

## **Decreased plasma adiponectin concentrations are closely related to steatosis in HCV-infected patients.**

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## ABSTRACT

*Objectives:* The mechanisms underlying steatosis during HCV infection are complex and multifactorial. Obesity is a well-recognized risk factor for the development of steatosis in chronic hepatitis C infection. The aim of our study was to investigate the role of adipocytokines in HCV-related steatosis. Therefore, we hypothesized that the endocrine function of adipose tissue could be in part responsible for HCV-related steatosis.

*Methods:* Seventy-one consecutive untreated chronic hepatitis C patients were studied to assess: the effects of adipocytokines, body mass index, age, and HCV genotype on steatosis. We used Enzyme-Linked-Immunosorbent-Assay to determine serum adiponectin, leptin, TNF receptors soluble I and II concentrations. *Results:* Steatosis was observed in 42 (59.1%) patients. BMI was significantly associated with leptin ( $r= 0.64$ ,  $p= 0.0001$ ) and was border significantly associated with adiponectin concentrations ( $r=-0.22$ ,  $p=0.06$ ). In univariate analyses, age, HCV genotype 3, BMI, increased leptin level, increased insulin level and decreased adiponectin concentration were associated with steatosis. In multivariate analysis, steatosis was significantly associated with low adiponectin concentration, age, and HCV genotype 3, ASAT level whereas leptin, insulin and BMI were not associated with steatosis. *In conclusion,* in chronic HCV patients hypoadiponectinemia is significantly associated with the development of liver steatosis. The fact that the plasma levels of adiponectin inversely correlate with steatosis in HCV-infected subjects suggests that hypoadiponectinemia may contribute to hepatic steatosis progression and liver injury in this population. One practical implication is that therapy to increase circulating adiponectin concentration, such as overweight reduction or thiazolidinediones, provides the potential to improve steatosis in chronic hepatitis C infection.

## **INTRODUCTION**

Steatosis is a common histological feature of HCV infection (1). The prevalence of steatosis in liver biopsy specimens from patients with chronic HCV infection has been reported at around 50% (1). Recent studies found a role for steatosis in the progression of chronic HCV (1, 2, 3). In addition, hepatic steatosis is an independent risk factor for hepatocarcinoma in patients with chronic HCV infection (4). The pathogenesis of steatosis in patients with HCV is not well understood. HCV genotype 3a has been linked to steatosis more strongly than with other genotypes (5). Moreover, HCV-related steatosis is not always virally related and other factors may coexist. Steatosis is associated with risk factors for NASH, particularly obesity, rather than with alcohol consumption (6). Obesity is a well-recognized risk factor for the development of steatosis and of fibrosis in HCV-infected patients (1,2,3,6). Visceral fat distribution rather than body mass index (BMI) proved to be associated with HCV-related steatosis (3). The mechanisms by which accumulation and anatomic distribution of adipose tissue may be related to the development of steatosis and fibrosis are under intense investigation. Adipose tissue has traditionally been considered an energy storage organ, but over the last decade, a new role has emerged for the adipose tissue as an endocrine organ (7, 8). Adipose tissues secrete a variety of hormones including adiponectin, and leptin which may contribute to the development of metabolic abnormalities (7, 8). In addition, adipose tissue produces and secretes inflammatory cytokines, for example TNF $\alpha$  and interleukin 6. There are few data on adipocytokines and liver function. There are some controversial data about the relationship between serum leptin levels and HCV-related steatosis (9, 10). Regarding adiponectin, its levels are associated in healthy humans with plasma concentrations of various liver function tests, however there is no data about its secretion during hepatitis C infection (11). Although the deleterious association between

obesity and HCV infection is well recognized, it has not been ascertained whether adipocytokines and in particular adiponectin may have a role in the development of steatosis in chronic hepatitis C.

