observed. The joint effects of BMI and *LIPC* genotype strongly influenced hepatic lipase activity and were significantly greater than the effects of either factor considered alone.

To accurately assess the correlation between BMI and hepatic lipase, the present study design included three sequential steps: *i*) a large sample size was used to ensure ample statistical power, *ii*) the potential influence of "outliers" in the data was carefully assessed, and *iii*) the findings were corroborated by analysis of an independent population. In both white men and African American men, a significant positive correlation was observed between BMI and hepatic lipase activity. This correlation was not an artifact of "outliers" in either population. Therefore these data provide strong evidence for a posi-

tive correlation between BMI and hepatic lipase activity in men.

To determine whether hepatic lipase activity is a determinant of BMI, we measured BMI in men with genetically defined differences in hepatic lipase activity. In both ethnic groups, mean BMI was similar in men with the CC, CT, and TT genotypes, indicating that genetic variation in hepatic lipase activity does not influence BMI. Therefore the correlation between BMI and hepatic lipase activity almost certainly is not due to an effect of hepatic lipase activity on adiposity.

A second possible explanation for the correlation observed is that an unidentified common factor(s) exerts independent effects on BMI and hepatic lipase activity. While we cannot formally exclude this possibility, we suggest that a third explanation is more likely, namely that one or more factors associated with high BMI leads to increased hepatic lipase activity. Increased BMI is associated with changes in circulating hormone levels including decreased circulating testosterone levels (22) and increased plasma insulin concentrations (23) that potentially affect hepatic lipase activity. Androgens stimulate hepatic lipase activity (5), therefore the reduced androgen levels associated with obesity are unlikely to account for the effects of BMI on the activity of the enzyme. Some studies have reported positive correlations between hepatic lipase activity and the plasma insulin response to oral glucose, an index of insulin resistance (14, 16, 18). Consequently, the association between BMI and hepatic lipase activity may reflect insulin resistance in the liver secondary to obesity.

Finally, the effects of BMI on hepatic lipase activity were observed in each of the three *LIPC* genotypes in this study. These data indicate that BMI and the sequence polymorphisms in the -514T allele influence hepatic lipase activity by different mechanisms. Their action appears to be additive, and independent rather than synergistic. This finding indicates that hepatic lipase activity is a multifactorial trait, determined in part by polymorphism within the *LIPC* gene as well as by factors that influence BMI. ■

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REFERENCES

1. Stahnke, G., R. Sprengel, J. Augustin, and H. Will. 1987. Human hepatic triglyceride lipase: cDNA cloning, amino acid sequence and expression in a cultured cell line. *Differentiation*. **35**: 45-52.
2. Datta, S., C. C. Luo, W. H. Li, P. VanTuinen, D. H. Ledbetter, M. A. Brown, S. H. Chen, S. W. Liu, and L. Chan. 1988. Human hepatic lipase. Cloned cDNA sequence, restriction fragment length polymorphisms, chromosomal localization, and evolutionary relationships

