Influence of Hepatic Steatosis (Fatty Liver) on Severity and Composition of Dyslipidemia in Type 2 Diabetes

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OBJECTIVE — The objective of this study was to examine the associations between the severity of hepatic steatosis and dyslipidemia in type 2 diabetes, including circulating apolipoprotein B100 (apoB) concentrations and lipoprotein particle size and numbers.

RESEARCH DESIGN AND METHODS — Computed tomography imaging was used to assess hepatic fat content and adipose tissue distribution in 67 men and women with type 2 diabetes, withdrawn from antidiabetic medications preceding the study. Fasting serum lipoprotein number and size was determined by nuclear magnetic resonance. Insulin sensitivity was measured with a glucose clamp and a [6,6-$^2H_2$]glucose isotope infusion.

RESULTS — Two-thirds of the cohort had fatty liver. Hepatic steatosis correlated with serum triglycerides ($r = 0.40$, $P < 0.01$) and lower HDL cholesterol ($r = -0.31$, $P < 0.05$). ApoB and LDL cholesterol did not, being virtually identical in those with or without steatosis. The association between serum triglycerides and hepatic steatosis was largely accounted for by greater triglyceride enrichment in VLDL particles, which were larger. Severe steatosis was also associated with 70% higher small, dense LDL concentrations. Visceral obesity did not fully explain these associations, and hepatic steatosis was better correlated with triglycerides than with hyperglycemia or hepatic insulin resistance ($P > 0.05$).

CONCLUSIONS — The presence of hepatic steatosis in type 2 diabetes does not appear to affect apoB levels, but potentially increases atherogenesis by increasing triglycerides, reducing HDL levels, and increasing small, dense LDL.

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Patients with type 2 diabetes typically have an atherogenic serum lipid profile that is characterized by hypertriglyceridemia, low HDL, small, dense LDL particles, and increased apolipoprotein B100 (apoB) concentrations (1–3). The central abnormality appears to be an increased rate of hepatic triglyceride synthesis and VLDL particle production, which results in secondary abnormalities of low HDL and increased LDL particle number and density. Insulin resistance is regarded as a major driving force for dyslipidemia, with one mechanism being an increase in free fatty acid (FFA) release, stimulating hepatic triglyceride output (2,4–7). Increased availability of FFAs within the liver also inhibits apoB degradation, stabilizing the formation of more VLDL (4).

In the current study, we were interested in the role of hepatic steatosis as an additional modifier and predictor of the severity of dyslipidemia in type 2 diabetes. Hepatic steatosis, also known as "fatty liver" or nonalcoholic fatty liver disease, is frequently observed in type 2 diabetes (8–11). In humans, it is recognized that hepatic steatosis is a predictor of insulin resistance (12–14), and it is also associated with hypertriglyceridemia in nondiabetic subjects (14). In type 2 diabetes, this association has also been reported (15). However, it remains unclear whether hepatic steatosis primarily induces an increased number of VLDL particles, as would be reflected in greater plasma apoB concentrations or primarily influences the triglyceride content of VLDL particles. The current study was undertaken to more precisely characterize the association between hepatic steatosis and the proatherogenic lipid profile in type 2 diabetes.

RESEARCH DESIGN AND METHODS

— The protocol was approved by the University of Pittsburgh Institutional Review Board, and research volunteers gave written informed consent. Participants were recruited for two separate clinical investigations, as earlier described (15). Briefly, inclusion criteria included a BMI >27 kg/m^2, a confirmed diagnosis of type 2 diabetes (<5 years of duration), and current treatment with a sulfonylurea, repaglinide, or metformin at submaximal doses (alone or in combination) or diet alone; good general health other than type 2 diabetes; no known cardiovascular disease; and a stable weight (<3 kg variation in the preceding 2 months). Exclusion criteria included elevations of serum transaminase or alkaline phosphatase, therapy with insulin, a thiazolidinedione, or fibrates, serum triglycerides >4.52 mmol/l (400 mg/dl), a history of hepatic disease, substance abuse, or daily consumption of more than one alcohol drink or the equivalent in beer and wine. Statin users were 13.4% of the cohort and uniformly distributed across tertiles of hepatic steatosis. Before metabolic and body composition measurements, participants were asked to withdraw from current diabetes medications for a 4-week baseline period. A dietitian met with participants to advise

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Abbreviations: apoB, apolipoprotein B100; EGP, endogenous glucose production; FFA, free fatty acid; IDL, intermediate-density lipoprotein; L/S ratio, ratio of liver to spleen; NMR, nuclear magnetic resonance; VAT, visceral adipose tissue.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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them to follow a weight maintenance nutritional plan with a balanced macronutrient composition. After this period, volunteers were admitted to the University of Pittsburgh General Clinical Research Center, where they received a standardized dinner (7 kcal/kg; 50% carbohydrate, 30% fat, and 20% protein) and fasted overnight before biochemical, metabolic, and body composition studies in the following morning.