The primary aim of our study was to investigate the role of adipocytokines in HCV-related steatosis. Therefore, we hypothesized that the relationship between obesity and HCV-related steatosis is the consequence of the endocrine function of adipose tissue. To address this issue, we analysed the role of adipocytokines of liver steatosis and fibrosis in 71 chronic hepatitis C patients by multivariable logistic regression

## **PATIENTS AND METHODS**

**Patients** – Seventy-one untreated chronic hepatitis C patients were hospitalized for liver biopsy. Collected data included age, gender, and alcohol use. BMI was calculated as body weight in kilograms divided by the square of height in meters ( $\text{kg}/\text{m}^2$ ). Information regarding average alcohol intake in grams per day was assessed by interview. Patients consuming more than 60 g per day of alcohol were considered as excessive drinkers. The study protocol had been approved by the Ethics Committees of the University of Dijon and subjects gave written consent to participate in the present study.

### **Laboratory determinations**

Venous blood samples were taken in the morning after 12 hours overnight fasting, the day when the liver biopsy was performed. Plasma glucose concentration was measured by a glucose oxidase method on a Vitros 750 analyzer (Johnson & Johnson Clinical Diagnostics, Rochester, NY, USA). Serum adiponectin, and serum leptin concentrations were measured by using a commercial Enzyme-Linked-Immunosorbent-Assay (Human Adiponectin ELISA-kit, and human leptin ELISA-Kit, Quantikine, R&D systems, Wiesbaden, Germany). Human soluble

tumor necrosis receptor I (sTNFRI) and human soluble tumor necrosis receptor II (sTNFR II) concentrations were measured by using a commercial Enzyme-Linked-Immunosorbent-Assay (Quantikine, R&D systems, Wiesbaden, Germany).

**Virology** - Serological testing for anti-HCV was carried out using a commercial microparticle enzyme immunoassay (AxSYM HCV version 3.0, Abbott laboratories, Chicago, IL) according to the manufacturer's instructions. Patients were tested for viral load by RT-PCR (Amplicor HCV, Roche) followed by reverse hybridization for genotyping (Inno-lipa HCVII, Innogenetics, Swigdrecht, Belgium).

**Histological data.** Fibrosis was evaluated using the Metavir scoring systems. The stages of fibrosis were modified to 2 categories as follows: mild fibrosis (stages 0 to 2), and advanced fibrosis (stages 3 and 4). Steatosis was graded by percentage of cells with fatty changes.

**Statistics** - Results were expressed as means  $\pm$  SD. Comparisons between groups were made using the Student's test or the Mann-Whitney U test for continuous variables and the  $\chi^2$  or Fisher exact probability test for categorical data. Comparisons between different groups were performed using analysis of variance (ANOVA). Adiponectin, leptin TNFRI, TNFR II concentrations and HCV viral load were analyzed after log<sub>10</sub> transformation to reduce skewness before t-test analysis. Statistical correlations were determined by the non-parametric Spearman test. Probability levels lower than 0.05 were considered significant. Probability levels lower than 0.15 for correlation with steatosis allowed inclusion of the factor in a logistic regression model. Age and sex were also included in the model. Non-significant factors were removed from the model

if this removal did not modify the coefficients of other factors by more than 20%. Otherwise, they were left in the model and considered as confounding factors.

## **RESULTS**

### **Characteristics of patients**

The main clinical and laboratory data are summarized in Table 1: 39 males (mean age: 40.8 $\pm$ 9.6) and 32 females (mean age: 45.8 $\pm$ 16.1 ) were studied. The mean BMI was 24.3  $\pm$  4.4 (range: 15.6 – 38.7 kg/m<sup>2</sup>) : 23.5  $\pm$  4.2 in males and 25.3  $\pm$  4.5 in females. Only 6 patients (8.45%) were considered as excessive drinkers. Steatosis was present in 42 patients (59.1%), 20 with grade 1 (<10%), 17 with grade 2 (10 to 30%) and 5 with grade 3 (>30). Fibrosis grade 0-1 was present in 40 patients (56.3%), grade 2 in 19 patients (26.7%) and grade 3-4 in 12 patients (16.9%). A significant correlation was found between BMI and steatosis graded by percentage of cells with fatty changes ( $r= 0.43$ ,  $p=0.0007$ ).

