Hepatic lipase activity influences high density lipoprotein subclass distribution in normotriglyceridemic men: genetic and pharmacological evidence

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Abstract **Several studies have reported an inverse relationship between hepatic lipase activity and plasma high density lipoprotein (HDL) cholesterol concentrations. The purpose of the present study was to determine whether genetic and pharmacological variation in hepatic lipase activity alters the distribution of HDL subclasses. Two independent analytical methods (nuclear magnetic resonance and gradient gel electrophoresis) were used to compare HDL subclass distributions in 11 homozygotes for the −514C allele of hepatic lipase and in 6 homozygotes for the −514T allele. Mean hepatic lipase activity was 45 ± 15 mmol·l^{-1}·hr^{-1} in −514C homozygotes and 20 ± 7 mmol·l^{-1}·hr^{-1} in −514T homozygotes. Both analytical methods indicated that HDL_{2b} was significantly higher and HDL_{3a} was significantly lower in −514T homozygotes than in −514C homozygotes. No differences were noted in the other HDL fractions (HDL_{2a}, HDL_{3b}, and HDL_{3c}). To determine the effects of increased hepatic lipase activity, 20 men were given the synthetic anabolic steroid, stanozolol. Stanozolol treatment increased hepatic lipase activity more than two-fold (38 ± 18 to 85 ± 25 mmol·l^{-1}·hr^{-1}), and markedly reduced the plasma concentrations of the larger HDL subclasses (HDL_{2b} and HDL_{2a}). The plasma concentrations of the smallest HDL subclasses (HDL_{3b} and HDL_{3c}) were unchanged by stanozolol treatment. Taken together, these genetic and pharmacological data indicate that variation in hepatic lipase activity has highly specific effects on the distribution of HDL subclasses in the circulation.—**Grundy, S. M., G. L. Vega, J. D. Otvos, D. L. Rainwater, and J. C. Cohen. **Hepatic lipase activity influences high density lipoprotein subclass distribution in normotriglyceridemic men: genetic and pharmacological evidence. *J. Lipid Res.* 1999.** 40: **229–234.**

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Human hepatic lipase is a 476 amino acid glycoprotein that exhibits phospholipase A1 and triglyceride hydrolase activities (1, 2). The enzyme is synthesized in hepatocytes, secreted, and bound to hepatocyte and hepatic endothelial surfaces (3), from which it can be released into the circulation by heparin. Data from several clinical, pharmacological, and genetic studies support an inverse relationship between hepatic lipase activity and the plasma concentrations of high density lipoprotein cholesterol (HDL-C) in humans. The activity of hepatic lipase in postheparin plasma appears to be inversely correlated with plasma HDL-C concentrations among unselected individuals (4–8). Drugs such as anabolic steroids that increase hepatic lipase activity decrease HDL concentrations (9), whereas hepatic lipase deficiency is associated with high plasma HDL levels in some patients (10). Studies of normolipidemic families indicated linkage between plasma HDL concentrations and *LIPC*, the gene encoding hepatic lipase (11, 12). DNA sequencing revealed an *LIPC* allele (designated −514T) containing four linked polymorphisms in the 5′ flanking region. This allele was associated with low hepatic lipase activity (13) and increased plasma HDL-C concentrations in white men (12). These findings suggest that hepatic lipase plays a role in the catabolism of HDL, and that normal variation in hepatic lipase activity is associated with inter-individual differences in plasma HDL concentration.

Some studies have indicated that hepatic lipase activity is correlated with the plasma concentrations of specific HDL subfractions. Kuusi, Saarinen, and Nikkila (4) reported that hepatic lipase activity was inversely correlated with plasma concentrations of HDL_2, but not HDL_3 determined by ultracentrifugation. Katzel et al. (14) found that postheparin plasma hepatic lipase activity was inversely correlated with the proportion of HDL in the HDL_{2b} sub-

Abbreviations: HDL-C, high density lipoprotein cholesterol; GGE, gradient gel electrophoresis; NMR, nuclear magnetic resonance.