Lipoprotein measurements

On the morning after admission, plasma and serum were obtained and stored at −70°C before biochemical analysis. Serum apoB concentration was determined by a variation of the Boehringer Mannheim turbidimetric procedure. HDL cholesterol was determined after selective precipitation by heparin-manganese chloride and removal by centrifugation of VLDL and LDL (16). Serum triglycerides (17) and total cholesterol (18) were determined as previously described. Plasma VLDL, LDL, and HDL particle number and size were determined by nuclear magnetic resonance (NMR) spectroscopy (NMR LipoProfile; LipoScience, Raleigh, NC). Particle subclasses were defined as follows: VLDL (>27–35 nm); intermediate-density lipoprotein (IDL) (23–27 nm); large LDL (21.2–23 nm); and small LDL (18–21.2 nm).

Body composition assessments

Fat and lean mass were determined by dual-energy X-ray absorptiometry (15,19). Computed tomography (CT) was used to measure hepatic steatosis and the cross-sectional area of adipose tissue in the abdomen and midthigh, as previously described in detail (15,19). Prior studies have shown a strong linear correlation between CT attenuation values in the liver and fatty infiltration measured by biopsy in a wide range of steatosis (20–23). The ratio of liver to spleen attenuation (L/S ratio) is a normalized index, with an L/S ratio <1 considered to represent fatty liver (20). Liver CT attenuations were determined by calculating the mean Hounsfield unit of three regions of interest of 120 mm^2 in the liver (two in the right lobe and one in the left lobe) and that of spleen also, on the basis of the mean Hounsfield unit of three regions of interest of 75 mm^2.

Insulin sensitivity. Euglycemic clamps were used to measure insulin sensitivity, as previously described (24). Insulin was infused at 40 $mU \cdot m^{-2} \cdot min^{-1}$ for 4 h. Plasma glucose was checked every 5 min and maintained at 100 mg/dl with a variable-rate dextrose infusion. To measure endogenous glucose production (EGP) (25), a primed (200 mg/m^2), continuous (2 $mg \cdot min^{-1} \cdot m^{-1}$) infusion of [6,6-$^2H_2$]glucose was given. [6,6-$^2H_2$]Glucose enrichment was determined by gas chromatography/mass spectrometry, as described previously (15). Plasma glucose was measured by the glucose-oxidase method (Yellow Springs Instruments, Yellow Springs, OH). FFAs were measured by an enzymatic method, and insulin was measured by a double-antibody radioimmunoassay (15).

Table 1—*Patient characteristics stratified by L/S ratio*

	L/S ratio tertiles		
	Upper: normal	Mid: moderate steatosis	Lower: severe steatosis
n	22	23	22
Range of L/S ratio	1.006–1.346	0.793–0.997	0.153–0.784
L/S ratio	1.16 ± 0.02	0.89 ± 0.01*	0.64 ± 0.03*†
Percent women	59	61	55
Age (years)	52.87 ± 1.71	50.34 ± 1.45	52.32 ± 1.84
A1C (%)	8.27 ± 0.30	8.45 ± 0.38	7.88 ± 0.28
Fasting glucose (mmol/l)	10.00 ± 0.48	10.23 ± 0.56	10.10 ± 0.61
Fasting insulin (pmol/l)	76.20 ± 17.52	109.4 ± 11.16‡	114.1 ± 10.50‡
Fasting FFAs (mmol/l)	0.64 ± 0.03	0.74 ± 0.03	0.67 ± 0.03
BMI (kg/m^2)	30.91 ± 0.91	36.52 ± 1.31‡	33.87 ± 0.85
Percent body fat	35.15 ± 2.07	40.90 ± 1.54	36.74 ± 1.36
STAT (cm^2)	113.77 ± 13.55	135.62 ± 13.74	102.49 ± 10.21
SAT (cm^2)	403.27 ± 30.35	459.24 ± 25.58	409.49 ± 25.06
VAT (cm^2)	176.38 ± 15.97	271.99 ± 19.35*	273.36 ± 16.03*

Data are means ± SE. *$P < 0.01$ vs. normal group. †$P < 0.05$ for marked vs. moderate steatosis groups. ‡$P < 0.05$ vs. normal group. SAT, subcutaneous abdominal (adipose) tissue; STAT, subcutaneous thigh adipose tissue.