### **Adipocytokines concentrations**

Women had significantly higher leptin and adiponectin levels than men (21561 $\pm$ 15115 vs 5230 $\pm$ 6141;  $p<0.0001$  and 14.28 $\pm$ 8.01 vs 9.01 $\pm$ 5.68;  $p=0.001$ , respectively). Leptin levels were significantly correlated with age ( $r= 0.37$ ,  $p= 0.001$ ), BMI ( $r= 0.64$ ,  $p< 0.0001$ ), and insulin ( $r= 0.53$ ,  $p< 0.0001$ ). No correlation were found between adiponectin levels and age ( $r= 0.20$ ,  $p= 0.09$ ), BMI ( $r= -0.22$ ,  $p= 0.06$ ), insulin ( $r= -0.10$ ,  $p= 0.37$ ), leptin ( $r= 0.10$ ,  $p= 0.36$ ), or TNFRII ( $r= 0.12$ ,  $p= 0.27$ ). TNFRII levels were significantly correlated with age ( $r= 0.33$ ,  $p= 0.004$ ), TNFRI ( $r=0.75$ ,  $p<0.0001$ ) and ASAT ( $r= 0.33$ ,  $p= 0.005$ ). No correlation was found between TNFRII and BMI ( $r= 0.06$ ,  $p= 0.60$ ).

The distribution of leptin, adiponectin, TNFRI, and TNFRII levels in relation to degree of hepatic

fibrosis has been evaluated. Only TNFRI and TNFRII levels were significantly higher in patients with advanced fibrosis (metavir 3-4) than in patients with mild fibrosis (metavir 0-2), (2073 $\pm$ 825 vs 1464 $\pm$ 0.22 pg/ml;  $p = 0.009$  and 4312 $\pm$ 967 vs 3397 $\pm$ 864 pg/ml;  $p=0.001$ ; respectively). We observed an increased level of adiponectin in patients with advanced fibrosis, but the difference was border significant (16.66 $\pm$ 11.30 vs 10.31 $\pm$ 5.72  $\mu$ g/ml,  $p=0.059$ ).

### **Factors associated with steatosis**

In univariate analysis, patients with genotype 3 had significantly more steatosis than other patients (84.6% versus 53.4%,  $p=0.05$ ). Patients with steatosis were older, had higher BMI and insulin level compared to patients without steatosis ( 46.7 $\pm$ 12.1 vs 37.7 $\pm$ 12.9,  $p=0.004$ ; 25.6 $\pm$ 4.6 vs 22.4 $\pm$ 3.3,  $p=0.004$ , and 10.89 $\pm$  9.00 vs 7.78  $\pm$  7.35,  $p=0.02$ ; respectively). The distribution of leptin, adiponectin, TNFRI, and TNFRII levels in relation to degree of hepatic steatosis is reported in Table 2. In univariate analysis, only adiponectin and leptin concentrations were significantly different in patients with steatosis compared to patients without steatosis (9.41 $\pm$ 5.92 vs 14.25 $\pm$ 8.16  $\mu$ g/ml;  $p = 0.005$  and 15013 $\pm$ 15097 vs 9082 $\pm$ 10783 pg/ml;  $p=0.03$ ). No significant association was found between alcohol consumption and steatosis ( $p=0.38$ ).

In multivariate analysis, the predictive variables for steatosis were HCV genotype 3 (OR, 3.83; 95% CI [1.29-11.33];  $p=0.015$ ), age (OR, 1.09; 95% CI [1.02-1.16];  $p=0.008$ ), elevated ASAT level (OR=7.73; 95% CI [1.40-42.58];  $p=0.019$ ) and adiponectin concentration (OR, 0.78; 95% CI [0.65-0.92];  $p=0.004$ ) (Table 3). BMI, insulin and leptin were not associated with steatosis.

