Published in final edited form as:

Metabolism. 2010 March ; 59(3): 430–432. doi:10.1016/j.metabol.2009.07.041.

Hepatic Fat and Inflammation in Type 2 Diabetes

Aramesh Saremi¹, Matthew Allison², Dominic Ditomasso², Ling Ge³, Robert Anderson^{3,4}, Tom E. Moritz³, William Duckworth¹, Carlos Abaira⁵, and Peter D. Reaven¹ for the VADT

¹ Phoenix VA Health Care System, Phoenix, AZ

² University of California San Diego, La Jolla, CA

³ Cooperative Studies Program Coordinating Center, Hines, IL

⁴ University of Illinois, Chicago, IL

⁵ Miami VA Medical Center, Miami, FL

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 (HS) as an independent predictor of chronic inflammation in 281 subjects with type 2 diabetes. Hepatic steatosis significantly ($p < 0.01$) correlated with CRP ($r = -0.16$) and adiponectin ($r = 0.23$). The association of HS with both CRP and adiponectin remained significant after adjustment for age, ethnicity, BMI (or WC), triglycerides, HDL, and total cholesterol. These data support the concept that accumulation of hepatic fat is related to enhanced inflammation in type 2 diabetes, independent of general measures of obesity and standard cardiovascular risk factors.

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 non-adipose 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 longstanding type 2 diabetes who characteristically have severe insulin resistance, high rates of hepatic steatosis, 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 IL-6, CRP and adiponectin in type 2 diabetes, and whether this association was independent of measures of adiposity such as BMI and WC and other potentially confounding variables.

Corresponding Author: Peter Reaven, MD, 650 E. Indian School Road (111E), Phoenix, AZ 85012-1892, Phone: 602-277-5551 x 6619, Fax: 602-200-6004, Peter.Reaven@va.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

MATERIALS AND METHODS

Data for this study derive from baseline examinations of participants with long standing type 2 diabetes in the Risk Factors, Atherosclerosis, and Clinical Events in Diabetes (RACED) study⁴, which is a sub-study 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 liver (Hounsfield units) and compared to that of the spleen (the L/S ratio). The lower the L/S ratio the greater the relative fat content, and a L/S < 1 is consistent with hepatic steatosis (HS)⁷. Plasma adiponectin, CRP and IL-6 levels were measured in duplicate with ELISA. Data are reported as means ($\pm$ 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) were 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.

RESULTS AND DISCUSSION

A total of 281 subjects (95% male), aged 40 years or older with a mean ($\pm$ 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, $p < 0.01$) and had diabetes for fewer years (10 ± 7 vs. 13 ± 8 , $p < 0.01$), than those without HS ($n=192$). There were no significant differences between groups (L/S < 1 vs. L/S ≥ 1) in gender, ethnicity, BMI, WC, HbA1c levels, or use of alcohol, tobacco, statins, thiazolidinediones or aspirin. Participants with HS had lower HDL cholesterol (35 ± 10 vs. 38 ± 10 mg/dl, $p=0.02$) and higher triglyceride (229 ± 135 vs. 186 ± 122 , mg/dl $p < 0.01$) 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, $p < 0.01$], and lower median adiponectin levels [4.5 (2.9 – 6.6) vs. 5.4 (3.6 – 9.5) ng/L, $p=0.02$] respectively. There were no significant differences in IL-6 levels between groups.

As shown in the figure 1 and 2, L/S values were significantly ($p < 0.01$) correlated with CRP ($r = -0.16$) and adiponectin ($r=0.23$). 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 ($\beta = 0.33$, SE =0.15, $p=0.03$) and CRP ($\beta = -0.57$ SE =0.28, $p=0.04$) respectively, and results were unchanged with replacement of BMI with WC. Further adjustment for diabetes duration and HbA1c did not change the results for adiponectin. However, the association between L/S and CRP ($\beta = -0.52$, SE =0.28, $p=0.07$) 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, 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 can not 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 transaminase (ALT) were elevated > 3 times above normal or serum bilirubin was above 1.9 mg/dl⁵, as would frequently occur in these more advanced stages of liver disease. Furthermore as shown in the figure 1 and 2, CRP and adiponectin 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.