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species determined by gradient gel electrophoresis (GGE) in 41 healthy men. Johansson et al. (15) reported that hepatic lipase was inversely correlated with HDL_{2b} and positively correlated with HDL_{3b} (determined by GGE) in 24 normolipidemic infarct survivors. These observations are consistent with in vitro studies indicating that hepatic lipase acts preferentially on large HDL particles (16), and suggest that hepatic lipase plays a role in the remodeling of circulating HDL. However, all of the clinical studies performed to date were based on correlations observed between two phenotypes, hepatic lipase activity and HDL subclass distribution. Such correlations are subject to confounding by secondary factors and do not constitute definitive evidence for a causal relationship between hepatic lipase activity and plasma HDL-C concentrations. For example, plasma triglyceride concentrations are positively correlated with hepatic lipase activity (5) and inversely related to plasma HDL-C concentrations (17–20). Therefore a correlation between hepatic lipase activity and plasma HDL size among unselected individuals may simply reflect the interactions of plasma triglyceride concentrations (or some other confounder) with both parameters.

Secondary confounding can be avoided by studying individuals with genetic (i.e., primary) variation in hepatic lipase activity. Detailed analysis of HDL subclass distribution in such subjects is limited to a single report by Knudsen et al. (20), who studied a compound heterozygote for two hepatic lipase gene mutations. Hepatic lipase activity in this individual was decreased by 70% compared to healthy control men. Density gradient ultracentrifugation of his HDL revealed markedly increased concentrations of the HDL_{2a} and HDL_{2b} subclasses. The present study was undertaken to determine whether variation in hepatic lipase activity alters the distribution of HDL subclasses. To minimize confounding by secondary factors, HDL subclass distributions were measured in normotriglyceridemic men with genetically defined differences in hepatic lipase activity. To ensure reliable estimation of HDL subclass distribution, two independent analytical methods (nuclear magnetic resonance and gradient gel electrophoresis) were used. To support the findings of this genetic approach, the effects of pharmacological stimulation of hepatic lipase activity on HDL size distribution was determined.

METHODS

Experimental design

Fasting blood samples were drawn from 21 normolipidemic white men. Six of the men were homozygous for the −514T allele of *LIPC*, four were −514C/−514T heterozygotes, and 11 were −514C homozygotes. Five of the −514T homozygotes and five of the −514C homozygotes had been matched for plasma HDL-C concentrations in a previous study. Aliquots of plasma were stored at 4°C for lipid and lipoprotein measurement, and at −80°C for HDL subclass analysis. Heparin injections for determination of postheparin plasma hepatic lipase activity were performed after an overnight fast. Twenty of the men then took 0.2 mg/kg per day of the oral synthetic anabolic steroid stanozolol (Winstrol, Winthrop, Pittsburg, PA) up to a maximum of 16 mg/day for 6 consecutive days. Compliance was monitored by pill counting. The drug was well tolerated by all of the men, and no side effects were reported. A second heparin injection was performed within 24 h after ingestion of the final dose of stanozolol.

Subjects

The study was approved by the Institutional Review Board at the University of Texas Southwestern Medical Center. Men aged 20 to 50 years who were apparently healthy, who had plasma triglyceride concentrations below 200 mg/dL, and who did not smoke or use lipid-lowering drugs or beta blockers were recruited by advertisements placed on notice boards at Southwestern Medical Center. Subjects were studied as outpatients on their ad libitum diets.

Hepatic lipase genotyping

Hepatic lipase genotypes were determined by PCR amplification and restriction enzyme digestion as described in detail previously (12).

