

# **The Association of Liver Fat with Inflammatory Markers in the Veterans Affairs Diabetes Trial (VADT)**

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# Abstract

Obesity is strongly related to the development of insulin resistance and type 2 diabetes (T2DM). It is increasingly recognized that tissue and systemic inflammation may be an important causal link between obesity and T2DM. The specific tissue location of fat and its' relevant metabolites may be particularly important as accumulation of fat in non-adipose tissue locations (i.e. “ectopic fat”), and in the liver particularly, may be uniquely proinflammatory. Computed tomography (CT) can be used to measure liver fat accumulation by comparing liver to spleen CT attenuation values (the L/S ratio). The lower the L/S ratio the greater the relative fat content with a  $L/S < 1$  being consistent with hepatosteatosis. The aim of this study was to determine whether liver fat was associated with two inflammatory markers (CRP and adiponectin) that have been linked to the development of insulin resistance and T2DM. A total of 281 subjects (94% male) participating in the VADT, aged 40 years or older with a mean diabetes duration of  $12.2 \pm 8$  years and HbA1c of  $9.2 \pm 1.3\%$  at baseline were included. Subjects with more liver fat ( $L/S < 1$ ) were significantly younger, had diabetes for fewer years, but had lower HDL cholesterol and higher triglycerides levels. There were no significant differences between groups ( $L/S < 1$  vs.  $L/S \geq 1$ ) in BMI, waist circumference (WC), gender, ethnicity, Hba1C levels, alcohol or medication use. Across decreasing tertiles of L/S ratio, there was a significant ( $p < 0.02$ ) stepwise increase in plasma CRP and a decrease in adiponectin. In multivariate linear regression models, the L/S ratio remained significantly associated with CRP and adiponectin levels, even after adjustment for age, BMI or WC, ethnicity, duration of diabetes, lipid levels, HbA1C, and other clinical characteristics. Conclusion: In individuals with T2DM, liver fat accumulation is significantly associated with CRP and adiponectin independent of other measures of body fat or traditional risk factors. These data are consistent with the hypothesis that accumulation of ectopic fat in the liver may be an important contributor to systemic inflammation and insulin resistance.

# Introduction

**Excess fat deposition in the liver (hepatic steatosis) occurs quite commonly in individuals with obesity, insulin resistance and/or type 2 diabetes. Accumulation of fat, or its metabolites, may be an inflammatory challenge to the liver, and is associated with increased plasma levels of pro-inflammatory cytokines such as CRP and IL-6 and decreased plasma levels of adiponectin. Systemic inflammation may be an important link between obesity, insulin resistance, type 2 diabetes and vascular complications.**

# AIM

**To determine whether hepatic steatosis was associated with several inflammatory markers in type 2 diabetes, and whether this association was independent of standard measures of fatness, such as BMI and/or waist circumference.**

# **Methods**

**A cross-sectional study of 281 subjects with type 2 diabetes, participating in the VADT.**

**Liver fat (ectopic fat) is assessed by comparing the computed tomography attenuation signal in the liver to that in spleen (the L/S ratio). The L/S ratio is a normalized index, with an  $L/S < 1$  considered to represent Hepatic Steatosis (HS).**

**Inflammatory markers: high sensitivity CRP, IL-6 and adiponectin were measured in duplicate by ELISA.**

## **Statistical analysis**

**Data are reported as mean and SD or median and 25<sup>th</sup> and 75<sup>th</sup> percentiles if continuous data, and as proportions if categorical.**

**Inflammatory markers with skewed distribution were natural log transformed.**

**Analysis of variance was used to compare the difference of inflammatory marker levels between subjects with ( $L/S < 1$ ) & without HS ( $L/S \geq 1$ ) across tertiles of BMI and waist circumference. Multivariable linear regression analyses was performed to assess the association of inflammatory markers with liver fat (L/S ratio).**

# Results

**Table-1. Clinical characteristics of the study population in those with ( $L/S < 1$ ) compared to those without HS( $L/S \geq 1$ ).**