Statistics

Data are presented as means ± SE, unless otherwise indicated. *P* values <0.05 were considered significant. ANOVA was used to test for differences across tertiles. Spearman analysis was used to determine correlations between variables.

RESULTS — The clinical characteristics of the research volunteers are shown in Table 1. The 67 participants were stratified according to tertiles of L/S ratio, which is inversely and linearly proportional to hepatic fat content (20,21). All subjects in the tertile with the lowest hepatic fat content had an L/S ratio >1.0, consistent with normal hepatic fat content (20), and thus this group is referred to as "normal" to denote the absence of fatty liver disorder. Subjects in the other tertiles had L/S ratios consistent with fatty liver and were labeled as "moderate" and "severe" to denote different degrees of hepatic steatosis.

Age and sex distributions were similar across the three groups. HbA_{1c} (A1C), fasting plasma glucose, and FFAs were also similar among groups. Fasting insulin was higher in moderate and severe steatosis. Mean BMI was in the obese range and higher in the moderate group, but the percentage of weight accounted for by fat mass (percent body fat) was similar across groups. In terms of regional fat distribution, visceral adipose tissue (VAT) was significantly greater in the moderate and severe groups, but the amounts of abdominal subcutaneous adipose tissue and thigh subcutaneous adipose tissue were similar across groups. VAT was correlated with the L/S ratio ($r = -0.47$, $P < 0.01$).

Serum lipid profiles

Compared with the normal group, a pattern of increased serum triglycerides and reduced HDL cholesterol was seen in those with moderate and severe steatosis (Table 2). Although there were small differences in triglycerides and HDL cholesterol between the moderate and severe groups, these differences were not statistically significant.

ApoB concentrations were similar across groups, indicating that although hepatic steatosis is associated with greater serum triglyceride concentrations, this is not accompanied by an increase in plasma

Table 2—*Serum lipoprotein profile, particle size, and numbers*

	Normal	Moderate steatosis	Severe steatosis
Serum lipids (mmol/l)			
Triglycerides	1.57 ± 0.12	2.07 ± 0.15*	2.28 ± 0.20†
HDL cholesterol	1.28 ± 0.06	1.12 ± 0.05*	1.01 ± 0.04†
LDL cholesterol	3.25 ± 0.16	3.38 ± 0.17	3.25 ± 0.16
Total cholesterol	5.12 ± 0.17	5.27 ± 0.19	5.05 ± 0.19
ApoB (g/l)	0.94 ± 0.05	1.04 ± 0.04	1.03 ± 0.05
Particle size (nm)			
VLDL	53.37 ± 1.40	58.73 ± 2.34*	60.88 ± 1.82*
LDL	21.23 ± 0.18	20.95 ± 0.26	20.35 ± 0.20*
HDL	8.74 ± 0.10	8.52 ± 0.09*	8.33 ± 0.05*‡
Particle concentration			
Chylomicrons (nmol/l)	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.01
VLDL (nmol/l)	71.20 ± 7.60	77.88 ± 5.43	78.77 ± 6.90
LDL (μmol/l)	1.22 ± 0.08	1.38 ± 0.07	1.49 ± 0.10
Small, dense LDL	0.65 ± 0.08	0.81 ± 0.11*	1.12 ± 0.12†
Large, buoyant LDL	0.51 ± 0.05	0.49 ± 0.08	0.30 ± 0.05*‡
IDL	0.06 ± 0.01	0.08 ± 0.01	0.08 ± 0.01
HDL (μmol/l)	23.34 ± 1.66	22.95 ± 1.95	23.19 ± 1.58

Data are means ± SE. *$P < 0.05$, †$P < 0.01$ vs. normal group; ‡$P < 0.05$ for severe vs. moderate steatosis groups.

apoB-containing lipoproteins. LDL particles account for the majority of circulating apoB-containing lipoproteins, and, as expected, LDL and total cholesterol were similar across groups too. ApoB concentrations in this cohort of individuals with type 2 diabetes were significantly greater than the mean value observed in lean, nondiabetic individuals. Even in the normal group, the mean apoB concentration (0.94 ± 0.05 g/l) was significantly greater than the mean obtained from a separate group of 12 lean, insulin-sensitive, nondiabetic subjects with a mean age of 41.6 ± 1.9 years (0.73 ± 0.04 g/l, $P <$ 0.05, not shown in the table).