Assay of hepatic lipase activity

Hepatic lipase activity was measured in postheparin plasma using a modification of the method reported by Baginsky and Brown (22) as described in detail previously (8). Briefly, patients received an intravenous injection of 75 IU of porcine heparin (Elkins-Sinn Inc.) per kg of body weight after a 12-h fast. Blood samples were drawn into tubes containing EDTA before and 15 min after injection. Plasma was separated immediately upon collection of blood and aliquots were frozen at −70°C. Hepatic lipase activity was measured by addition of 0.025 mL postheparin plasma to 0.475 mL medium (pH 8.8) containing NaCl (0.75 m), a gum arabic-stabilized emulsion of triolein (final concentration 15 mmol/L) labeled with [^{3}H]triolein (0.5 μCi), and fatty acid-free bovine serum albumin (final concentration 50 g/L). The free [^{3}H]oleate generated during the hydrolysis reactions was extracted using the method of Belfrage and Vaughan (23). The intra- and inter-assay coefficients of variation were <5%.

Assay of plasma lipids and lipoproteins

Plasma triglyceride, cholesterol, and HDL-C concentrations were measured using standard enzymatic assays as described previously (12). Plasma LDL-C concentrations were calculated using the Friedewald formula.

Analysis of HDL size and subclass distribution

HDL size and subclass distributions were measured using two independent methods, gradient gel electrophoresis and nuclear magnetic resonance.

Gradient gel electrophoresis (GGE). Aliquots of plasma (drawn before heparin injection) were stored at −80°C and shipped on dry ice to Dr. Rainwater's laboratory. Lipoproteins in plasma were subjected to electrophoresis in nondenaturing composite gradient gels as described previously (24). After electrophoresis, lipoprotein cholesterol was stained using Sudan black B, and absorbance profiles were determined with an LKB-Ultroscan XL laser densitometer. HDL particle sizes were calibrated using a standard curve that included albumin, lactate dehydrogenase, ferritin, and thyroglobulin (Pharmacia). Estimates of median HDL diameter were obtained from the absorbance profiles by determining the particle diameter at which half of the absorbance comes from larger and half from smaller HDL particles. The relative cholesterol content of the HDL subclasses was determined by dividing the lipoprotein absorbance profile into five size intervals: HDL_{3c}, 7.2–7.8 nm; HDL_{3b}, 7.8–8.2 nm; HDL_{3a}, 8.2–8.8 nm;

HDL_{2a}, 8.8–9.7 nm; and HDL_{2b}, 9.7–12.9 nm. Each sample was repeated at least twice on different gels and the average values were analyzed.

Nuclear magnetic resonance (NMR). Aliquots of plasma drawn before heparin injection were stored at −80°C and shipped on dry ice to Dr. Otvos' laboratory. Lipoprotein subclass profiles were measured by proton NMR spectroscopy as described previously (25, 26). This method exploits the fact that each lipoprotein particle in plasma broadcasts a distinct lipid NMR signal, the intensity of which is proportional to its lipid mass concentration. By acquiring NMR spectra of each plasma specimen (0.7 ml) on a dedicated 360 MHz spectrometer (Analogic Corp.) under defined conditions (47°C), and then deconvoluting the composite lipid methyl group signal envelope that appears in the spectrum at ~0.8 ppm, the concentrations of 15 subclasses of VLDL, LDL, and HDL are derived simultaneously. The diameter ranges of the five HDL subclasses quantified by NMR were determined by calibration using purified HDL subclasses characterized by GGE. The names given to the NMR-derived HDL subclasses and their average diameters are as follows: H1, 7.5 ± 0.2 nm; H2, 8.0 ± 0.2 nm; H3, 8.5 ± 0.3 nm; H4, 9.4 ± 0.6 nm; H5 11.5 ± 1.5 nm. The size ranges of these subclasses match those measured by GGE, therefore they will be referred to as HDL_{3c}, HDL_{3b}, HDL_{3a}, HDL_{2a}, and HDL_{2b} respectively. Concentrations of the HDL subclasses are expressed in units of cholesterol (mg/dL) and mean HDL particle size was determined by weighting the relative percentage of each HDL subclass by its diameter.

Statistical methods

Correlations between hepatic lipase activity, plasma triglyceride concentrations, HDL size, subclass concentrations, and relative subclass distributions were determined using Spearmans rank procedure. The mean and median values of each measured parameter in −514C and −514T homozygotes were compared using unpaired *t*-tests and Wilcoxon rank tests, respectively. The effects of stanozolol treatment on the mean and median values of each parameter were assessed using paired *t*-tests and paired Wilcoxon rank tests.

