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Plasma apelin levels in subjects with nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD), one of the most common forms of chronic liver disease, is closely associated with obesity and insulin resistance. Recent studies suggest that apelin, a newly described adipokine, is associated with hyperinsulinemia and inflammation. The aim of the study was to investigate plasma apelin concentrations in biopsy-proven NAFLD patients who had no metabolic confounders and also to search for the association of apelin with adiponectin, body mass index (BMI), and insulin sensitivity. Fifty male patients with NAFLD and 30 healthy male controls were enrolled. Apelin was measured along with BMI, lipids, glucose, insulin, adiponectin, and homeostasis model assessment (HOMA) of insulin resistance indexes. Plasma apelin levels were significantly higher and adiponectin levels were lower in NAFLD patients when compared with the controls ($P < .001$ and $P = .013$, respectively). In multivariate analysis adjusted for BMI and HOMA indexes, the differences in apelin and adiponectin disappeared in the 2 groups ($P = .3$ and $P = .1$, respectively). In addition, apelin levels were positively correlated with BMI ($r = 0.29$, $P = .05$) and HOMA indexes ($r = 0.4$, $P = .008$) in subjects with NAFLD. The results of this preliminary study suggest that plasma apelin levels are not altered in nondiabetic and normotensive male subjects with NAFLD.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) represents a wide spectrum of diseases, ranging from simple steatosis (SS) through steatosis with inflammation (nonalcoholic steatohepatitis [NASH]) to cirrhosis. Nonalcoholic fatty liver disease, which is strongly associated with obesity, insulin resistance, and type 2 diabetes mellitus (T2DM), is now well recognized as being part of the metabolic syndrome. The pathogenesis of NAFLD is thought to be related mainly with insulin resistance (IR) syndrome and oxidative stress; the latter resulting from mitochondrial fatty acids oxidation, nuclear factor-κB–dependent inflammatory cytokine expression, and adipocytokines may promote hepatocellular damage, inflammation, fibrosis, and progressive liver disease. Adipocytokines and other recognized cytokines produced partially by inflammatory cells infiltrating adipose tissue play an important role in the pathogenesis of IR and NAFLD through complex and interactive paracrine and endocrine mechanisms [1,2].

Apelin is a new member of adipose tissue–derived peptides, which is also produced in the endothelial cells in various parts of the body. It affects through a cell surface G protein–coupled receptor called *APJ*, which has structural similarity with angiotensin type I receptor [3]. Apelin synthesis in adipocytes is stimulated by insulin, and plasma apelin level markedly increases in obesity associated with IR and hyperinsulinemia [4]. We have recently reported decreased circulating apelin levels in patients with dyslipidemia and also newly diagnosed and untreated T2DM [5,6]. In recent years, apelin has also been reported to be associated with inflammation and angiogenesis [7].

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To our knowledge, there are no human data regarding the role of apelin in the pathogenesis of NAFLD. Therefore, in the present study, we aimed to investigate plasma apelin concentrations in subjects with biopsy-proven NAFLD who had no additional disorder such as morbid obesity, T2DM, and hypertension. In addition, the relationship of apelin with adiponectin, body mass index (BMI), and insulin sensitivity were also searched.

2. Subjects and methods

2.1. Subjects

A total of 50 subjects with NAFLD (n = 36 for NASH and n = 14 for SS) were enrolled the study. This was a selected sample of male outpatients recruited among 120 patients with biopsy-proven NAFLD who attended the outpatient clinic of the Gastroenterology Division, Gulhane School of Medicine, Ankara, Turkey. Inclusion criteria were as follows: persistently (at least 6 months) elevated aminotransferases, ultrasonographic presence of bright liver without any other liver or biliary tract disease, and liver histology compatible with a diagnosis of NASH or SS. Exclusion criteria were as follows: a history of alcohol consumption of 40 g/wk, as assessed by a detailed interview extended to family members; a BMI of at least 35 kg/m^2; positive serum markers of viral, autoimmune, or celiac disease; abnormal copper metabolism or thyroid function test results; a diagnosis of diabetes mellitus (fasting plasma glucose ≥126 mg/dL or ≥200 mg/dL at 2 hours on a standard oral glucose tolerance test); serum total cholesterol of at least 250 mg/dL; serum triglycerides of at least 400 mg/dL; or exposure to occupational hepatotoxins or drugs known to be steatogenic or to affect glucose and lipid metabolism. The control group, who was matched for age, consisted of 30 male healthy volunteers with normal liver ultrasonography results and normal liver function test results.