Lipoprotein composition

To further examine the relationship between hepatic steatosis and lipoprotein composition and concentration, NMR spectroscopy of lipoprotein subfractions was performed in 19 randomly selected subjects from each group (Table 2). VLDL particles were larger in the moderate and severe steatosis groups compared with the normal group. Yet, the number of circulating VLDL particles was statistically indistinguishable across groups. This suggests that the association between hepatic steatosis and hypertriglyceridemia in type 2 diabetes is mostly accounted for by increased triglyceride content per VLDL particle. The increase in serum triglycerides with hepatic steatosis could not be attributed to chylomicrons, as these were nearly absent. We also observed that smaller LDL and HDL particles were associated with hepatic steatosis. However, the numbers of LDL and HDL particles did not differ. As noted in Table 2, severe steatosis was also associated with a 70% greater small, dense LDL particle concentration than in the normal group, and this was associated with a reciprocal lowering in large LDL. IDL subclass was not influenced by severity of hepatic steatosis.

Strength of correlation between serum lipids and hepatic steatosis

As shown in Fig. 1*A*, the L/S ratio, which is inversely proportional to hepatic fat, was significantly correlated with serum triglycerides ($r = -0.40$, $P < 0.01$). It also correlated with HDL cholesterol ($r =$ 0.31, $P < 0.05$) but not with LDL cholesterol or apoB ($P > 0.05$). VAT also correlated with serum triglycerides ($r = 0.51$, $P < 0.01$) and weakly with apoB ($r =$ 0.26, $P < 0.05$), although not with HDL cholesterol.

As shown in Fig. 1*A*, CIs in the association between hepatic steatosis and plasma triglyceride were greater with more severe steatosis. In support of this notion, the correlation between the L/S ratio and serum triglycerides within the tertile with minimal or no steatosis (normal), was substantially more robust ($r = -0.69$, $P < 0.01$) and is plotted in Fig. 1*B*. In this group, the association between serum triglycerides and VAT was no longer statistically significant ($r = 0.37$, $P = 0.08$).

Insulin resistance

We found that systemic insulin resistance was more severe in the moderate and severe groups of hepatic steatosis (Fig. 2). This was evident both by lower glucose disposal rates measured by the euglycemic clamp technique and by higher values for the fasting homeostasis model assessment of insulin resistance index. In contrast, fasting rates of EGP and insulin-suppressed EGP were equivalent across groups, indicating similar levels of hepatic insulin resistance.

CONCLUSIONS — Both hepatic steatosis and dyslipidemia commonly occur in obesity and type 2 diabetes. Fatty liver reflects hepatic oversupply of lipids, which we postulate marks a metabolic state conducive to hyperlipidemia. One of the main purposes of the current study was to examine whether hepatic steatosis is a predictor of a more severe dyslipidemia in type 2 diabetes. We found this to be the case. The presence of hepatic steatosis was associated with elevated serum triglycerides; small, dense LDL; and reduced HDL. A further observation concerns apoB. Insulin resistance is associated with

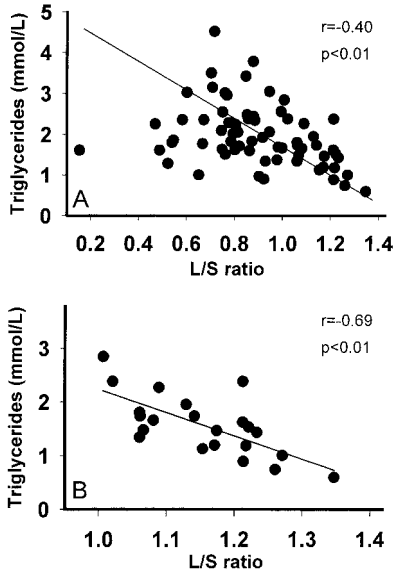

**Figure 1—*Correlation between serum triglycerides and hepatic steatosis (L/S ratio).* A: *Across the whole spectrum of steatosis* (r = *−0.40,* P < *0.01).* B: *Correlation analyzed within the group with none or minimal steatosis, i.e., L/S ratios ≥1.0* (r = *−0.69,* P < *0.01).*