RESULTS

Hepatic lipase genotype and HDL size distribution

Hepatic lipase activity was significantly lower in the 6 men who were −514T homozygotes than in the 11 men who were −514C homozygotes (**Table 1**). Plasma triglyceride, cholesterol, and HDL cholesterol concentrations were similar in the two groups (Table 1). HDL sizes tended to be larger in −514T than in −514C homozygotes, but the differences did not reach statistical significance (**Table 2**). Both NMR and GGE indicated that the proportion of HDL-C in the largest HDL subclass (HDL_{2b}) was significantly higher, and the proportion of HDL-C in the HDL_{3a} subclass was significantly lower in homozygotes for the −514T allele than in homozygotes for the −514C allele (Table 2).

TABLE 1. Plasma lipid and lipoprotein concentrations in normolipidemic men with the −514C or −514T hepatic lipase genotypes

Variable	−514C (n = 11)	−514T (n = 6)
Body Mass Index ($kg \cdot m^{-2}$)	26 ± 3	25 ± 2
Hepatic lipase activity ($mmol \cdot h^{-1} \cdot l^{-1}$)	45 ± 15[a]	20 ± 7
Total cholesterol (mg/dl)	162 ± 26	173 ± 25
Total triglyceride (mg/dl)	95 ± 36	78 ± 30
LDL-C (mg/dl)	107 ± 28	120 ± 31
HDL-C (mg/dl)	40 ± 7	43 ± 7

Plasma total cholesterol, triglyceride, LDL, and HDL concentrations were determined by standard enzymatic methods. Very similar estimates of these parameters were obtained by NMR spectroscopy (not shown).

[a] $P < 0.01$ for −514C versus −514T (Wilcoxon ranked sum test).

TABLE 2. HDL size and subclass distributions in normolipidemic men with the −514C or −514T hepatic lipase genotypes

	−514C (n = 11)	−514T (n = 6)	*P* value
NMR			
HDL size (nm)	8.7 ± 0.4	9.0 ± 0.4	0.36
HDL_{2b} (mg/dl)	4.6 ± 2.5	7.4 ± 3.2	0.04
HDL_{2a} (mg/dl)	6.6 ± 7.8	6.3 ± 4.7	0.95
HDL_{3a} (mg/dl)	11.0 ± 3.5	6.1 ± 2.1	0.01
HDL_{3b} (mg/dl)	9.4 ± 5.7	8.8 ± 6.3	0.88
HDL_{3c} (mg/dl)	7.6 ± 4.8	10.9 ± 6.1	0.26
GGE			
HDL size (nm)	8.6 ± 0.3	8.9 ± 0.3	0.14
HDL_{2b} (%)	17 ± 8	33 ± 7	0.005
HDL_{2a} (%)	22 ± 6	20 ± 3	0.60
HDL_{3a} (%)	32 ± 6	24 ± 3	0.007
HDL_{3b} (%)	22 ± 8	17 ± 6	0.21
HDL_{3c} (%)	7 ± 5	7 ± 5	0.90

P values were determined by Wilcoxon rank sum test.

Effects of stanozolol on hepatic lipase activity and HDL size distribution

Stanozolol administration markedly increased hepatic lipase activity and decreased HDL-C concentrations in all 20 men. Plasma triglyceride and LDL-C concentrations were not significantly altered by the drug (**Table 3**). Mean HDL size was reduced in all of the men. NMR spectroscopy revealed that the decrease in HDL size reflected a decrease in the plasma concentrations of the larger HDL subclasses (HDL_{2b} and HDL_{2a}) (**Table 4**). The plasma concentrations of the smallest HDL subclasses (HDL_{3b} and HDL_{3c}) were not affected by stanozolol treatment.

