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Plasma adiponectin—an independent indicator of liver fat accumulation

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ABSTRACT

Proinflammatory cytokines and adipokines have a significant role in the development and progression of fatty liver. The aim of our current study was to explore the major indicators for hepatic fat determined as liver brightness. In addition to peptide hormones, several known cardiovascular and metabolic risk factors were included in the model. This is a population-based, epidemiological, cross-sectional study where 1200 subjects (600 men and 600 women, aged 40-59 years) were randomly selected, half of them having hypertension and half of them being controls. The severity of liver adiposity was measured by ultrasound and based on the brightness of the liver estimated as a numerical value ranging from 0 to 2. With respect to the studied peptide hormones, the associations between liver brightness and plasma adiponectin ($P < .001$), leptin ($P < .001$), ghrelin ($P = .005$), and highly sensitive C-reactive protein concentrations ($P < .001$) were significant before adjustments. When several other risk factors (age, sex, body mass index, waist circumference, quantitative insulin sensitivity check index, smoking, and alcohol consumption) and novel risk markers (adiponectin, leptin, ghrelin, and highly sensitive C-reactive protein concentrations) were considered simultaneously, of the peptide hormones, adiponectin remained the strongest independent indicator of the brightness of the liver ($P = .025$). Adiponectin is a very strong predictor for liver brightness, even after adjustment for the numerous other metabolic risk factors, markers of inflammation, and novel obesity-related peptide hormones. Whether low adiponectin levels predict to liver fat accumulation remains to be explored in a future prospective follow-up of this cohort.

1. Introduction

The prevalence of obesity is dramatically increasing in the Western world, taking on the characteristics of an epidemic. Obesity is currently regarded as a low-grade inflammation and is associated with multiple metabolic risk factors for cardiovascular disease, including insulin resistance, diabetes, and dyslipidemia [1,2]. The metabolic syndrome (MS) is a range of risk factors that increase an individual's propensity for developing atherosclerotic cardiovascular disease, type 2 diabetes mellitus, and chronic kidney disease. Evident risk factors are abdominal obesity, atherogenic dyslipidemia, hypertension, and elevated plasma glucose leading to prothrombic and a proinflammatory state [3]. Chronic inflammation is frequent-

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ly associated with MS, and proinflammatory cytokines such as highly sensitive C-reactive protein (hs-CRP) result in advanced insulin resistance and lipolysis of adipose tissue triglyceride stores [3]. Adipose tissue inflammation contributes to reduced plasma adiponectin levels in obesity [4], and adiponectin levels are known to be lower in patients with the MS [3]. Most obese people have high endogenous leptin levels indicating complete or relative leptin resistance [5]. Circulating levels of ghrelin, a peptide hormone secreted mainly from the stomach [6], are low in human obesity where insulin resistance is the most likely reason for low fasting ghrelin concentrations [7].

Nonalcoholic fatty liver disease (NAFLD) is a hepatic manifestation of the MS [8]. It is the most common liver disease because its prevalence is estimated to be 10% to 25% in the general population of Western countries [9]. *Nonalcoholic fatty liver disease* refers to a spectrum of liver disorders resulting from abnormal fat deposition in the liver, which ranges in severity from simple hepatic steatosis with no inflammation to steatohepatitis (nonalcoholic steatohepatitis [NASH]) that can progress to liver cirrhosis [10,11]. Patients with NAFLD display a larger prevalence of metabolic abnormalities, and the prevalence of MS is more than doubled [12]. Today, the presence of fatty liver is considered as an early and sensitive indicator of insulin resistance; and it has been reported to be present in 46% of NASH patients [13].

Adipokines, cytokines, and many other peptides may play a pivotal role in the pathogenesis and severity of NAFLD [14]. Several investigators have discovered serum adiponectin levels to be inversely associated with the severity of NAFLD [15-17]. Studies in *ob/ob* mice have indicated that leptin prevents fatty liver both directly and indirectly [5], having spontaneous mutations that diminish production of leptin [18]. Nevertheless, patients with obesity have fatty liver despite the presence of elevated leptin levels, suggesting that these patients have hepatic leptin resistance [4]. Obese *ob/ob* mice and *fa/fa* rats exhibit insulin resistance, hyperglycemia, and fatty livers, as do most obese humans [19].