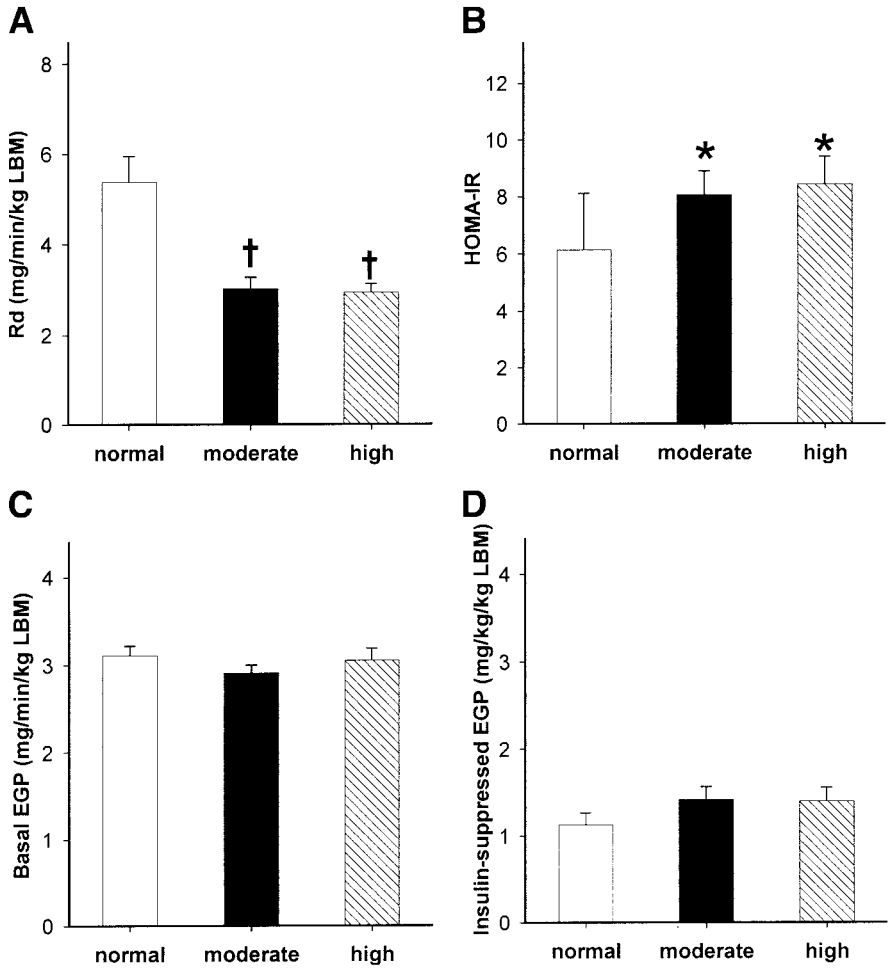

Figure 2—*Measurements of insulin sensitivity in groups with normal, moderate, and severe steatosis.* A*: Insulin sensitivity measured by the euglycemic-hyperinsulinemic clamp. LBM, lean body mass; Rd, systemic glucose disposal rate.* B*: Estimated insulin resistance under fasting conditions by the homeostasis model assessment of insulin resistance (HOMA-IR).* C*: Fasting EGP rates.* D*: Insulin-suppressed EGP rates during the euglycemic clamp.* *P $<$ *0.05,* †P $<$ *0.01 vs. normal group.*

increased apoB, and in general, there is higher apoB in type 2 diabetes (26,27). Levels of apoB were increased in the type 2 diabetic subjects of the current study but did not appear to be associated with the severity of hepatic steatosis or systemic insulin resistance. Our findings indicate that hepatic steatosis in type 2 diabetes is associated with increased triglyceride content per VLDL particle, rather than more increases in total VLDL particle numbers. Although we did not study apoB secretion kinetics, our findings are consistent with a model in which hepatic triglyceride content is not the only and perhaps not the principal determinant of hepatic VLDL-apoB secretion rate (28) but a more substantial determinant of triglyceride content in VLDL.