	L/S < 1 (n=89)	L/S ≥ 1 (n=192)	P-value
<b>Age (years)</b>	<b>59 ± 9</b>	<b>62 ± 9</b>	<b>&lt; 0.01</b>
<b>Male (%)</b>	<b>95</b>	<b>92</b>	<b>0.24</b>
<b>Non-Hispanic White (%)</b>	<b>66</b>	<b>66</b>	<b>0.98</b>
<b>BMI (kg/m2)</b>	<b>31.6 ± 4.3</b>	<b>31.0 ± 4.3</b>	<b>0.36</b>
<b>Waist Circumference (cm)</b>	<b>111 ± 15</b>	<b>111 ± 8</b>	<b>0.98</b>
<b>Diabetes Duration (year)</b>	<b>10 ± 7</b>	<b>13 ± 8</b>	<b>&lt; 0.01</b>
<b>Hypertension (%)</b>	<b>76</b>	<b>77</b>	<b>0.90</b>
<b>Statin use (%)</b>	<b>53</b>	<b>63</b>	<b>0.10</b>
<b>TZD use (%)</b>	<b>9</b>	<b>10</b>	<b>0.81</b>
<b>Ever Smoker (%)</b>	<b>74</b>	<b>69</b>	<b>0.40</b>
<b>Alcohol (abstinence) (%)</b>	<b>60</b>	<b>49</b>	<b>0.15</b>
<b>HbA1c (%)</b>	<b>9.3 ± 1.4</b>	<b>9.2 ± 1.3</b>	<b>0.56</b>
<b>Total cholesterol (mg/dl)</b>	<b>180 ± 32</b>	<b>179 ± 40</b>	<b>0.24</b>
<b>Triglycerides (mg/dl)</b>	<b>229 ± 135</b>	<b>186 ± 122</b>	<b>&lt; 0.01</b>
<b>LDL (mg/dl)</b>	<b>104 ± 29</b>	<b>104 ± 31</b>	<b>0.80</b>
<b>HDL (mg/dl)</b>	<b>35 ± 10</b>	<b>38 ± 10</b>	<b>0.02</b>
<b>TC / HDL</b>	<b>6 ± 2</b>	<b>5 ± 2</b>	<b>0.02</b>
<b>Adiponectin (ng/mL)</b>	<b>4493 (2885 -6564)</b>	<b>5405 (3550 - 9464)</b>	<b>0.02</b>
<b>CRP (mg/L)</b>	<b>3.9 (2.1-8.5)</b>	<b>2.5 (1.7-4.8)</b>	<b>&lt; 0.01</b>
<b>IL-6 (pg/ml)</b>	<b>3.2 (2.3 – 5.1 )</b>	<b>3.1 (2.1 -4.5)</b>	<b>0.45</b>