Reduced ghrelin levels have been reported in patients with NAFLD also after correction for sex, age, and body mass index (BMI) [7]. The elevation of the serum hs-CRP level has been observed in patients with NASH compared with that in those with simple steatosis [20].

Although adipokines and other obesity-related peptide hormones have been individually related to liver fat, very few studies have considered all these factors together. The aim of our study was to explore the important independent indicators for increased liver brightness in a large randomized population-based cohort. In addition to peptide hormones, we included several known cardiovascular and metabolic risk factors in the same model to explain ultrasound-determined liver adiposity.

2. Patients

This study is a part of the Oulu Project Elucidating Risk of Atherosclerosis, which is a population-based, epidemiological, cross-sectional study designed to address the risk factors and disease end points of atherosclerotic cardiovascular diseases. Briefly, this cohort included 600 men and 600 women, aged 40 to 59 years, randomly selected from the national register. Half of them were patients with hypertension, with the other half being controls [21]. Body mass index was calculated as [mass (kilograms)]/{[height (meters)]^2}. Waist circumference was measured to the nearest 0.5 cm with a tape measure midway between the lower rib margin and the iliac crest in light expirium. Blood pressure was measured according to the recommendations of the American Society of Hypertension in sitting position from the right arm with an oscillometric device (Dinamap† model 18465X; Criticon, Ascot, UK) after an overnight fast and after a 10- to 15-minute rest. Three measurements were made at 1-minute intervals, and the means of the last 2 were used in the analyses.

Altogether, 1045 subjects volunteered to participate in the study, which was approved by the Ethical Committee of the Faculty of Medicine, University of Oulu.

3. Determination of liver adiposity

The determination of liver adiposity was based on liver-kidney contrast measured with ultrasonography [22,23] by one single trained radiologist with extensive experience in abdominal ultrasound examinations. The ultrasound examination was carried out using a Toshiba SSA 270 ultrasound system (Toshiba, Tokyo, Japan) with a scanning frequency of 5 MHz. The whole scanning procedure was captured on a video film of Super-VHS videocassette recorder (Panasonic, Osaka, Japan), and the videotapes were analyzed later. The severity of liver adiposity was based according to the brightness of the liver estimated as a numerical value ranging from 0 to 2 (0 = normal brightness, indicating a nonfatty liver; 1 = medium brightness, moderate lipid accumulation; and 2 = intensely bright, severe lipid accumulation and fatty liver).

4. Laboratory methods

Plasma adiponectin concentrations were measured with an enzyme-linked immunosorbent assay technique devised in our laboratory [24]. Monoclonal anti-human adiponectin antibody (R&D Systems, Minneapolis, MN, catalog no. MAB10651) was used as the capture antibody; and biotinylated monoclonal anti-human adiponectin antibody (R&D Systems, catalog no. BAM1065), as the detection antibody. Both antibodies were used in a concentration of 2 μg/mL. For the detection of biotin-labeled detection antibody, we used alkaline phosphatase–labeled NeutrAvidin diluted in a ratio of 1:18 000 (Pierce, Rockford, IL, catalog no. 31 002) and 30% Lumiphos530 (Lumigen, Southfield, MI, catalog no. P-501). The standard curve from 1.56 to 100 ng/mL was prepared from human recombinant adiponectin (Biovendor, Modrice, Czech Republic, catalog no. RD172023100). Plasma samples were diluted in the ratio of 1:500, and the concentrations were measured in duplicate. The intraassay variation of the method was 13.9%, and the interassay variation was 15.9% before and 6.5% after correction.

