Fatty Liver Is Independently Associated With Alterations in Circulating HDL2 and HDL3 Subfractions

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Fatty liver is associated with insulin resistance, atherosclerosis, and the metabolic syndrome (1–7) and predicts future cardiovascular events (4–8). The pro-atherogenic serum lipid profile in subjects with fatty liver is characterized by elevated levels of triglycerides, low HDL cholesterol, and an increase in small dense LDL particles (9–12). Regarding HDL, not only quantitative, but also qualitative and compositional alterations are related to its antiatherogenic properties (13–16). In particular, circulating HDL_2 was found to protect from atherosclerosis (17,18). In the present study, we first investigated whether fatty liver is associated with altered circulating HDL cholesterol subfractions and second whether this relationship is independent of insulin sensitivity, thus possibly representing a direct link between fatty liver and cardiovascular disease.

RESEARCH DESIGN AND METHODS

— In our ongoing study on the pathophysiology of type 2 diabetes, ~300 Caucasians were carefully characterized for fatty liver and its associated metabolic characteristics (19). To select a subgroup that was representative for the percentage of subjects having fatty liver (liver fat >5.56%) (20) in our large cohort (~40%) and in whom enough sample volume was available to measure the HDL cholesterol subfractions, we randomly selected 8 men and 8 women with fatty liver and 24 control subjects.

The subjects underwent a 75-g oral glucose tolerance test to exclude diabetes and calculate insulin sensitivity (21). Total body fat was measured by bioelectrical impedance, visceral fat by magnetic resonance (MR) tomography, and liver fat by ^{1}H-MR spectroscopy (19). Serum total, HDL, and LDL cholesterol, as well as triglyceride concentrations, were measured by standard colorimetry, plasma adiponectin by radioimmunoassay, and apolipoprotein (apo)B_{100} and apoA-I by immunonephelometry. Circulating HDL_2 and HDL_3 cholesterol levels were quantified after ultracentrifugation of 1.5 ml serum (22), and the fatty acid pattern of these subfractions was determined by gas chromatography (23) within the entire particle, thereafter, in a subgroup of 34 subjects in whom sufficient sample volume was available after ultracentrifugation and chemical derivatization. A total of 15 subjects had fatty liver, and 19 were control subjects. Intima-media thickness (IMT) of the carotid artery was measured by high-resolution ultrasound. Relationships between parameters were tested using univariate correlations, and multivariate linear regression models were used to investigate independent relationships. $P \leq 0.05$ was considered statistically significant.

RESULTS

— Altogether, 25 women and 15 men were studied. While age ($P = 0.08$) and total body fat ($P = 0.23$) were not different between subjects with fatty liver and control subjects, individuals with fatty liver had higher body weight ($P = 0.02$), BMI ($P = 0.002$), waist circumference ($P = 0.003$), and visceral fat ($P < 0.0001$), as well as higher IMT ($P = 0.04$). Furthermore, they had higher fasting ($P = 0.006$) and 2-h ($P = 0.01$) glycemia and insulinemia (both $P < 0.0001$) and lower plasma adiponectin levels and insulin sensitivity (Table 1).

As expected, subjects with fatty liver had higher circulating triglyceride and $apoB_{100}$ and lower HDL cholesterol levels. Interestingly, the largest differences between subjects with fatty liver and control subjects were found for circulating HDL_2 cholesterol and the HDL_2-to-HDL_3 cholesterol ratio. No difference in HDL_3 cholesterol levels was observed between the groups (Table 1).

We next investigated whether the differences in the HDL subfractions between the two groups were independent of insulin sensitivity and adiponectin. Only circulating HDL_2 cholesterol and the HDL_2-to- HDL_3 cholesterol ratio remained statistically different between the groups after adjustment for these determinants (insulin sensitivity [Table 1] and adiponectin, $P = 0.036$ and $P = 0.01$, respectively). Power calculations revealed that we had a power of 94% to detect a difference in circulating HDL_2 cholesterol between the subjects with fatty liver and control subjects at the α level of 0.05. Nevertheless, to further validate our novel findings and to exclude a type 1 error that frequently may occur when using dichotomous analyses, we also used continuous variables for analyses. The strongest relationships, and the only ones that remained statistically significant after additional adjustment for insulin sensitivity (Table 1), as well as adiponectin, were those of liver fat with the HDL_2 cholesterol levels and the HDL_2-to-HDL_3 cholesterol ratio (insulin sensitivity [Table 1]

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Abbreviations: apo, apolipoprotein; IMT, intima-media thickness.