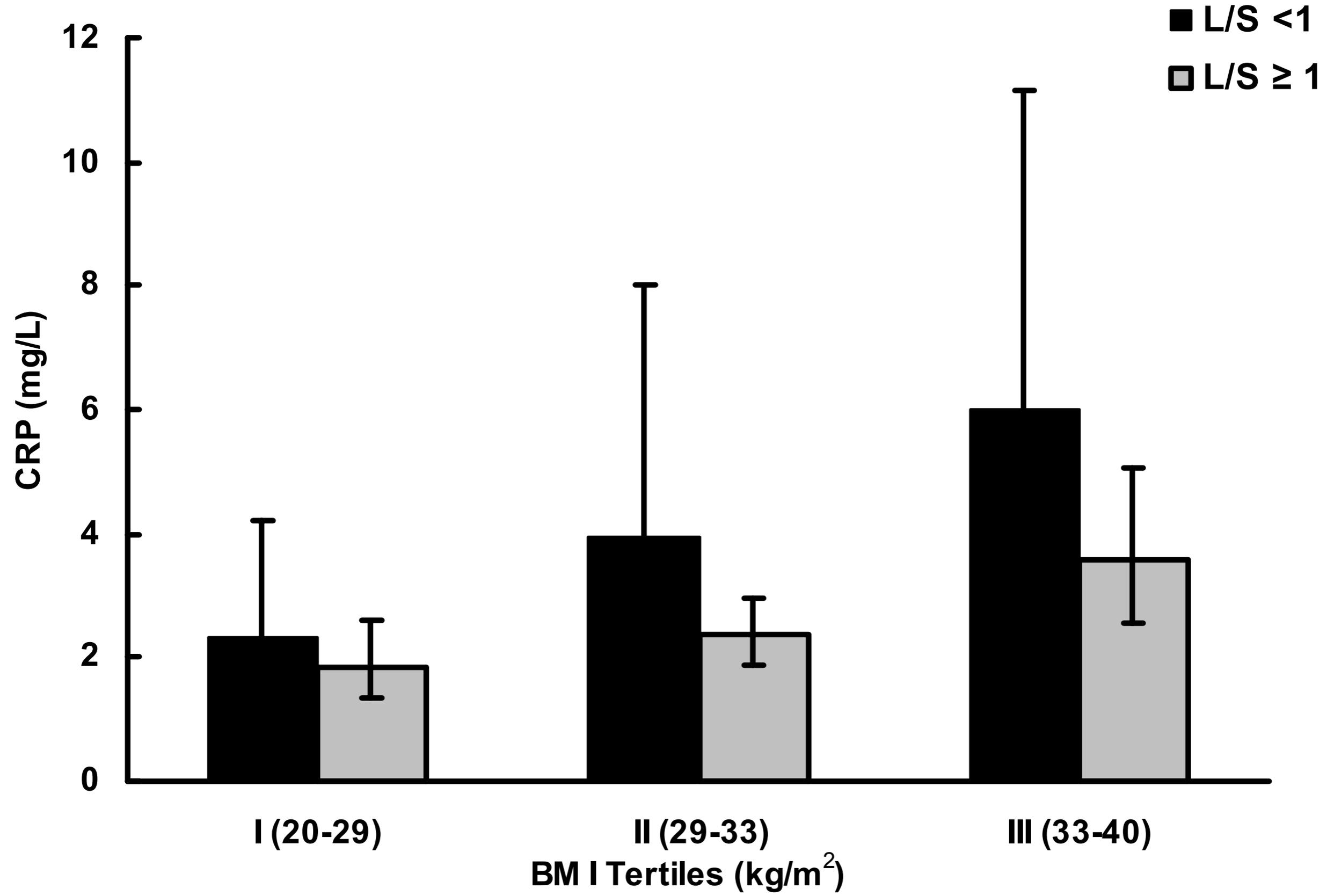
**Numbers are means ± SD, Median (25%-75%) or prevalence (%)**

**Subjects with HS ( $L/S < 1$ ) were significantly younger and had diabetes for fewer years. Although BMI and waist circumference (WC) were not significantly different among the two groups, subjects with HS had significantly lower HDL cholesterol and adiponectin levels and higher triglycerides and CRP levels respectively.**

**Figures 1-2. Plasma levels of inflammatory markers in those with (L/S < 1) & without HS (L/S ≥ 1) according to BMI & WC categories.**