Fasting plasma leptin concentrations were measured using a commercial double-antibody radioimmunoassay (Human

Leptin RIA Kit; Linco Research, St Charles, MO) with an intraassay coefficient of variation of 3.4% to 8.3% and an interassay coefficient of variation of 3.0 to 6.2%. Fasting ghrelin concentrations were measured from plasma samples stored at –20°C using a commercial radioimmunoassay kit (Phoenix Pharmaceuticals, Belmont, CA) [25], which recognizes both acylated and desacylated ghrelin and uses ^{125}I-labeled bioactive ghrelin as a tracer molecule and a polyclonal antibody raised in rabbits against full-length octanoylated human ghrelin. All assays included 3 control samples from one fresh plasma sample that was aliquoted and frozen at the beginning of the study and used to normalize each test for interassay variability. Highly sensitive CRP was measured using commercially available enzyme-linked immunosorbent assay kits with a detection limit of 0.31 ng/mL (Diagnostic Systems Laboratories, Webster, TX).

After fasting blood had been drawn, the subjects were given a 75-g glucose load. Both 1-hour and 2-hour glucose and insulin concentrations were determined, except for previously known insulin-treated diabetic patients. The glucose concentrations were measured with the glucose dehydrogenase method (Diagnostica; Merck, Darmstadt, Germany). The serum insulin levels were measured using a 2-site immunoenzymometric assay (AIA-PACK IRI; Tosoh, Tokyo, Japan). Insulin sensitivity was assessed via fasting plasma insulin concentrations and a quantitative insulin sensitivity check index {QUICKI = 1/[log (fasting insulin)+log (fasting glucose)]} [26].

Very low-density lipoprotein (VLDL, $d < 1.006$ g/mL^{-1}) was isolated by centrifuging plasma in a Kontron TFT 45.6 (Kontron AG, Zurich, Switzerland) rotor at 105 000*g* and 15°C for 18 hours [21]. A half milliliter of the VLDL-free fraction was mixed with 25 μL of 2.8% (wt/vol) heparin and 25 μL of 2 mol/L manganese chloride. After centrifugation at 1000*g* and 4°C for 30 minutes, aliquots of the supernatant were taken for analysis of the high-density lipoprotein (HDL) concentration. The low-density lipoprotein concentration was then calculated by subtracting the cholesterol concentration in HDL from that in the VLDL-free fraction. The concentrations of total cholesterol and triglycerides in the plasma and lipoprotein fractions were determined by enzymatic colorimetric methods (kits of Boehringer Diagnostica, Mannheim, Germany; catalog nos. 236691 and 701912), respectively, using a Kone Specific Selective Chemistry Analyser (Kone Instruments, Espoo, Finland).

5. Statistical analysis

Statistical analysis was performed by using SPSS (Chicago, IL) version 16.0. Analysis of variance was used to compare the means of the variables measured. Statistical significances between percentages were measured by using χ^2 test. The possible interaction between adiponectin and liver fat accumulation was assessed by analysis of covariance. The following variables were entered into the multivariate models as covariates: sex; age; BMI; waist circumference; QUICKI; smoking; alcohol consumption; and leptin, ghrelin, and hs-CRP concentrations. *P* value < .05 was regarded as significant. Post hoc tests were performed using the Tukey method. Multiple logistic regression analysis was performed to investigate the associations of the different variables associated to liver brightness (fat).

6. Results

The main characteristics of the study population are shown in Table 1. Male sex was more prevalent among the subjects with severe (56.8%) and moderate (65.9%) liver fatness than in those with normal brightness of the liver (45%) ($P < .001$). Smoking status differed between the groups, with those subjects with grade 1 liver fat accumulation having the highest and the subjects with no fat accumulation the lowest smoking prevalence. Alcohol consumption rates were lowest among subjects with normal brightness of the liver and highest among subjects with moderate liver fat accumulation ($P = .01$).

When the controls and hypertensive patients were considered, 57.8%, 33.3%, and 28.1% of the control subjects belonged to the group with normal, moderate, and severe brightness of the liver, whereas the same figures among hypertensive patients were 42.2%, 66.7% and 71.9%, respectively ($P < .001$).