- with lipoprotein lipase and pancreatic lipase. *J. Biol. Chem.* **263**: 1107-1110.
3. Shirai, K., R. L. Barnhart, and R. L. Jackson. 1981. Hydrolysis of human plasma high density lipoprotein 2-phospholipids and triglycerides by hepatic lipase. *Biochem. Biophys. Res. Commun.* **100**: 591-599.
4. Jansen, H., and W. C. Hulsmann. 1985. Enzymology and physiological role of hepatic lipase. *Biochem. Soc. Trans.* **13**: 24-26.
5. Tikkanen, M. J., and E. A. Nikkila. 1987. Regulation of hepatic lipase and serum lipoproteins by sex steroids. [Review]. *Am. Heart J.* **113**: 562-567.
6. Packard, C. J., J. Shepherd, G. M. Lindsay, A. Gaw, and M. R. Taskinen. 1993. Thyroid replacement therapy and its influence on post-heparin plasma lipases and apolipoprotein-B metabolism in hypothyroidism. *J. Clin. Endocrinol. Metab.* **76**: 1209-1216.
7. Baynes, C., A. D. Henderson, W. Richmond, D. G. Johnston, and R. S. Elkeles. 1992. The response of hepatic lipase and serum lipoproteins to acute hyperinsulinaemia in type 2 diabetes. *Eur. J. Clin. Invest.* **22**: 341-346.
8. Beshyah, S. A., A. Henderson, C. Baynes, D. Copping, W. Richmond, and D. G. Johnston. 1995. Post-heparin plasma lipase activity in hypopituitary adults. *Horm. Res.* **44**: 69-74.
9. Taskinen, M. R., E. A. Nikkila, R. Pelkonen, and T. Sane. 1983. Plasma lipoproteins, lipolytic enzymes, and very low density lipoprotein triglyceride turnover in Cushing's syndrome. *J. Clin. Endocrinol. Metab.* **57**: 619-626.
10. Perusse, L., T. Rice, J-P. Despres, J. Bergeron, M. A. Province, J. Gagnon, A. S. Leon, D. C. Rao, J. S. Skinner, J. H. Wilmore, and C. Bouchard. 1997. Familial resemblance of plasma lipids, lipoproteins and postheparin lipoprotein and hepatic lipases in the HERITAGE family study. *Arterioscler. Thromb. Vasc. Biol.* **17**: 3263-3269.
11. Kuusi, T., Y. A. Kesaniemi, M. Vuoristo, T. A. Miettinen, and M. Koskenvuo. 1987. Inheritance of high density lipoprotein and lipoprotein lipase and hepatic lipase activity. *Arteriosclerosis*. **7**: 421-425.
12. Guerra, R., J. P. 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.
13. 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 than in white American men: effects of 5' flanking polymorphism in the hepatic lipase gene. *J. Lipid. Res.* **39**: 228-232.
14. Katznel, L. I., P. J. Coon, M. J. Busby, S. O. Gottlieb, R.M. Krauss, and A.P. Goldberg. 1992. Reduced HDL₂ cholesterol subspecies and elevated postheparin hepatic lipase activity in older men with abdominal obesity and asymptomatic myocardial ischemia. *Arterioscler. Thromb.* **12**: 814-823.
15. 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.
16. Cominacini, L., U. Garbin, A. Davoli, M. Campagnola, S. A. De, Pasini, A. M. Pastorino, and O. Bosello. 1993. High-density lipoprotein cholesterol concentrations and postheparin hepatic and lipoprotein lipases in obesity: relationships with plasma insulin levels. *Ann. Nutr. Metab.* **37**: 175-184.
17. Sorbris, R., B. G. Pettersson, and P. Nilsson-Ehle. 1981. Effects of weight reduction on plasma lipoproteins and adipose tissue metabolism in obese subjects. *Eur. J. Clin. Invest.* **11**: 491-498.
18. Pollare, T., B. Vessby, and H. Lithell. 1991. Lipoprotein lipase activity in skeletal muscle is related to insulin sensitivity. *Arterioscler. Thromb.* **11**: 1192-1203.
19. Reitman, J. S., F. C. Kosmakos, B. V. Howard, M. R. Taskinen, T. Kuusi, and E. A. Nikkila. 1982. Characterization of lipase activities in obese Pima Indians. Decreases with weight reduction. *J. Clin. Invest.* **70**: 791-797.
20. Blades, B., G. L. Vega, and S. M. Grundy. 1993. Activities of lipoprotein lipase and hepatic triglyceride lipase in postheparin plasma of patients with low concentrations of HDL cholesterol. *Arterioscler. Thromb.* **13**: 1227-1235.
21. SAS Institutes Inc. 1989. SAS/STAT® User's Guide. Version 6, Fourth Edition, Volume 2. SAS Institute Inc., Cary, NC. 891-993.
22. Vermeulen, A. 1996. Decreased androgen levels and obesity in men. *Ann. Med.* **28**: 13-15.
23. Cooney, G. J., and L. H. Storlien. 1994. Insulin action, thermogenesis and obesity. *Baillieres. Clin. Endocrinol. Metab.* **8**: 481-507.