Correlation between HDL sizes determined by NMR and GGE

The values of the mean HDL diameters obtained using the two methods were similar in magnitude (means and standard deviations were 8.8 ± 0.4 and 8.7 ± 0.3 nm for NMR and GGE, respectively) and were highly correlated ($r = 0.88$). The percentages of HDL in each subclass

TABLE 3. Effects of stanozolol on plasma lipid and lipoprotein concentrations in 20 normolipidemic men

	Hepatic Lipase	Cholesterol	Triglyceride	LDL-C	HDL-C
	mmol·l⁻¹·h⁻¹		*mg/dl*		
Baseline	38 ± 18[a]	164 ± 29	117 ± 48	111 ± 29	39 ± 7[a]
Stanozolol	87 ± 25	154 ± 44	106 ± 47	118 ± 41	23 ± 5

Values in the table are means ± standard deviations from 20 men given stanozolol (0.2 mg/kg/day to a maximum of 16 mg/day) orally for 6 days.

[a] $P < 0.001$ (paired Wilcoxon ranked sum tests).

TABLE 4. Effects of stanozolol on HDL size and subclass distributions in 20 normolipidemic men

	Baseline	Stanozolol	*P* Value
NMR			
HDL size (nm)	8.8 ± 0.4	8.4 ± 0.3	0.0001
HDL_{2b} (mg/dl)	4.9 ± 2.8	1.9 ± 1.5	0.0001
HDL_{2a} (mg/dl)	7.1 ± 6.7	0.8 ± 1.4	0.0003
HDL_{3a} (mg/dl)	9.5 ± 3.2	6.1 ± 3.2	0.0014
HDL_{3b} (mg/dl)	10.4 ± 5.4	9.1 ± 5.1	0.25
HDL_{3c} (mg/dl)	7.1 ± 4.2	5.0 ± 4.0	0.25
GGE			
HDL size (nm)	8.7 ± 0.3	8.3 ± 0.2	0.0001
HDL_{2b} (%)	19 ± 10	15 ± 5	0.14
HDL_{2a} (%)	22 ± 7	12 ± 7	0.0001
HDL_{3a} (%)	32 ± 7	24 ± 8	0.003
HDL_{3b} (%)	21 ± 9	36 ± 9	0.0001
HDL_{3c} (%)	6 ± 4	13 ± 6	0.0001

Values in the table are means ± standard deviations from 20 men given stanozolol (0.2 mg/kg/day to a maximum of 16 mg/day) orally for 6 days. *P* values were determined using paired Wilcoxon ranked sum tests.

(HDL_{2b}, HDL_{2a}, HDL_{3a}, HDL_{3b}, HDL_{3c}) determined using NMR were 14 ± 7, 14 ± 13, 24 ± 9, 25 ± 15, and 23 ± 16, respectively. The corresponding percentages determined using GGE were 22 ± 10, 21 ± 6, 31 ± 7, 20 ± 8, and 7 ± 4, respectively.

Correlations between hepatic lipase activity, plasma lipid and lipoprotein concentrations, and HDL size distribution

Hepatic lipase activity was negatively correlated with HDL-C but the correlation coefficient observed did not reach statistical significance at the 5% confidence level (**Table 5**). Strong, statistically significant negative correlations were observed between hepatic lipase activity and the plasma concentrations of the larger HDL subclasses (HDL_{2b} and HDL_{2a}) (Table 5). Hepatic lipase activity was not significantly correlated with the concentrations of small HDL (HDL_{3b} and HDL_{3c}) determined by NMR but was positively correlated with the percentage of HDL in these subclasses as determined by GGE. Plasma triglyceride concentrations were significantly negatively correlated with HDL-C concentration, and with HDL size and subclass distributions (Table 5). Hepatic lipase activity was positively correlated with plasma triglyceride concentrations, but not with plasma or LDL cholesterol concentrations.