As expected, BMI and waist circumference were higher among ($P < .001$) patients with moderate or severe lipid accumulation than in those with normal lipid accumulation of the liver. The differences were significant also in the comparison between moderate and severe fat accumulation groups (waist circumference, $P < .05$; BMI, $P < .01$).

Triglyceride levels were higher and HDL concentrations were lower in the groups with lipid accumulation than in those with normal liver brightness ($P < .001$). Fasting insulin levels were more than 2 times higher in the group with severe liver fatness compared with patients with no fatty liver ($P < .001$). In addition, the difference in fasting insulin levels between groups with normal and moderate brightness and moderate and severe brightness of the liver reached statistical significance ($P < .001$).

The QUICKI level decreased as one moved from the normal to moderate to severe fat accumulation groups.

6.1. *Peptide hormones and liver brightness*

The associations between plasma adiponectin ($P < .001$), leptin ($P < .001$), ghrelin < .01, and hs-CRP concentrations ($P < .001$) and liver brightness were significant before adjustments (Table 1). Subjects with a severe lipid accumulation had the lowest adiponectin and ghrelin, at the same time having the highest hs-CRP and leptin concentrations.

After adjustments for age, sex, and BMI, the association between plasma adiponectin levels and liver brightness remained strongly statistically significant ($P < .001$). Associations between plasma leptin ($P = .013$) and hs-CRP ($P < .001$) levels remained statistically significant as well.

After further adjustment for age; sex; BMI; waist circumference; QUICKI; alcohol consumption; smoking; and ghrelin, leptin, and CRP concentrations, adiponectin levels still remained statistically significantly related to liver brightness ($P = .025$) (Fig. 1). When all these subjects were added as

Table 1 – The main characteristics of study group as means (SD) or frequencies (percentages, n = number of patients) grouped by liver brightness

Grade of liver brightness	0 (n = 747)	1 (n = 135)	2 (n = 146)	P	[a]P	[b]P	[c]P
Age (y)	51.03 (6.04)	52.09 (6.14)	51.60 (5.54)	NS	NS	NS	NS
Male	45.0% (n = 336)	65.9% (n = 89)	56.8% (n = 83)	<.001	–	–	–
Smoking (pack-years)	11.01 (13.60)	14.26 (14.97)	13.90 (14.54)	<.01	<.05	NS	NS
Alcohol consumption (g/wk)	50.99 (82.35)	90.98 (115.28)	82.10 (104.52)	<.05	<.05	NS	NS
BMI (kg/m^2)	26.49 (3.91)	29.95 (4.88)	31.77 (4.92)	<.001	<.001	<.01	<.001
Waist circumference (cm)	86.89 (11.90)	98.02 (11.65)	102.06 (11.84)	<.001	<.001	<.05	<.001
Hypertensive	42.2% (n = 315)	66.7% (n = 90)	71.9% (n = 105)	<.001	–	–	–
Total serum cholesterol (mmol/L)	5.65 (1.04)	5.85 (1.06)	5.78 (1.08)	NS	NS	NS	NS
HDL (mmol/L)	1.41 (0.38)	1.22 (0.34)	1.18 (0.35)	<.001	<.001	NS	<.001
Triglycerides (mmol/L)	1.38 (0.83)	1.90 (1.02)	2.22 (1.35)	<.001	<.001	NS	<.001
Fasting insulin (mmol/L)	10.80 (7.60)	18.43 (10.56)	23.73 (17.56)	<.001	<.001	<.001	<.001
QUICKI	0.64 (0.11)	0.54 (0.09)	0.50 (0.07)	<.001	<.001	<.001	<.001
Adiponectin (μg/mL)	16.54 (6.33)	14.09 (5.35)	12.24 (5.34)	<.001	< .001	<.05	<.001
Leptin (ng/mL)	9.77 (7.53)	12.04 (9.67)	13.57 (9.15)	<.001	<.05	NS	<.001
Ghrelin (pg/mL)	682.27 (240.06)	632.65 (226.48)	622.53 (260.33)	<.01	NS	NS	<.05
hs-CRP (ng/mL)	3147.13 (6832.83)	4600.87 (10362.07)	6087.52 (6593.80)	<.001	<.001	<.01	<.001