NMR spectroscopy was used to confirm these findings on lipoprotein composition. There was an increase in mean VLDL particle size but not number associated with hepatic steatosis, indicating the presence of triglyceride-enriched VLDL. Increased serum triglycerides were also strongly related to an increased proportion of small, dense LDL and decreased HDL. Although the total atherogenic particle number was not increased with hepatic steatosis, as noted by no increase in apoB, the risk of atherosclerosis might be raised by the greater numbers of small, dense LDL and perhaps even more significantly by reductions in HDL cholesterol and size that were noted.

There are three major methods to characterize lipoprotein subclasses: ultracentrifugation, gradient gel electrophoresis, and NMR. They differ not only in methodology but also in how what is measured is expressed. We chose the NMR methodology because it measures both particle numbers and size and has been successfully used in studies with insulin-resistant patients as well as in type 2 diabetes (29,30). It is important to note that in our study, the lipoprotein data from NMR were of a confirmatory nature, as the result can be anticipated from the measurements of triglycerides and apoB; a significant increase in triglycerides with no significant change in apoB should approximately equate to a higher triglyceride enrichment of VLDL.

Hepatic steatosis has not been traditionally regarded as a predictor of the severity of diabetic dyslipidemia, although the results of our study indicate that it should merit consideration. In fact, our study demonstrated that hepatic steatosis in type 2 diabetes is a better predictor of dyslipidemia severity than hyperglycemia or hepatic insulin resistance. Factors already regarded as influencing severity of diabetic dyslipidemia include hyperglycemia, increased plasma FFAs, and hyperinsulinemia; each of which is related to insulin resistance (2–5). Modest reductions in lipoprotein lipase activity may also contribute (31,32). It is of interest that in the current study, fasting hyperglycemia, A1C, and plasma FFA were quite similar across tertiles of hepatic steatosis, yet as earlier described, the severity of dyslipidemia differed. Although plasma insulin was higher in those with more steatosis, insulin was a much weaker correlate of serum triglycerides than hepatic steatosis. Together, these observations indicate the potential additional influence of hepatic steatosis as a predictor of the severity of diabetic dyslipidemia.

As in any association study, causality between variables is difficult to establish. However, the plausibility of causality should be considered. Because steatosis reflects increased lipid availability in hepatocytes, it is quite conceivable that hepatic steatosis reflects a state of increased hepatic FFA flux that stimulates VLDL triglyceride output. In agreement with this notion, liver-specific overexpression of lipoprotein lipase in mice, a manipulation intended to increase hepatic FFA uptake, causes a twofold increase in liver triglyceride content and fasting hypertriglyceridemia (33). The importance of VAT should also be considered (34). A limitation of our study is that the relative contributions of hepatic steatosis and VAT upon dyslipidemia cannot be easily separated because VAT and he-

patic steatosis are tightly associated with each other. In fact, VAT and hepatic steatosis can be seen as components of the same pathophysiological process, one in which visceral adiposity promotes hepatic lipid overload. The portal circulation is uniquely exposed to visceral adipokine secretions and FFA flux from insulin-resistant visceral adipocytes (7,35). However, it is also important to recognize that the hepatic lipid content is not only a result of FFA delivery and lipogenesis but also depends on hepatic fatty acid disposal by mitochondrial oxidation or secretion in the form of VLDL triglyceride. Thus, hepatic steatosis may be more proximally connected to VLDL metabolism than VAT because it directly reflects the metabolic state of hepatic fat overload. This hypothesis could explain why serum triglycerides were observed to be better correlated with hepatic fat content than VAT, plasma insulin, or plasma FFAs. This hypothesis remains speculative though because kinetic studies of VLDL synthesis and clearance were not performed. Of interest, a subtle characteristic of the relationship between hepatic steatosis and hypertriglyceridemia was apparent in this cohort, with this relationship being stronger among subjects with minor grades of hepatic fat content and being weaker among subjects with moderate to severe steatosis. This observation points to a loss of proportionality between hepatic steatosis and serum triglycerides once more severe steatosis is present and may indicate that, perhaps, the incorporation of triglycerides into VLDL also has a finite capacity.

One additional limitation of our study is that the influence of estrogen was not stringently controlled for. Nonetheless, this influence was probably minor because men and women were uniformly represented among groups, and most women were postmenopausal and of similar age.

In summary, we have observed that hepatic steatosis is a determinant of the severity of the dyslipidemia in type 2 diabetes, an association that is more evident when mild-to-moderate levels of steatosis are present. The association between hepatic steatosis and dyslipidemia is not attributable to greater apoB levels but to changes in the composition of VLDL particles and associated reductions in HDL and increases in small, dense LDL.

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