## **DISCUSSION**

The results of our study indicate that hypoadiponectinemia is strongly associated with the presence of steatosis in patients with chronic hepatitis C. These findings were independent of age, gender, viral characteristics, leptin or insulin concentration and BMI. The present observation that serum adiponectin is one of only 4 independent predictors of hepatic steatosis in hepatitis C chronic infection is novel and interesting.

The pathogenesis of steatosis in patients with HCV is not well understood. Our study is in accordance with previous work showing that HCV genotype 3a is linked to steatosis more strongly than other genotypes (5). However, HCV-related steatosis is not always virally related and other factors may coexist. Steatosis is associated with risk factors for NASH, particularly obesity, rather than alcohol consumption (6).

The mechanisms by which obesity is related to HCV steatosis remain unclear. BMI correlated independently with steatosis in several studies suggesting that obesity has a role in the pathogenesis of HCV steatosis (2, 6). Adinolfi et al. show that HCV-steatosis is strictly correlated with abdominal fat mass rather than total fat mass (3). Therefore visceral obesity rather than total fat mass seems to play a role in the pathogenesis of HCV steatosis (1, 3). The endocrine function of adipose tissue is probably one explanation of the relationship between obesity and liver injury in chronic hepatitis C infection. Several studies have evaluated the role of leptin in HCV steatosis (9, 10). However, some controversial data were obtained, leptin was found associated with steatosis in some studies but not all (9, 10). Giannini et al. have found no relationship between leptin levels and severity of steatosis (9). In contrast, a recent study observed that hepatic steatosis was associated with leptin, body mass index, percentage of body fat, and visceral obesity (10). In our work, we observed that adiponectin rather other adipocytokines is associated with HCV-steatosis. Adiponectin is an adipose-specific secreted protein (7, 8). Unlike other

adipocytokines, such as leptin and TNF-[alpha], plasma adiponectin is inversely correlated with body mass index (BMI), intra-abdominal fat, and indices of insulin resistance. Two receptors of adiponectin have been cloned (12). Adiponectin receptor 1 is abundantly expressed in skeletal muscle, whereas Adiponectin receptor 2 is predominantly expressed in the liver (12). Growing evidence suggests that adiponectin can regulate lipid and glucose metabolism and lipid fat content in hepatocyte (13).

Recently, in a mouse model, Xu et al. showed that circulating concentrations of adiponectin decreased significantly following chronic consumption of high-fat ethanol-containing food (14). In addition, they observed that delivery of recombinant adiponectin into these mice dramatically alleviated hepatomegaly and steatosis (14). In mouse models of altered insulin sensitivity, it has been demonstrated that adiponectin decreases insulin resistance by decreasing triglyceride content in muscle and liver (13). This effect results from increased expression of molecules involved in both fatty-acid combustion and energy dissipation in muscle (13). Likewise, it has been demonstrated that adiponectin had an antifibrogenic effect (15).

In our study, the fact that the plasma levels of adiponectin inversely correlate with steatosis in HCV-infected subjects suggests that hypoadiponectinemia is at least partly responsible for hepatic steatosis and liver injury in this population. The mechanism of this association needs to be clarified, however it is probably related with the effect of adiponectin on lipid metabolism.

Insulin is not an adipocytokine, but its secretion is related to visceral obesity. Insulin resistance already occurs in the early stages of the course of HCV infection, before the development of liver cirrhosis (16). The association between obesity, and steatosis has suggested that hyperinsulinemia and diabetes mellitus may play a role in the pathogenesis of obesity-related steatosis in chronic HCV infection (1, 6). However, our data do not confirm this hypothesis.

Further study needs to explore the respective role of insulin and adiponectin in the pathogenesis of HCV-related steatosis.