Data are means ± SD or percentages. Differences between groups were assessed by the analysis of variance test and Tukey post hoc test. Statistical significances between percentages were measured by using the χ^2 test. *P* values for the difference between whole group.
[a] *P* values for differences between groups 0 and 1.
[b] *P* values for differences between groups 1 and 2.
[c] *P* values for the differences between groups 0 and 2.

covariates, the other factors reaching statistical significance (in addition to adiponectin) were waist circumference ($P = .044$) and QUICKI ($P < .001$).

Logistic regression analysis was performed to identify independent indicators of the liver brightness. Patients were divided into 2 groups (no fat in the liver vs fatty liver). The same covariates were added as explanatory factors into this logistic regression analysis as in the analysis of covariance.

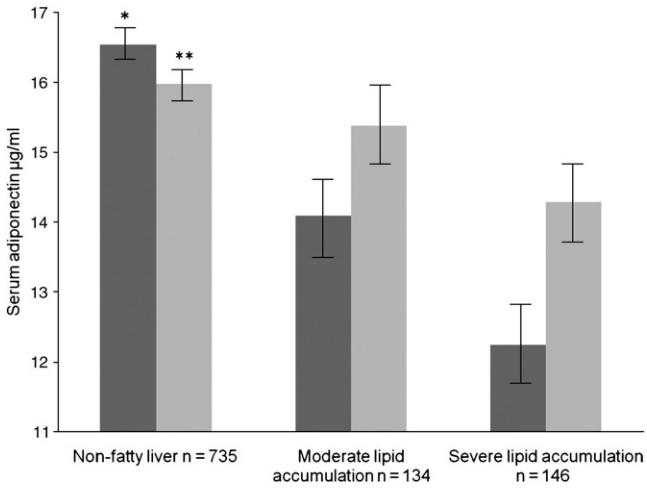

Fig. 1 – Black (left) panels: serum adiponectin level (mean and SE) and severity of liver adiposity without adjustments. Grey (right) panels: serum adiponectin level and severity of liver adiposity after adjustment for age, sex, BMI, waist circumference, alcohol consumption, smoking, serum leptin level, serum hs-CRP level, serum ghrelin level, and QUICKI. *P value significant ($P < .01$) between nonfatty liver and severe lipid accumulation groups. **P values significant ($P < .05$) between all groups.

QUICKI was multiplied by 10 to reach better logistic regression analysis resolution capacity. Adiponectin ($P = .02$), QUICKI ($P < .001$), and alcohol consumption ($P = .006$) remained statistically significant risk factors for liver brightness (Table 2). When these variables were added into the logistic regression analysis independently, adiponectin reached a Nagelkerke R^2 value of 0.089; QUICKI, 0.366; and alcohol consumption, 0.013 .

7. Discussion

Nonalcoholic fatty liver disease is one of the most common liver diseases affecting both adults and children in the Western world. It is strongly associated with the MS and

Table 2 – Multiple logistic regression analysis for independent indicators of hepatic brightness (fat)

Source	B	Odds ratio (with a 95% confidence interval)	P value
Age	0.026	1.027 (0.997-1.058)	.080
Sex	-0.013	1.013 (0.472-2.174)	.973
BMI	0.054	1.055 (0.969-1.148)	.214
Waist circumference	0.014	1.014 (0.979-1.051)	.438
Adiponectin	-0.038	0.963 (0.933-0.994)	.020
Ghrelin	0.000	1.000 (0.999-1.000)	.284
Leptin	0.203	1.226 (0.408-3.685)	.717
hs-CRP	0.231	1.260 (0.870-1.825)	.222
QUICKI	-1.522	0.218 (0.154-0.310)	.000
Smoking (pack-years)	0.003	1.003 (0.990-1.017)	.620
Alcohol consumption	0.188	1.207 (1.056-1.380)	.006
Nagelkerke R^2 0.426			

