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Preliminary report: Hepatic fat and inflammation in type 2 diabetes mellitus

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Abstract

Although the association between inflammation and hepatic fat is fairly established, it remains unclear whether this association is independent of general measures of obesity and standard cardiovascular risk factors. Therefore, the aim of this study was to investigate the contribution of hepatic steatosis as an independent predictor of chronic inflammation in 281 subjects with type 2 diabetes mellitus. Reduced hepatic steatosis significantly ($P < .01$) correlated with C-reactive protein ($r = -0.16$) and adiponectin ($r = 0.23$). The association of hepatic steatosis with both C-reactive protein and adiponectin remained significant after adjustment for age, ethnicity, body mass index (or waist circumference), triglycerides, high-density lipoprotein, and total cholesterol. These data support the concept that accumulation of hepatic fat is related to enhanced inflammation in type 2 diabetes mellitus independent of general measures of obesity and standard cardiovascular risk factors.
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The link between inflammation and general obesity is well established. However, the specific tissue location of triglyceride accumulation and/or related bioactive precursors or metabolites may be particularly important, as accumulation of these products in nonadipose tissue locations (ectopic fat), such as the liver, may be proinflammatory. Recent evidence suggests a strong correlation between inflammation and hepatic fat [1-3]. However, it remains unclear whether enhanced inflammation is just a marker of other metabolic abnormalities commonly associated with hepatic fat (such as generalized obesity, insulin resistance, hyperglycemia, or dyslipidemia) or a unique consequence of hepatic fat, and/or its metabolites, on inflammation. Studying these relationships in individuals with long-standing type 2 diabetes mellitus who characteristically have severe insulin resistance, high rates of hepatic steatosis (HS), obesity, and inflammation provides an opportunity to clarify the nature of these relationships. The aim of this study was to determine whether hepatic fat was associated with the inflammatory markers interleukin-6 (IL-6), C-reactive protein (CRP), and adiponectin in type 2 diabetes mellitus and whether this association was independent of measures of adiposity such as body mass index (BMI) and waist circumference (WC) and other potentially confounding variables.

1. Materials and methods

Data for this study were derived from baseline examinations of participants with long-standing type 2 diabetes mellitus in the Risk Factors, Atherosclerosis, and Clinical Events in Diabetes study [4], which is a substudy of the Veterans Affairs Diabetes Trial. Methods for these studies have been described in detail previously [5,6]. Hepatic fat content was estimated by computed tomography using the attenuation signal in several locations in the right lobe of the

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liver (Hounsfield units) and compared with that of the spleen (the L/S ratio). The lower the L/S ratio is, the greater is the relative fat content; and an L/S ratio less than 1 is consistent with HS [7]. Plasma adiponectin, CRP, and IL-6 levels were measured in duplicate with enzyme-linked immunosorbent assay. Data are reported as means (±SD) or medians (25th-75th percentiles) if continuous and as proportions if categorical. Inflammatory markers with skewed distribution were natural log transformed. The relationship between inflammatory markers and liver fat content (L/S) was determined by Pearson correlation analysis. Multivariable linear regression analyses were performed to assess the association between inflammatory markers and L/S ratio independent of confounding variables.

2. Results and discussion

A total of 281 subjects (95% male) 40 years or older with a mean (±SD) diabetes duration of 12 ± 8 years were included. Subjects (n = 89) with HS (L/S <1) were significantly younger (59 ± 9 vs 62 ± 9 years, $P < .01$) and had diabetes for fewer years (10 ± 7 vs 13 ± 8, $P < .01$) than those without HS (n = 192). There were no significant differences between groups (L/S <1 vs L/S ≥1) in sex, ethnicity, BMI, WC, hemoglobin A_{1c} (HbA_{1c}) levels, or use of alcohol, tobacco, statins, thiazolidinediones, or aspirin. Participants with HS had lower high-density lipoprotein (HDL) cholesterol (35 ± 10 vs 38 ± 10 mg/dL, $P = .02$) and higher triglyceride (229 ± 135 vs 186 ± 122 mg/dL, $P < .01$) levels. Individuals with HS also had significantly higher median CRP levels (3.9 [2.1-8.5] vs 2.5 [1.7-4.8] mg/L, $P < .01$) and lower median adiponectin levels (4.5 [2.9-6.6] vs 5.4 [3.6-9.5] ng/L, $P = .02$), respectively. There were no significant differences in IL-6 levels between groups.