Table 1—*Serum lipids, insulin sensitivity, and circulating adiponectin in subjects with fatty liver and in control subjects, as well as correlations of these characteristics with liver fat as a continuous variable*

	Fatty liver (n = 16)	Control subjects (n = 24)	P	P*	Liver fat			
					r	P	r*	P*
Triglyceride (mg/dl)	171 ± 29	92 ± 8	0.003	0.21	0.47	0.002	0.22	0.18
Cholesterol (mg/dl)								
Total	194 ± 9	179 ± 6	0.16	0.66	0.18	0.25	0.005	0.98
LDL	127 ± 8	111 ± 5	0.09	0.76	0.26	0.11	0.03	0.86
HDL	45 ± 2	55 ± 3	0.02	0.22	−0.44	0.005	−0.31	0.06
HDL_2	4.7 ± 0.4	8.5 ± 0.9	0.0008	0.049	−0.54	0.0003	−0.37	0.02
HDL_3	22.2 ± 1.0	22.7 ± 0.9	0.70	0.23	−0.12	0.46	0.11	0.51
HDL_2-to-HDL_3	0.21 ± 0.01	0.37 ± 0.03	0.0001	0.004	−0.58	<0.0001	−0.48	0.002
ApoAI (mg/dl)	139 ± 4	150 ± 5	0.12	0.28	−0.28	0.08	−0.23	0.17
ApoB (mg/dl)	102 ± 5	85 ± 3	0.009	0.49	0.41	0.009	0.11	0.51
Insulin sensitivity (AU)	7.5 ± 0.9	19.1 ± 1.4	<0.0001	—	−0.77	<0.0001	—	—
Adiponectin (μg/ml)	9.3 ± 0.7	14.2 ± 1.0	0.0002	0.28	−0.48	0.002	−0.03	0.84

Data are means ± SD. *Additionally adjusted for insulin sensitivity. AU, arbitrary units.

and adiponectin, $P = 0.01$ and $P = 0.005$, respectively). The association of liver fat with total HDL cholesterol remained statistically significant after adjustment for adiponectin ($P = 0.02$) but not for insulin sensitivity (Table 1).

IMT, an early marker of atherosclerosis, correlated strongly with HDL_2 cholesterol ($r = -0.51$, $P = 0.001$) and weaker with total HDL cholesterol ($r = -0.47$, $P = 0.003$), HDL_3 cholesterol ($r = -0.42$, $P = 0.008$), and the HDL_2-to-HDL_3 cholesterol ratio ($r = -0.45$, $P = 0.005$). These correlations were independent of insulin sensitivity (HDL_2 cholesterol, $r = -0.44$, $P = 0.005$; total HDL cholesterol, $r = -0.41$, $P = 0.01$; HDL_3 cholesterol, $r = -0.37$, $P = 0.02$; and HDL_2-to-HDL_3 cholesterol ratio, $r = -0.38$, $P = 0.02$) or adiponectin (HDL_2 cholesterol, $r = -0.53$, $P = 0.0007$; total HDL cholesterol, $r = -0.46$, $P = 0.004$; HDL_3 cholesterol, $r = -0.41$, $P = 0.01$; and HDL_2-to-HDL_3 cholesterol ratio, $r = -0.46$ $P = 0.003$). Finally, we addressed potential mechanisms that may render HDL_2 more potent in the prevention of atherosclerosis than HDL_3. Besides lipoprotein-associated phospholipase A_2 and its product lysophosphatidylcholine (24), the enzyme paraoxonase 1 has a critical role in the antiatherosclerotic properties of HDL particles (13). Because the latter enzyme is inactivated by saturated but not unsaturated fatty acids (25), we investigated whether the fatty acid pattern within the HDL subfractions differed. Indeed, the HDL_2 subfraction contained a higher percentage of unsaturated fatty acids than the HDL_3 subfraction ($P < 0.0001$). Similar results were obtained when we performed these analyses separately within the subjects with fatty liver and the control subjects (both $P < 0.0001$). Since we analyzed fatty acids in the entire HDL particle, and lipid subgroups differ between HDL_2 and HDL_3, it remains to be established whether the fatty acid pattern differs within the isolated lipid subgroups.

CONCLUSIONS — In the present study, we provide novel information that liver fat correlates stronger with circulating HDL_2 cholesterol and the HDL_2-to-HDL_3 cholesterol ratio than with total HDL cholesterol. The correlations of liver fat with HDL_2 cholesterol and the HDL_2-to-HDL_3 cholesterol ratio remained statistically significant even after adjustment for whole-body insulin resistance, the major underlying abnormality that drives dyslipidemia (11,12), and circulating adiponectin, which is associated with both dyslipidemia and liver fat. In contrast, the associations of liver fat with triglycerides and circulating total HDL cholesterol, as well as circulating apoB, were rendered nonsignificant after correction for insulin resistance. Thus, the present findings provide evidence that circulating HDL_2, which is the more potent antiatherogenic HDL subfraction (17,18), and upon our preliminary analyses contained lipids with a higher amount of unsaturated fatty acids than HDL_3, is more suppressed than total HDL in subjects with fatty liver. These data further imply that determination of HDL_2 cholesterol, in addition to total HDL cholesterol, may more precisely predict the actual risk for cardiovascular events in subjects with fatty liver.

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