insulin resistance. The exact mechanisms by which NAFLD develops into steatohepatitis and potentially leads to cirrhosis are still unknown. The main result of our study is that, if one considers the peptide hormones, then adiponectin is the strongest independent indicator of the brightness of the liver even after adjustment for several risk factors (age; sex; BMI; waist circumference; QUICKI; smoking; alcohol consumption; and leptin, ghrelin, and hs-CRP concentrations).

Our data indicated that BMI, waist circumference, triglyceride level, fasting insulin concentration, and hs-CRP and leptin levels increased, whereas HDL level, QUICKI, and adiponectin and ghrelin levels decreased, when the magnitude of liver brightness increased. It is known that obesity increases dramatically the risk of steatohepatitis [27]. The reported prevalence of obesity in several series of patients with nonalcoholic fatty liver disease has varied between 30% and 100% [10]. There are several obesity-related peptide hormones; and of those, the associations between liver brightness and plasma adiponectin and hs-CRP concentrations were significant even after adjustment for BMI in our study. The relationship between ghrelin and leptin levels and liver fat accumulation seems to relate only to obesity. Ghrelin levels are inversely related to BMI, which might represent a compensatory response to the sustained positive energy balance [28]. In the study of Marchesini et al [7], ghrelin levels were reduced in NAFLD patients after correction for sex, age, and BMI. In our study, the relationship between ghrelin levels and liver fat accumulation was not statistically significant after adjustment for BMI. Most obese humans have increased leptin levels [5]. In the report of Krawzyk et al [15], serum concentration of leptin showed no significant difference between patients with NASH and the control group. In patients with obesity-associated NAFLD, serum levels of leptin were increased and the liver became resistant to the "antisteatotic" effects of leptin [17]. In patients with more advanced inflammation and fibrosis, the mean serum concentration of leptin was reported to be significantly higher than that in patients with steatosis and less advanced inflammation and fibrosis [29].

In the study of Targher et al [30], hs-CRP concentrations were lowest in healthy controls, intermediate in overweight nonsteatotic patients, and the highest in those subjects with NASH. In another study, the plasma hs-CRP level was markedly higher in subjects with nonalcoholic hepatic steatosis compared with those without hepatic steatosis [31]. However, the number of subjects in the latter study was rather small (n = 100). In our study, with its large sample size, the difference between normal, intermediate, and severe fat accumulation groups was statistically significant after adjustment for BMI ($P < .001$). The pathogenesis of steatohepatitis is generally explained by the "2-hit theory" whereby the first hit consists of free fatty acids accumulation in the liver. An excess of free fatty acids predisposes the liver to the second hit, which is evoked by the oxidative stress and proinflammatory cytokines [32].

Serum adiponectin concentrations have been reported to correlate inversely with BMI, fasting insulin levels, and plasma triglycerides concentration [16]; and our study confirms these hypotheses. Recent studies have demonstrated that adiponectin levels are low in NAFLD patients [9,12,15,30]. Adiponectin levels are decreased from 20% to 40% during the development of NAFLD [17]. The mean adiponectin levels in the controls were reported by Hui et al [33] to be about twice as high as the levels in subjects with NASH. Therefore, it not surprising that our patients with no fat in the liver had significantly higher adiponectin concentrations than the patients with the highest liver brightness. Thus, our study suggests that adiponectin is a strong independent marker of liver brightness. Notably, the association of adiponectin and liver brightness was still evident after considering numerous other metabolic risk factors, markers of inflammation, and novel obesity-related peptide hormones at the same time. One could speculate that the low adiponectin concentration in the patients with the highest liver brightness may be due to decreased adipose tissue release, increased adiponectin degradation, or both.