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, animal studies have shown direct exposure of hepatic cells to fatty acids can induce hepatic cell inflammation and insulin resistance^{8;9}, both which may in turn further alter levels of inflammatory markers¹⁰. 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 steatohepatitis¹¹.

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.

Reference List

1. Yoneda M, Iwasaki T, Fujita K, et al. Hypoadiponectinemia plays a crucial role in the development of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus independent of visceral adipose tissue. *Alcohol Clin Exp Res* 2007;31:S15–S21. [PubMed: 17331160]
2. Iwasaki T, Nakajima A, Yoneda M, et al. Relationship between the serum concentrations of C-reactive protein and parameters of adiposity and insulin resistance in patients with type 2 diabetes mellitus. *Endocr J* 2006;53:345–356. [PubMed: 16714840]
3. McKimmie RL, Daniel KR, Carr JJ, et al. Hepatic steatosis and subclinical cardiovascular disease in a cohort enriched for type 2 diabetes: the Diabetes Heart Study. *Am J Gastroenterol* 2008;103:3029–3035. [PubMed: 18853970]
4. Reaven PD, Sacks J. Coronary artery and abdominal aortic calcification are associated with cardiovascular disease in type 2 diabetes. *Diabetologia* 2005;48:379–385. [PubMed: 15688207]
5. Abaira C, Duckworth W, McCarren M, et al. Design of the cooperative study on glycemic control and complications in diabetes mellitus type 2: Veterans Affairs Diabetes Trial. *J Diabetes Complications* 2003;17:314–322. [PubMed: 14583175]
6. Duckworth W, Abaira C, Moritz T, et al. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med* 2009;360:129–139. [PubMed: 19092145]
7. Oliva MR, Morteale KJ, Segatto E, et al. Computed tomography features of nonalcoholic steatohepatitis with histopathologic correlation. *J Comput Assist Tomogr* 2006;30:37–43. [PubMed: 16365570]
8. Joshi-Barve S, Barve SS, Amancherla K, et al. Palmitic acid induces production of proinflammatory cytokine interleukin-8 from hepatocytes. *Hepatology* 2007;46:823–830. [PubMed: 17680645]
9. Cai D, Yuan M, Frantz DF, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med* 2005;11:183–190. [PubMed: 15685173]
10. Schwartz EA, Reaven PD. Molecular and signaling mechanisms of atherosclerosis in insulin resistance. *Endocrinol Metab Clin North Am* 2006;35:525–49. viii. [PubMed: 16959584]
11. Greco D, Kotronen A, Westerbacka J, et al. Gene expression in human NAFLD. *Am J Physiol Gastrointest Liver Physiol* 2008;294:G1281–G1287. [PubMed: 18388185]

Figure 1. Association between liver fat content (L/S) and log transformed adiponectin (Figure-1) and CRP (Figure-2) levels. Correlation coefficient and p-values determined by Pearson correlation analysis.

Figure 2. Association between liver fat content (L/S) and log transformed adiponectin (Figure-1) and CRP (Figure-2) levels. Correlation coefficient and p-values determined by Pearson correlation analysis.

Table

Independent association of L/S with Adiponectin and CRP

	Dependent Variable			
	Log (Adiponectin)		Log (CRP)	
L/S	$\beta \pm SE$	p-value	$\beta \pm SE$	p-value
Model-1	0.48 ± 0.16	0.002	-0.65 ± 0.28	0.022
Model-2	0.33 ± 0.15	0.029	-0.57 ± 0.28	0.041
Model-3	0.34 ± 0.16	0.033	-0.52 ± 0.28	0.069

Model-1 adjusted for age and ethnicity

Model-2 adjusted for model 1 +BMI (or WC), triglycerides, HDL, total cholesterol

Model-3 adjusted for model 2 + diabetes duration, HbA1C