Relation of Nonalcoholic Hepatic Steatosis to Early Carotid Atherosclerosis in Healthy Men

Role of visceral fat accumulation

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Nonalcoholic fatty liver disease (NAFLD) is a clinicopathological syndrome that is closely associated with obesity, dyslipidemia, insulin resistance, and type 2 diabetes, thus suggesting that NAFLD represents another component of the metabolic syndrome (1,2). Because it is now recognized that individuals with the metabolic syndrome are at increased cardiovascular risk (3–5), it is possible to hypothesize that NAFLD patients might portend a greater cardiovascular disease (CVD) risk. Ultrasonographically measured carotid intima-media thickness (CIMT), as a reliable index of subclinical atherosclerosis (6), can be used to characterize the CVD risk of patients with NAFLD. However, the available data on this specific topic are lacking.

We compared CIMT values in subjects with and without nonalcoholic hepatic steatosis (HS) and examined whether such differences were mediated by one or more metabolic disorders typically clustering in these subjects.

RESEARCH DESIGN AND METHODS — We studied 85 male volunteers who were randomly selected among those who agreed to have an evaluation of their liver and intra-abdominal fat by ultrasonography and computed tomography (CT) scan, respectively. The exclusion criteria were as follows: age >50 years combined with an alcohol consumption ≥20 g/day, taking any drugs, and a history of recent acute illness or clinical evidence suggestive of any cardiovascular events, diabetes, or kidney or liver disorders. All individuals were clinically healthy. Most subjects were abstainers ($n = 68$) or drank minimally ($n = 17$) (i.e., alcohol consumption <20 g/day). Plasma liver function tests and other biochemical blood measurements were determined by an automatic colorimetric method (Hitachi; Boehringer Mannheim, Mannheim, Germany). Most subjects had normal liver function tests. All participants had normal glucose tolerance during an oral glucose tolerance test; their degree of insulin resistance was calculated from the homeostasis model assessment (HOMA) (7). Ultrasound CIMT measurements were made bilaterally in the 1-cm segment proximal to the dilation of the carotid bulb and always in plaque-free segments (8). Repeated measurements on the same subjects gave coefficients of variation (CVs) <5%. HS was diagnosed by ultrasonography, according to conventional criteria (1,9). Repeated measurements on the same subjects gave CVs <1%. In all participants, the diagnosis of HS was confirmed by CT (Siemens Somatom CT scanner; Siemens, Concord, CA). Total, subcutaneous, and visceral abdominal fat (VF) areas were measured by a single CT scan made at the level of the L4-L5 vertebrae (10).

Statistical analysis

Data are presented as means ± SD. The following statistical tests were used: ANOVA, ANCOVA, and Pearson's product-moment correlation. Because of skewness and kurtosis of the distributions, CT-measured VF, plasma triglycerides, and insulin concentrations were logarithmically transformed for statistical analyses. A two-factor ANOVA was performed to separately discriminate the adverse effects of HS and VF on CIMT and metabolic syndrome variables. In that analysis, the population was dichotomized into those with high and those with low values of VF, according to the 75th percentile value of control subjects (i.e., 87 cm^2). We have arbitrarily chosen this cutoff point because very few of our subjects had a cutoff point of VF area that was clinically related to an increased cardiovascular risk (i.e., ≥130 cm^2) (11).

RESULTS — The baseline characteristics of participants are shown in Table 1. Subjects with HS had significantly greater (~20%) CIMT measurements than those without HS. The former also had higher values for BMI, VF, diastolic blood pressure, plasma insulin, and triglycerides and lower HDL cholesterol concentration. Accordingly, subjects with HS had higher HOMA insulin resistance (3.57 ± 1.4 vs. 2.28 ± 1; $P < 0.01$). Age, behavioral variables, plasma glucose, and LDL cholesterol levels did not differ between the groups.