It has been postulated that adiponectin can increase glucose uptake in skeletal muscle and may protect the hepatocytes from triglyceride accumulation, conceivably by increasing β-oxidation of free fatty acids and/or decreasing de novo free fatty acid synthesis in both skeletal muscle and liver [34]. The activation of the adenosine monophosphate kinase pathway may represent the mechanistic link for these effects of adiponectin [34]. Furthermore, adiponectin might play a role in suppressing inflammation and macrophage activity [16]. It is clear that adipokines play an important role and may represent a link between obesity, insulin resistance, and NAFLD [27]. There are also several inflammatory factors that regulate adiponectin levels in patients with NAFLD [27].

In this study, the grade of liver brightness was measured by ultrasound. The invasive diagnostic technique of liver biopsy is necessary for the diagnosis of NASH [20]. Real-time ultrasound using a combination of sonographic findings has a high specificity, but the problem is that it underestimates the prevalence of hepatic steatosis when there is less than 20% fat [35]. It is obvious that taking liver biopsies from large groups of symptomless subjects is ethically unjustifiable. Therefore, we chose to use this noninvasive ultrasound method. The research groups that have taken biopsies have had access to rather small patient samples.

The results of the present study can be enlarged to relate to the whole population aged 40 to 59 years, at least in Finland. This was a population-based, epidemiological, cross-sectional cohort study with the subjects being randomly selected from the national register. Therefore, the selection bias can be regarded as minimal.

In conclusion, adiponectin is a very strong predictor for liver brightness, even after adjustment for numerous other metabolic risk factors, markers of inflammation, and novel obesity-related peptide hormones. Whether the low adiponectin level predicts liver fat accumulation remains to be explored in a future prospective follow-up of this cohort. In the clinical setting, adiponectin could serve as a potential marker in the diagnosis of liver fat accumulation; and its potential therapeutic usefulness for the treatment and/or prevention of NAFLD should also be tested. In addition, detailed mechanistic studies are needed to unravel the specific metabolic pathways of adiponectin in relation to the pathogenesis of NAFLD.