All participants provided a medical history and underwent a clinical examination. The weight and height of the participants were measured with a calibrated scale after the patients had removed their shoes and any heavy clothing. Body mass index was calculated by dividing weight in kilograms by height in meters squared. Waist circumference (WC) was measured as the midpoint between the lower costal margin and the level of the anterior superior iliac crests. Blood pressure was measured with a standard manometer.

Written informed consent was obtained from all participants. The protocol was approved by the local ethical committee.

2.2. Biological measurements

All blood samples were collected from an antecubital vein between 8:00 and 9.00 AM after an overnight fasting. The samples were centrifuged for 15 minutes at 3000 rpm, aliquoted, and immediately frozen at −80°C for analyses until examination. All samples were run in the same assay.

Glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and γ-glutamyl transpeptidase levels were measured by the enzymatic colorimetric method with Olympus AU2700 autoanalyzer using reagents from Olympus Diagnostics (Hamburg, Germany). Low-density lipoprotein cholesterol (LDL-C) was calculated by the formula of Friedewald et al [8]. The serum basal insulin level was measured in duplicate by the chemiluminescence method (Roche Diagnostics, Osaka, Japan). Insulin resistance was calculated by homeostasis model assessment (HOMA) [9] using the following formula: HOMA-IR = fasting insulin (in microunits per milliliter) × fasting glucose (in milligrams per deciliter)/405. This index has been shown to be well correlated with the results of the euglycemic-hyperinsulinemic clamp method to determine IR [10]. Low HOMA-IR values indicate high insulin sensitivity, whereas high HOMA-IR values indicate low insulin sensitivity.

Plasma apelin-12 levels were determined by enzyme-linked immunosorbent assay (ELISA) (Human Apelin-12 ELISA Kit, catalog no. EK-057-23; Phoenix Pharmaceuticals, Belmont, CA) (sensitivity [minimum detectable concentration] = 0.15-0.25 ng/mL, intraassay coefficient of variation [CV]: 5%, and interassay CV: 14%). Plasma adiponectin concentration was measured in duplicate by ELISA (Human Adiponectin ELISA Kit, catalog no. HADP-61K; Linco Research, St Charles, MO) (sensitivity [minimum detectable concentration] = 1 ng/mL, intraassay CV: 3.59%, and interassay CV: 9.25%).

2.3. Pathologic examination

An experienced hepatopathologist blinded to subjects' details scored liver biopsy specimens using the classification of Kleiner et al [11]. Briefly, liver tissues were stained with hematoxylin-eosin, reticulin, and Gomori trichrome stains and scored. All cases showed macrovesicular steatosis affecting at least 5% of hepatocytes, and these were classified as steatosis. In addition to steatosis, the minimum criteria for the diagnosis of steatohepatitis included the presence of lobular inflammation and either ballooning cells or perisinusoidal/pericellular fibrosis in zone 3 of the hepatic acinus. Steatosis was graded as follows: grade 1, more than 5% and less than 33% of hepatocytes affected; grade 2, 33% to 66% of hepatocytes affected; or grade 3, more than 66% of hepatocytes affected. Grades 2 and 3 were combined for statistical analysis (high grade); grade 1 was regarded as low grade.

2.4. Statistical analysis

Results are reported as the mean ± SD and median (minimum-maximum). Kolmogorov-Smirnov test was used to determine the distribution characteristics of variables, and

Levene test was used to evaluate the equality of variance. Differences between groups were tested for significance by independent-samples t test and Mann-Whitney U test, as appropriate. The relationship between variables was analyzed by Spearman ρ correlation. Variables that were significantly different between 2 groups were analyzed on multivariate analysis. In addition, multivariate logistic regression analysis was used to assess the association between apelin, adiponectin, and histopathologic findings. Differences and correlations were considered significant at $P < .05$.

3. Results

Table 1 shows the characteristics and the laboratory data of the patients and the controls. Age was similar between the 2 groups. Body mass index and WC levels were higher in NAFLD group when compared with the healthy controls.

Insulin levels and HOMA indexes were significantly higher in subjects with NAFLD ($P < .001$ for both). Plasma apelin levels were higher and adiponectin levels were lower in the NAFLD group compared with controls ($P < .001$ and $P = .013$, respectively). However, in multivariate analysis adjusted for BMI and HOMA indexes, the differences in apelin and adiponectin plasma concentrations disappeared ($P = .3$ and $P = .1$, respectively).

We also searched for associations between apelin and the other parameters in both groups. In NAFLD group, apelin levels were positively correlated with BMI ($r = 0.458$, $P = .002$) and HOMA indexes ($r = 0.305$, $P = .039$) (Figs. 1 and 2, respectively). No correlation was found between apelin and WC levels and also histopathologic findings. In addition, adiponectin levels were negatively correlated with total cholesterol ($r = -0.43$, $P = .015$) and LDL-C ($r = -0.37$, $P = .043$) and positively correlated with HDL-C levels ($r = 0.44$, $P = .022$). There was only a negative association independent of the age and BMI between hepatic steatosis and adiponectin levels (odds ratio = 0.720, 95% confidence interval = 0.523-0.991, $P = .044$). However, in control group, there was no association between apelin and the other parameters.