Levels of soluble TNF receptors were not significantly correlated with HCV-steatosis. By contrast, their levels were significantly correlated with the severity of the disease such as fibrosis grade. This finding is in line with previous observations that elevated circulating TNFRs in HCV-infected patients were significantly correlated with liver injury (17). Another surprising result of our study is the lack of a correlation between adiponectin level and BMI or insulin concentration in patients with HCV infection. Recently, it has been demonstrated that plasma adiponectin levels are elevated in patients with cirrhosis and do not correlate with any of the established parameters influencing adiponectin levels in normal controls or obese or diabetic populations (18). Instead, liver function seems to be a major determinant of the circulating adiponectin levels in patients with established liver cirrhosis (18). Our findings support these observations and show that adiponectin level is higher in patients with severe fibrosis (metavir A 3-4) than in others (metavir A 0-2). Tietge et al. suggest that the liver may play an important role in the catabolism of adiponectin and that the elevated plasma levels in cirrhosis are, at least in part, due to decreased hepatic catabolism (18).

There are several limitations to our study. The first one is the number of subjects included. Mainly, our results concerning the relationship between fibrosis and adipocytokines should be interpreted cautiously, because only 12 patients presented a severe fibrosis. The second one is that we did not use objective methods to evaluate visceral obesity or body composition.

Our study gives new support to the argument that adiponectin, or treatment leading to an increase of the circulating adiponectin level such as thiazolidinedione, might represent a novel treatment strategy in order to decrease steatosis during HCV infection. Indeed, in type 2 diabetic patients, treatment with Pioglitazone an insulin-sensitizing agent, causes a 3-fold increase in plasma

adiponectin concentration (19). This increase in plasma adiponectin is strongly associated with a decrease in hepatic fat content (19). In NASH patients, Pioglitazone leads to improvement in biochemical and histological features of NASH (20). This kind of treatment could be an interesting alternative to decrease the risk of development of steatosis and to prevent liver damage during HCV infection.

In conclusion, this study demonstrates that hypoadiponectinemia in HCV-infected patients correlates with hepatic steatosis. One practical implication is that therapy to increase circulating adiponectin concentration, such as overweight reduction or thiazolidinediones, provides the potential to improve steatosis in chronic hepatitis C infection.

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Table 1. Clinical and epidemiological characteristics of HCV patients.

	<i>All patients</i>	<i>men</i>	<i>women</i>	<i>p (men vs women)</i>
n	71	39	32	
Age	43.07+/-13.14	40.81+/-9.69	45.81+/-16.15	0.18
BMI	24.33+/-4.43	23.52+/-4.23	25.33+/-4.53	0.03
Insulin	9.57+/-8.42	7.70+/-4.67	11.96+/-11.23	0.24
Leptin (pg/ml)	12590+/-13734	5230+/-6141	21561+/-15115	<0.0001
Adiponectin (µg/ml)	11.39+/-7.27	9.01+/-5.68	14.28+/-8.01	0.001
TNFR1 (pg/ml)	1567+/-517	1471+/-368	1684+/-642	0.22
TNFR2 (pg/ml)	3351+/-940	3399+/-763	3737+/-1103	0.35
Genotype				
3	13	9	4	0.35
non 3	58	30	28	
Viral load (log)	729785+/-490959	658515+/-306481	813334+/-640023	0.64

Table 2: Adipocytokines levels according to steatosis grade.

	Without Steatosis	With Steatosis	p
n	29	42	
Age	37.7+/-12.9	46.71+/-12.1	0.004
BMI	22.4+/-3.2	25.6+/-4.6	0.004
Adiponectin (µg/l)	14.25+/-8.16	9.41+/-5.92	0.005
Leptin (pg/ml)	9082+/-10783	15013+/-15097	0.03
TNFR1 (pg/ml)	1490+/-553	1620+/-490	0.14
TNFR2 (pg/ml)	3376+/-885	3672+/-968	0.13

Table 3: Logistic regression analysis independent association of clinical and biological characteristics with steatosis

variables		coefficient	SE	z	p
Adiponectin		0.77	0.07	-2.88	0.004
Genotype	3	3.82	2.11	2.43	0.015
	non 3	1			
age		1.09	0.03	2.66	0.008
Leptin		1.00	0.00	1.13	0.26
BMI		1.16	0.16	1.06	0.29
Asat level	normal	1			
	elevated	7.73	6.73	2.35	0.019