As shown in Figs. 1 and 2, L/S values were significantly ($P < .01$) correlated with adiponectin ($r = 0.23$) and CRP ($r = -0.16$), but not with IL-6. Furthermore, in multivariable linear regression models adjusted for age, ethnicity, BMI, triglycerides, HDL, and total cholesterol, L/S ratio remained significantly and independently associated with adiponectin

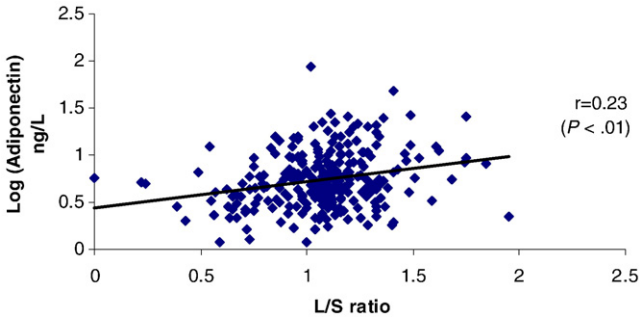

Fig. 1. Association between liver fat content (L/S) and log-transformed adiponectin levels. Correlation coefficient and *P* values were determined by Pearson correlation analysis.

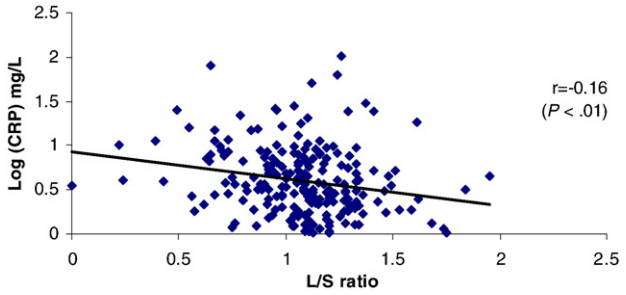

Fig. 2. Association between liver fat content (L/S) and log-transformed CRP levels. Correlation coefficient and *P* values were determined by Pearson correlation analysis.

($\beta = 0.33$, SE = 0.15, $P = .03$) and CRP ($\beta = -0.57$, SE = 0.28, $P = .04$), respectively (Table 1); and results were unchanged with replacement of BMI with WC. Further adjustment for diabetes duration and HbA_{1c} did not change the results for adiponectin. However, the association between L/S and CRP ($\beta = -0.52$, SE = 0.28, $P = .07$) was not significant after the addition of these additional covariates.

Insulin resistance has been postulated as a potential regulator of both hepatic fat and inflammation, thus raising the possibility that differences in this condition could account for these study results. Although insulin resistance was not directly measured and is a limitation of this study, this cohort consisted primarily of obese individuals with long-standing type 2 diabetes mellitus; and marked insulin resistance would be present in nearly all participants, thereby making it very unlikely that this variable could confound the relationship between hepatic fat and inflammation. We also cannot exclude the possibility that the relatively less common conditions of steatohepatitis or fibrosis (a consequence of hepatic fat and known inducer of CRP) were present in some individuals. However, participants were excluded from the study if alanine transaminase was elevated more than 3 times higher than normal or if serum bilirubin was greater than 1.9 mg/dL [5], as would frequently occur in these more advanced stages of liver disease. Furthermore, as shown in Figs. 1 and 2, adiponectin and CRP were correlated to L/S throughout the range of hepatic fat; and this correlation was not the result of a few outliers with more severe disease.

Table 1
Independent association of L/S with adiponectin and CRP

	Dependent variable			
	Log (adiponectin)		Log (CRP)	
L/S	β ± SE	*P* value	β ± SE	*P* value
Model 1	0.48 ± 0.16	.002	−0.65 ± 0.28	.022
Model 2	0.33 ± 0.15	.029	−0.57 ± 0.28	.041
Model 3	0.34 ± 0.16	.033	−0.52 ± 0.28	.069

Model 1: adjusted for age and ethnicity. Model 2: adjusted for model 1 + BMI (or WC), triglycerides, HDL, and total cholesterol. Model 3: adjusted for model 2 + diabetes duration and HbA_{1c}.

The results of this study suggest that there is a unique and independent association between hepatic fat and CRP and adiponectin. Consistent with this notion, animals studies have shown that exposure of hepatic cells to fatty acids can induce hepatic cell inflammation and insulin resistance [8,9], both of which may in turn further alter levels of inflammatory markers [10]. Moreover, a recent study demonstrated a broad increase in inflammatory gene expression in hepatic tissue from individuals with biopsy-proven HS in the absence of steatohepatitis [11].

Although these data suggest that accumulation of fat in liver is an important and independent correlate of inflammation, it is recognized that an alternative explanation may be that abnormal levels of inflammatory markers may also directly or indirectly drive hepatic fat accumulation. Prospective follow-up of this cohort may help clarify the temporal sequence of these events.

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