TABLE 5. Correlations between hepatic lipase activity, plasma lipid and lipoprotein concentrations, and HDL size and subclass distribution determined by NMR and GGE in 21 normolipidemic men

	Hepatic Lipase Activity	Plasma Triglyceride Concentration
Plasma total cholesterol	0.16	0.44
Plasma triglyceride	0.56	1
LDL-C	0.07	0.29
HDL-C	−0.33	−0.57
NMR		
HDL size	−0.64	−0.71
HDL_{2b}	−0.64	−0.55
HDL_{2a}	−0.41	−0.69
HDL_{3a}	0.49	0.29
HDL_{3b}	0.17	0.20
HDL_{3c}	0.04	0.39
GGE		
HDL size	−0.70	−0.72
HDL_{2b}	−0.62	−0.59
HDL_{2a}	−0.47	−0.74
HDL_{3a}	0.37	0.19
HDL_{3b}	0.64	0.75
HDL_{3c}	0.59	0.68

Correlation coefficients greater than 0.37 or less than −0.37 are statistically significant ($P < 0.05$).

DISCUSSION

HDL is a heterogenous group of lipoproteins that differ in size and composition (27–29). Several factors including gender, diet, and hypolipidemic drugs preferentially influence specific HDL subclasses, but the metabolic determinants of HDL subclass distribution have not been fully elucidated. In the present study, the relationship between hepatic lipase activity and HDL subclass distribution was investigated using proton NMR and GGE techniques. The study included two components. First, the subclass distribution of HDL was compared in normotriglyceridemic white men who were homozygotes for either the −514T or the −514C allele of hepatic lipase. Homozygotes for the −514T allele, who have genetically low hepatic lipase activity (13), had higher plasma concentrations of the largest HDL subclass, HDL_{2b}, and lower plasma concentrations of a smaller HDL subclass, HDL_{3a}, than did homozygotes for the −514C allele. These differences were independent of plasma triglyceride and total HDL concentrations, which were similar in −514T and −514C homozygotes as a consequence of the selection procedure. This finding provides direct evidence that primary variation in hepatic lipase activity influences HDL subclass distribution. Second, the synthetic anabolic steroid stanozolol was used to increase hepatic lipase activity. Stanozolol treatment markedly decreased the plasma concentrations of the largest HDL subclasses (HDL_{2b} and HDL_{2a}) but did not affect concentrations of the smallest HDL subclasses (HDL_{3b} and HDL_{3c}). Taken together, our data indicate that variation in hepatic lipase activity within the normal range of the population has highly specific effects on HDL size distribution among normotriglyceridemic men.

The results of the present study support and extend the findings of previous studies in two respects. First, by comparing normotriglyceridemic men with genetically defined differences in hepatic lipase activity, the problem of confounding by secondary factors is avoided. The mean hepatic lipase activity of the −514T homozygotes in this study corresponds to the 10th percentile for white men studied in our laboratory, whereas the mean value for the −514C homozygotes is equivalent to the 65th percentile (J. C. Cohen, unpublished results). This difference in hepatic lipase activity is due exclusively to DNA sequence differences between the −514T and −514C al-

leles, and is independent of secondary factors such as obesity or insulin resistance. Plasma triglyceride concentrations, which have a major influence on lipoprotein size distributions (30, 31), were similar in the two genotype groups. Therefore the differences in HDL size distribution between −514T and −514C homozygotes are almost certainly the direct result of genetic differences in their hepatic lipase activities.

Second, most previous studies have examined the relationship between hepatic lipase and the two major HDL subclasses, HDL_2 and HDL_3 (4, 6, 32). The methods used in the present study allowed further resolution of HDL into five subclasses, and indicated that primary variation in hepatic lipase activity within the normal range of the population is associated with highly specific differences in HDL subclass distribution. Homozygotes for the −514T allele had higher plasma concentrations of HDL_{2b} and lower concentrations of HDL_{3a} compared with −514C homozygotes. Highly consistent results were obtained using two independent methods (NMR and GGE) therefore it is extremely unlikely that this result is an artifact of the analytical procedure. Accordingly, this finding indicates that low hepatic lipase activity leads to high plasma concentrations of the HDL_{2b} subclass, and a relative decrease in the concentration of the HDL_{3a} subclass. The plasma concentrations of the other three HDL subclasses (HDL_{2a}, HDL_{3b}, and HDL_{3c}) do not appear to be significantly affected by a low hepatic lipase activity.