In subgroup analysis, there were no significant differences regarding the levels of apelin and adiponectin between patients with low- (n = 30) and high-grade (n = 20) steatosis and also between patients with SS (n = 14) and NASH (n = 36).

Table 1
The characteristics of the subjects with NAFLD and controls

	NAFLD (n = 50)	Controls (n = 30)	P
Age (y)	32.1 ± 6.3	31.4 ± 5.6	.62[a]
BMI (kg/m^2)	27.9 ± 2.9	23.7 ± 2.4	<.001[a]
SBP (mm Hg)	117.2 ± 9.6	116.5 ± 8.4	.82
DBP (mm Hg)	73.4 ± 5.9	74.2 ± 4.9	.57
WC (cm)	95.9 ± 6.2	86.7 ± 6.2	<.001[a]
FPG (mg/dL)	92.1 ± 14	79.5 ± 9.3	<.001[a]
TC (mg/dL)	195.8 ± 37.8	181.3 ± 30.3	.09[a]
TG (mg/dL)	143 (22-377)	111.5 (51-290)	.05[b]
HDL-C (mg/dL)	41.5 ± 5.9	44.9 ± 8.1	.05[a]
LDL-C (mg/dL)	122 (24-187)	108 (60-171)	.27[b]
ALT (IU/L)	81 (44-164)	17 (6-35)	<.001[b]
AST (IU/L)	39 (20-89)	20 (14-35)	<.001[b]
GGT (IU/L)	62.4 ± 33.1	22.8 ± 9.9	<.001[a]
Insulin (mU/mL)	11.5 (2.1-44.9)	6.54 (3.1-18.5)	<.001[b]
HOMA-IR	2.6 (0.3-12,4)	1.29 (0.6-4.1)	<.001[b]
Adiponectin (μg/mL)	8.86 (4.2-14.7)	10.36 (5.5-22.8)	.013[b]
Apelin (ng/mL)	0.26 (0.09-0.5)	0.21 (0.2-0.4)	<.001[b]

The data are presented as the mean ± SD or median (minimum-maximum). SBP indicates systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase.

[a] Independent-sample t test (mean ± SD).

[b] Mann-Whitney U test (median).

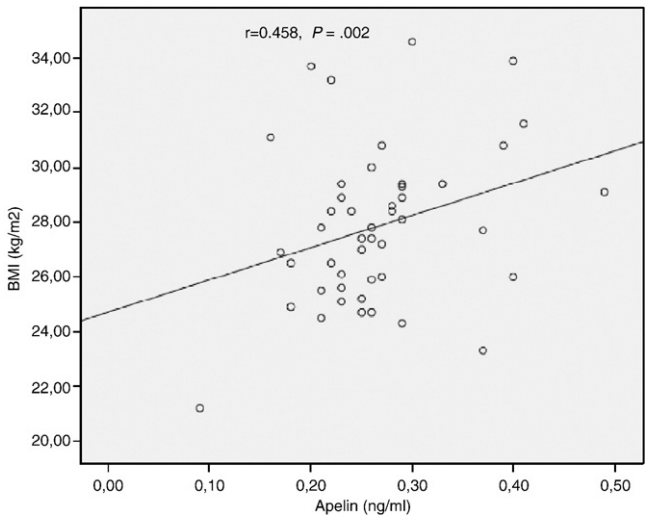

Fig. 1. Correlation between apelin and BMI levels.

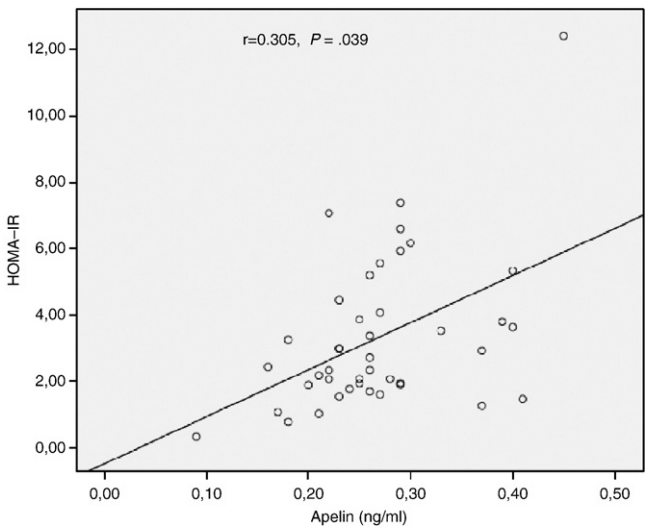

Fig. 2. Correlation between apelin and HOMA-IR index.