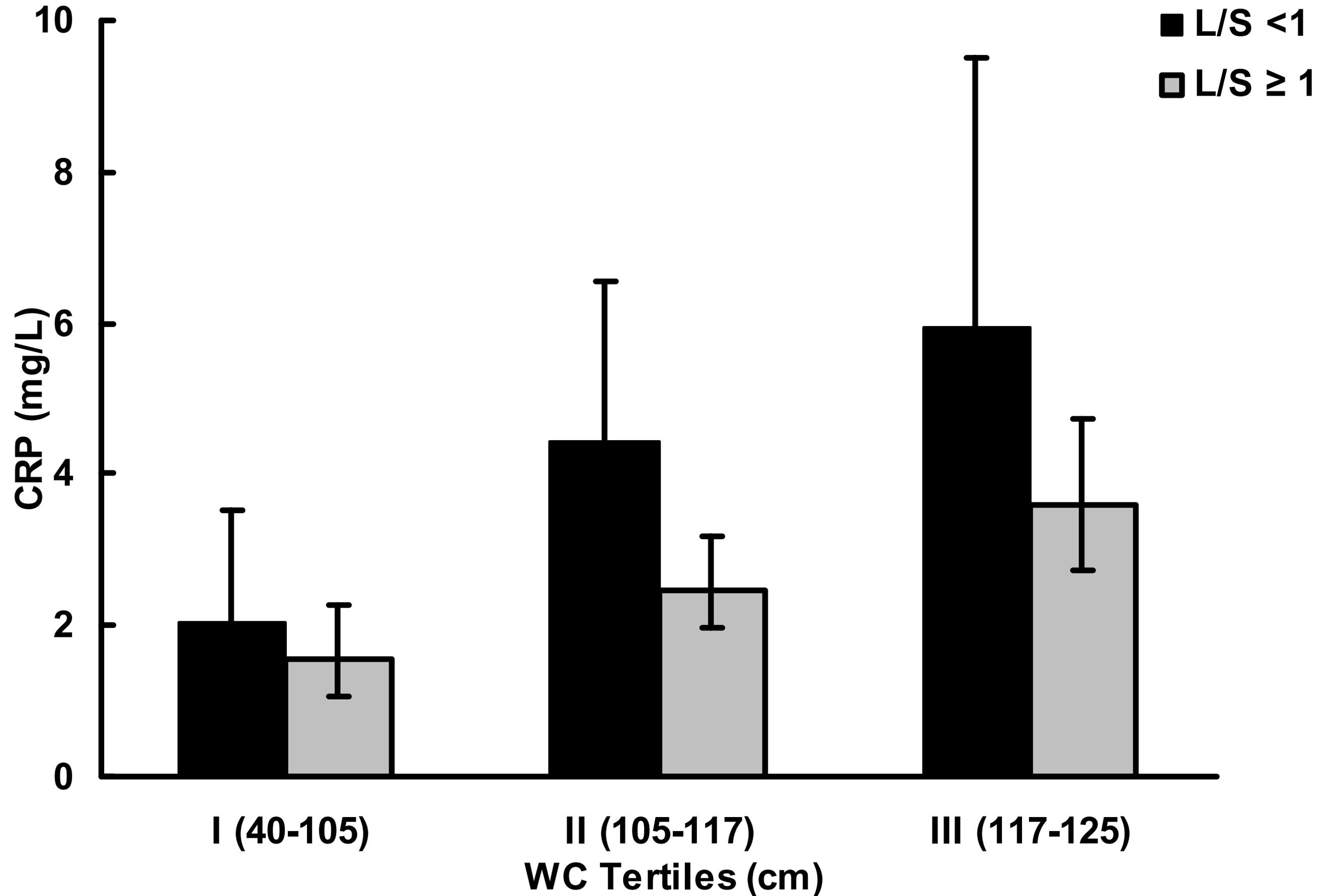
**Geometric means & 95% CI are shown in all figures.**