To date no hepatic lipase allele associated with markedly elevated hepatic lipase activity has been identified. Accordingly we analyzed the effects of pharmacological stimulation of hepatic lipase activity on HDL subclass distribution using the synthetic anabolic steroid, stanozolol. After 6 days of stanozolol treatment, mean hepatic lipase activity increased more than 2-fold, reaching the upper end of the normal range (>95th percentile) for white men measured in our laboratory. Mean plasma HDL-C decreased by more than 40%. NMR spectroscopy indicated that the major decrease occurred in the larger HDL subclasses (HDL_{2b} and HDL_{2a}) with a smaller decrease in HDL_{3a} and no change in the plasma concentrations of the smallest HDL subfractions, HDL_{3b} and HDL_{3c}. This resulted in a decrease in the proportion of lipid staining in the larger HDL, and an increase in the proportion of lipid staining in smaller HDL, as indicated by GGE. The observation that the plasma concentrations of the smallest HDL subclasses (HDL_{3b} and HDL_{3c}) were not affected by stanozolol treatment despite a 2-fold increase in hepatic lipase activity clearly indicates that variation in hepatic lipase activity does not affect these lipoprotein subclasses. This finding is consistent with the observation that plasma concentrations of HDL_{3b} and HDL_{3c} were similar in men with genetically defined differences in hepatic lipase activity. The decreased concentrations of the larger HDL subclasses during stanozolol treatment are consistent with the notion that high hepatic lipase activity leads to a decrease in the plasma concentrations of large HDL. However, a recent study in two hepatic lipase-deficient patients indicates that the effect of stanozolol on plasma HDL concentrations and subclass distribution cannot be fully accounted for by changes in hepatic lipase activity (33). In that study, Bausserman, Saritelli, and Herbert (33) found that 7 days of stanozolol treatment (a duration similar to that used in the present study) led to a decrease in HDL_2 concentrations in one patient but not in the other. After 14 days of stanozolol treatment, plasma concentrations of HDL_2 decreased by 40% in one patient and by 59% in the other, compared with a 90% decrease in normal control subjects. Although the plasma lipid and lipoprotein concentrations of these two hepatic lipase-deficient patients differed considerably from those of the men in the present study (both patients were hypertriglyceridemic and both had a marked preponderance of HDL_2 in their HDL fraction), these findings suggest that the effects of stanozolol on the HDL_{2b} and HDL_{2a} subclasses observed in the present study may not be entirely due to increased hepatic lipase activity.

The HDL_{2b} subclass identified by GGE is equivalent to the HDL_2 subclass isolated by zonal ultracentrifugation (34, 35). This subclass is enriched in all lipid constituents (free and unesterified cholesterol, phospholipids, and triglycerides) and has a lower protein content relative to HDL_3 (35, 36). Interestingly, the apolipoprotein A-II content of HDL_2 is markedly reduced relative to HDL_3 (36). The results of the present study, therefore, suggest that large, lipid-enriched HDL that are relatively deficient in apolipoprotein A-II accumulate in men with low hepatic lipase activity, and are preferentially catabolized in men with high hepatic lipase activity.

In summary, the results of the present study provide direct evidence that normal variation in hepatic lipase activity is associated with specific differences in the subclass distribution of HDL among normolipidemic men. Low hepatic lipase activity leads to a specific increase in the largest HDL subclass, HDL_{2b}. Our data suggest that high hepatic lipase activity leads to low plasma concentrations of the HDL_{2b} and HDL_{2a} subclasses, although direct evidence for this association must await the identification of a hepatic lipase allele that confers genetically high hepatic lipase activity. Finally, our data clearly indicate that wide variation in hepatic lipase activity has little effect on the plasma concentrations of the smallest HDL particles, HDL_{3b} and HDL_{3c}.

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