4. Discussion

The findings of this preliminary study indicate that circulating apelin levels are not altered in male subjects with NAFLD. However, apelin concentration is associated with the degree of IR and body composition. These results are new and of importance concerning the specifically selected group of NAFLD patients who had no additional disorder or condition associated with alterations in plasma adipokine levels.

Apelin, a newly described adipokine, has various effects in many organ systems including regulation of blood pressure and vascular tone, cardiac contractility, heart rate, regulation of food intake, anterior pituitary functions, angiogenesis, apoptosis, and inflammation. Concerning its role as an adipokine, plasma apelin concentrations were found to be higher in obese animals and humans. In addition, it appears that there is a strong relationship between apelin and insulin secretion [3]. Boucher et al [12] have reported up-regulation of apelin synthesis and secretion from the adipose tissue by insulin. They showed an increase in plasma apelin levels as well as its messenger RNA concentrations in the adipocytes in parallel with the body fat content and hyperinsulinemia. In the present study, we did not find any difference regarding the apelin plasma concentrations between male subjects with NAFLD and healthy male controls when the findings were adjusted according to the BMI levels and HOMA indexes. Thus, in correlation analysis, there were statistically significant correlations between apelin and BMI levels and HOMA indexes. Therefore, it seems that apelin concentrations are closely related to the body composition and insulin sensitivity; and apelin per se may not be involved in the pathogenesis of NAFLD.

Recent studies also suggest a role for apelin in inflammation and angiogenesis because its expression is regulated by tumor necrosis factor–α. Tumor necrosis factor–α increases adipose tissue apelin expression and also circulating apelin levels [13]. Therefore, it could be conceivable that in adipocytes there is substantial regulation of apelin synthesis exerted by tumor necrosis factor–α, leading to sustained apelin secretion in obesity. In our opinion, there is only one report that investigates the role of apelin in human liver diseases [14]. In this study, Principe et al [14] assessed the circulating apelin concentrations in subjects with liver cirrhosis and showed a marked increase in apelin levels in patient group when compared with healthy controls. In our work, no significant difference was observed in the circulating apelin between subjects with NASH and SS, which are clearly different with regard to liver histology. In addition, apelin was not associated with hepatic steatosis/necroinflammation or fibrosis in 2 groups. These findings may be caused by relatively low grade of inflammation and fibrosis and also the absence of cirrhosis in our patients. Therefore, we think that hepatic inflammation associated with NAFLD might not contribute to the regulation of apelin system in this clinically relevant condition.

We also measured plasma adiponectin, a well-known adipokine [15], and did not find any difference between the 2 groups (NAFLD and controls) when the results were adjusted to the BMI and insulin sensitivity. Plasma adiponectin levels have been reported to be lower in subjects with NAFLD when compared with healthy controls [16]. However, there are conflicting reports about the relation of adiponectin and hepatic histologic findings in NAFLD. One study reported that NAFLD patients had lower adiponectin levels and that hypoadiponectinemia is independently associated with the degree of steatosis/necroinflammation [17]. On the contrary, other studies showed that NAFLD subjects had lower adiponectin levels, but failed to find significant associations between hypoadiponectinemia and liver histology [18,19]. Like circulating adiponectin concentrations, there are also contradictory studies reporting the increased and decreased expression of adiponectin and its receptors in liver [20-22]. We think that the different results of these studies might be caused by the potential confounding factors that are closely associated with NAFLD such as obesity, hypertension, and diabetes. Hence, it is well known that adipokine levels may be easily affected by these confounders [23,24] and by the drugs that are commonly prescribed for these metabolic problems [5].

There are 3 limitations of the present study. Firstly, because of the sample size and the strict inclusion criteria, the findings obtained are not representative for all subjects with NAFLD. However, we think that the design of our study was a requirement for the goals to be achieved. Secondly, all participants were men; and it remains to be determined if these results are similar also in women. Lastly, although it is simple, noninvasive, and known to be correlated well with clamp test, the HOMA formula used to calculate insulin sensitivity in this work is only an estimate and cannot be as accurate as the euglycemic-hyperinsulinemic clamp method.

In conclusion, this study shows that plasma apelin levels are not changed in nondiabetic and normotensive male subjects with NAFLD. In addition, apelin seems to be associated with body composition and IR in this condition. Further studies with larger populations may provide more information regarding the role of apelin system in subjects with NAFLD.

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