**Figure -1A**



**P-value for the difference in CRP levels between those with & without HS adjusted for BMI=0.008**

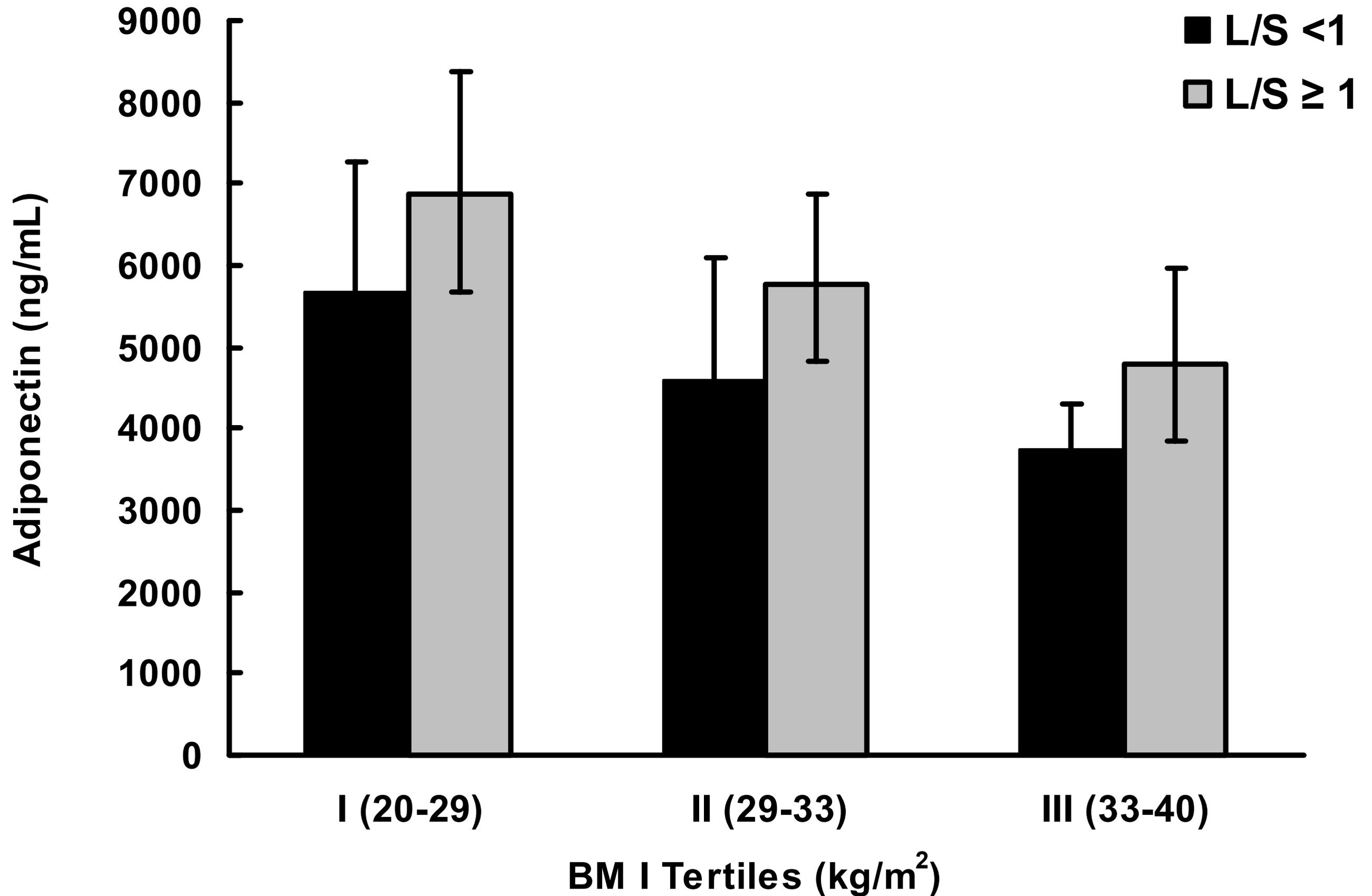
1B



**P-value for the difference in CRP levels between those with & without HS adjusted for WC=0.003**

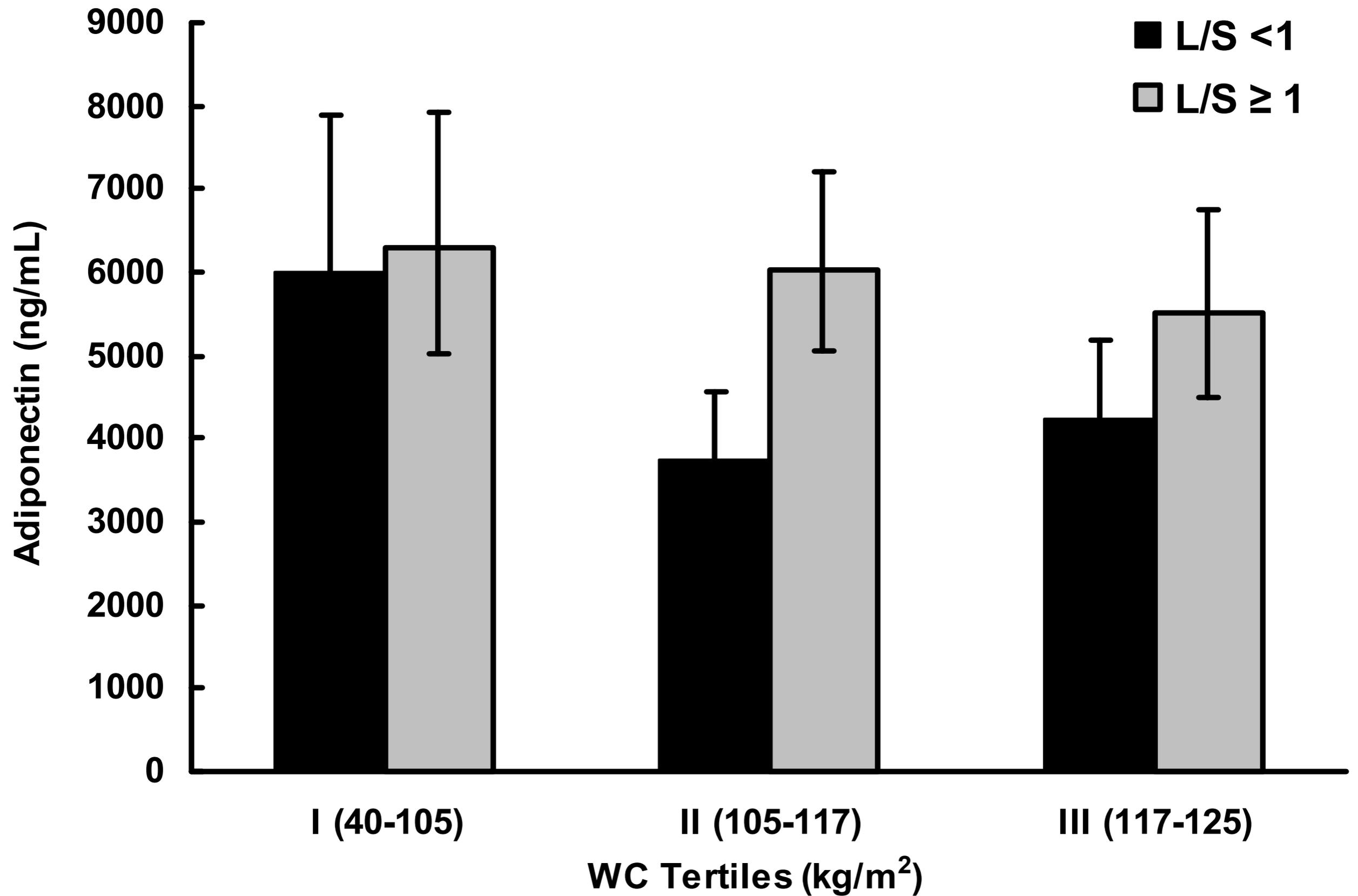
**Plasma CRP levels were in general higher in those with HS compared to those without HS in all BMI or WC categories, even in those in the highest BMI & WC categories. This suggests that the effect of HS on inflammation is independent of, and in addition to, general obesity.**