Importantly, while the differences in CIMT were little affected by adjustment for age, BMI, lipids, blood pressure, and HOMA insulin resistance (or postload insulinemia), they were abolished after controlling for VF. These results remained unchanged even when subjects who drank minimally ($n = 17$) were excluded

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Abbreviations: CIMT, carotid intima-media thickness; CT, computed tomography; CVD, cardiovascular disease; HOMA, homeostasis model assessment; HS, hepatic steatosis; NAFLD, nonalcoholic fatty liver disease; VF, visceral abdominal fat.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1 —*Clinical and biochemical characteristics of male study subjects grouped according to HS status*

	Without HS	With HS	*P*
n	40	45	—
Age (years)	43 ± 6	41 ± 6	NS
BMI (kg/m^2)	24 ± 2	26 ± 2	<0.001
Waist circumference (cm)	87 ± 5	92 ± 9	<0.001
Daily alcohol intake (g/day)	5.0 ± 3	5.3 ± 4	NS
Number of smokers	6	5	NS
Systolic blood pressure (mmHg)	129 ± 9	134 ± 10	NS
Diastolic blood pressure (mmHg)	81 ± 6	86 ± 7	<0.01
Triglycerides (mmol/l)	1.26 ± 0.7	2.23 ± 1.0	<0.001
HDL cholesterol (mmol/l)	1.37 ± 0.3	1.27 ± 0.5	<0.05
LDL cholesterol (mmol/l)	3.75 ± 0.8	3.74 ± 1.0	NS
Fasting glucose (mmol/l)	5.1 ± 0.4	5.4 ± 0.6	NS
1-h glucose (mmol/l)	6.8 ± 0.7	7.1 ± 0.7	NS
2-h glucose (mmol/l)	5.2 ± 0.6	5.6 ± 0.6	NS
Fasting insulin (pmol/l)	10 ± 5	15 ± 6	<0.01
1-h insulin (pmol/l)	65 ± 28	101 ± 39	<0.01
2-h insulin (pmol/l)	29 ± 21	56 ± 31	<0.01
CT-measured total fat area (cm^2)	197 ± 62	315 ± 148	<0.005
CT-measured VF (cm^2)	75 ± 30	112 ± 49	<0.001
CT-measured subcutaneous fat area (cm^2)	121 ± 36	203 ± 76	<0.01
CIMT (mm)	0.94 ± 0.12	1.18 ± 0.14	<0.001

Data are means ± SD.

from analyses. When CT-measured VF was replaced as a covariate by waist circumference, CIMT retained a significant difference between the groups ($P = 0.03$).

In a two-factor ANOVA, increased VF, but not HS, independently ($P < 0.01$) predicted CIMT, the greatest values being in those with HS and high VF ($n = 34$, 1.32 ± 0.16 mm) and the lowest values in those without HS and low VF ($n = 30$, 0.91 ± 0.14 mm). Similarly, in that analysis, increased VF was also the major determinant of many features of the metabolic syndrome.

In pooled subjects, CIMT significantly ($P < 0.05$–0.001) correlated to CT-measured VF ($r = 0.58$), BMI ($r = 0.46$), postload insulinemia ($r = 0.45$), HOMA insulin resistance ($r = 0.42$), waist circumference ($r = 0.41$), age ($r = 0.39$), and systolic and diastolic blood pressure ($r = 0.38$ for both) and marginally to LDL cholesterol. CIMT did not correlate to smoking, plasma triglycerides, HDL cholesterol, or any of the liver function tests.

CONCLUSIONS — These results demonstrate that in the presence of normal or moderately increased body weight, abdominal VF accumulation (probably through its multiple secreted factors, i.e., free fatty acids, adiponectin, tumor necrosis factor-α, or other adipocytokines) is a key mediator for the relationship linking nonalcoholic HS to increased CIMT. Consistent with this finding, several cross-sectional studies (12–14) reported a positive association between CIMT and abdominal fat distribution. More importantly, interventional studies showed a beneficial impact of weight loss, known to primarily reduce VF depots (11), on several coronary risk factors and the progression rate of early carotid atherosclerosis in obese individuals (15,16). Similarly, a gradual weight reduction significantly improved liver test results and liver biopsy features in most NAFLD patients (1). All of these findings, therefore, strongly emphasize the role of avoiding abdominal obesity to prevent atherosclerotic diseases and to improve NAFLD and its potential complications. This conclusion was also validated by the recent prospective results of the Insulin Resistance Atherosclerosis Study (17).

Because liver biopsies were not available in our subjects, we obviously cannot exclude the possibility of a differential detrimental relationship of the broad spectrum of NAFLDs to CIMT and metabolic syndrome variables. Notably, in this study we used two imaging techniques (ultrasound and CT) for diagnosis of HS, and CT was used for VF measurement, which is the best technology actually available (11). Moreover, because our sample included healthy individuals who were clinically free of traditional coronary risk factors, we believe that it enhances the validity of our findings.

In conclusion, these results demonstrate that in healthy, nonobese male subjects, the significant increase of CIMT in the presence of nonalcoholic HS is largely mediated by increased VF accumulation.

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