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REFERENCES

[1] Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. Am J Clin Nutr 2006;83: 461S-5S.
[2] Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology 2010;51:679-89.
[3] Paschos P, Paletas K. Nonalcoholic fatty liver disease and metabolic syndrome. Hippokratia 2009;13:9-19.
[4] Marra F, Bertolani C. Adipokines in liver diseases. Hepatology 2009;50:957-69.
[5] Mantzoros CS. The role of leptin in human obesity and disease: a review of current evidence. Ann Intern Med 1999;130:671-80.
[6] Boguszewski CL, Paz-Filho G, Velloso LA. Neuroendocrine body weight regulation: integration between fat tissue, gastrointestinal tract, and the brain. Endokrynol Pol 2010;61: 194-206.
[7] Marchesini G, Pagotto U, Bugianesi E, De Iasio R, Manini R, Vanni E, et al. Low ghrelin concentrations in nonalcoholic fatty liver disease are related to insulin resistance. J Clin Endocrinol Metab 2003;88:5674-9.
[8] Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003;37:917-23.
[9] Tsochatzis EA, Papatheodoridis GV, Archimandritis AJ. Adipokines in nonalcoholic steatohepatitis: from pathogenesis to implications in diagnosis and therapy. Mediators Inflamm 2009;2009:831670.
[10] Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346:1221-31.
[11] Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology 2006;43(2 Suppl 1): S99-112.
[12] Bugianesi E, Pagotto U, Manini R, Vanni E, Gastaldelli A, de Iasio R, et al. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. J Clin Endocrinol Metab 2005;90:3498-504.
[13] Tsochatzis EA, Manolakopoulos S, Papatheodoridis GV, Archimandritis AJ. Insulin resistance and metabolic syndrome in chronic liver diseases: old entities with new implications. Scand J Gastroenterol 2009;44:6-14.
[14] Jarrar MH, Baranova A, Collantes R, Ranard B, Stepanova M, Bennett C, et al. Adipokines and cytokines in non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2008;27:412-21.
[15] Krawczyk K, Szczesniak P, Kumor A, Jasinska A, Omulecka A, Pietruczuk M, et al. Adipohormones as prognostic markers in patients with nonalcoholic steatohepatitis (NASH). J Physiol Pharmacol 2009;60(Suppl 3):71-5.
[16] Tsochatzis E, Papatheodoridis GV, Archimandritis AJ. The evolving role of leptin and adiponectin in chronic liver diseases. Am J Gastroenterol 2006;101:2629-40.
[17] Wang Y, Zhou M, Lam KS, Xu A. Protective roles of adiponectin in obesity-related fatty liver diseases: mechanisms and therapeutic implications. Arq Bras Endocrinol Metabol 2009;53:201-12.
[18] Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, et al. Effects of the obese gene product on body weight regulation in *ob/ob* mice. Science 1995;269:540-3.
[19] Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. N Engl J Med 2000;343:1467-76.
[20] Yoneda M, Mawatari H, Fujita K, Iida H, Yonemitsu K, Kato S, et al. High-sensitivity C-reactive protein is an independent clinical feature of nonalcoholic steatohepatitis (NASH) and also of the severity of fibrosis in NASH. J Gastroenterol 2007;42:573-82.
[21] Rantala AO, Kauma H, Lilja M, Savolainen MJ, Reunanen A, Kesaniemi YA. Prevalence of the metabolic syndrome in drug-treated hypertensive patients and control subjects. J Intern Med 1999;245:163-74.
[22] Sampi M, Veneskoski M, Ukkola O, Kesaniemi YA, Horkko S. High plasma immunoglobulin (ig) A and low IgG antibody titers to oxidized low-density lipoprotein are associated with markers of glucose metabolism. J Clin Endocrinol Metab 2010;95:2467-75.
[23] Yajima Y, Ohta K, Narui T, Abe R, Suzuki H, Ohtsuki M. Ultrasonographical diagnosis of fatty liver: significance of the liver-kidney contrast. Tohoku J Exp Med 1983;139:43-50.
[24] Santaniemi M, Kesaniemi YA, Ukkola O. Low plasma adiponectin concentration is an indicator of the metabolic syndrome. Eur J Endocrinol 2006;155:745-50.
[25] Poykko SM, Kellokoski E, Horkko S, Kauma H, Kesaniemi YA, Ukkola O. Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. Diabetes 2003;52:2546-53.
[26] Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85:2402-10.
[27] Tilg H, Hotamisligil GS. Nonalcoholic fatty liver disease: cytokine-adipokine interplay and regulation of insulin resistance. Gastroenterology 2006;131:934-45.
[28] Dostalova I, Haluzik M. The role of ghrelin in the regulation of food intake in patients with obesity and anorexia nervosa. Physiol Res 2009;58:159-70.
[29] Javor ED, Ghany MG, Cochran EK, Oral EA, DePaoli AM, Premkumar A, et al. Leptin reverses nonalcoholic steatohepatitis in patients with severe lipodystrophy. Hepatology 2005;41:753-60.
[30] Targher G, Bertolini L, Rodella S, Lippi G, Franchini M, Zoppini G, et al. NASH predicts plasma inflammatory biomarkers independently of visceral fat in men. Obesity (Silver Spring) 2008;16:1394-9.
[31] Targher G, Bertolini L, Scala L, Zoppini G, Zenari L, Falezza G. Non-alcoholic hepatic steatosis and its relation to increased plasma biomarkers of inflammation and endothelial dysfunction in non-diabetic men. role of visceral adipose tissue. Diabet Med 2005;22:1354-8.
[32] Hijona E, Hijona L, Arenas JI, Bujanda L. Inflammatory mediators of hepatic steatosis. Mediators Inflamm 2010;2010:837419.
[33] Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? Hepatology 2004;40:46-54.
[34] Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288-95.
[35] Dasarathy S, Dasarathy J, Khiyami A, Joseph R, Lopez R, McCullough AJ. Validity of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. J Hepatol 2009;51:1061-7.