**Figure- 2A**



**P-value for the difference in adiponectin levels between those with & without HS adjusted for BMI=0.01**

2B



**P-value for the difference in adiponectin levels between those with & without HS adjusted for WC=0.005**

**Plasma adiponectin levels were in general lower in those with HS compared to those without HS, and decreased across BMI tertiles but not WC tertiles.**

**Table-2 Multivariable linear regression models for the association of CRP and adiponectin with L/S.**

Independent Variables	Dependent Variable: Ln (CRP)			Dependent Variable: Ln (Adiponectin)		
	$\beta$	SE	P-Value	$\beta$	SE	P-Value
<b>L/S ratio</b>	<b>-0.58</b>	<b>0.28</b>	<b>0.04</b>	<b>0.33</b>	<b>0.15</b>	<b>0.03</b>
<b>Age (years)</b>	-0.002	0.009	0.8	<b>0.01</b>	<b>0.005</b>	<b>&lt; 0.01</b>
<b>NHW (vs. others)</b>	-0.19	0.16	0.25	<b>-0.32</b>	<b>0.08</b>	<b>&lt; 0.01</b>
<b>BMI (kg/m<sup>2</sup>)</b>	<b>0.07</b>	<b>0.01</b>	<b>0.002</b>	-0.0007	0.01	0.49
<b>Triglycerides (mg/dl)</b>	-0.0004	0.0008	0.62	-0.004	-0.81	0.42
<b>HDL mg/dl)</b>	-0.008	0.009	0.41	<b>0.02</b>	<b>0.005</b>	<b>&lt;0.01</b>
<b>Total cholesterol(mg/dl)</b>	0.002	0.002	0.47	0.00002	0.001	0.98
Replacing WC for BMI did not change the results.						

**After adjustment for other covariates L/S remained significantly associated with CRP and adiponectin.**

# Summary

## **These data suggest:**

**That hepatic fat is a closely associated with CRP and adiponectin, independent of BMI and WC, in obese individuals with diabetes.**

**Simultaneous occurrence of general obesity and hepatic fat increases the likelihood of having higher CRP, and lower adiponectin levels compared to either of those risk factors alone, suggesting additive effects.**

# **CONCLUSION**

**Accumulation of fat and/or it's metabolites in the liver may be an important source of systemic inflammation which may in turn contribute to the development or worsening of insulin resistance/diabetes and/or vascular complications.**

# **ACKNOWLEDGEMENTS**

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