

# Serum concentration of adiponectin, leptin and resistin in obese children with non-alcoholic fatty liver disease

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## ABSTRACT

**Purpose:** Obesity, insulin resistance and dyslipidemia are the most significant risk factors of non-alcoholic fatty liver disease (NAFLD) but the role of adipokines in pathomechanism of this disease is not clear. The aim of the study was to evaluate the serum levels of leptin, adiponectin and resistin in obese children with NAFLD.

**Material/Methods:** The fasting serum levels of adipokines were determined in 44 consecutive obese children with suspected liver disease and in 24 lean controls. The degree of the ultrasound liver steatosis was graded according to Saverymuttu.

**Results:** The fatty liver was confirmed in 33 children by ultrasonography (16 of them also showed an increased ALT activity). The serum leptin level was significantly higher and adiponectin level was lower in the obese children with NAFLD when compared to controls. Only adiponectin correlated with homeostasis model assessment of insulin resistance (HOMA-IR). Significant negative correlations were found between the ultrasonographic grades of liver steatosis and adiponectin and resistin levels. Serum adiponectin and resistin levels were lower in children with an advanced liver steatosis (grade 3, n=10) compared to patients with a mild steatosis (grade 1-2, n=23). The ability of serum adiponectin and resistin to differentiate children with an advanced liver steatosis from those with mild steatosis was significant.

**Conclusions:** These data suggest a role of both adiponectin and resistin in the pathogenesis of NAFLD in obese children and confirm the association between adiponectin and insulin resistance. Adiponectin and resistin may be suitable serum markers in predicting an advanced liver steatosis in children with NAFLD.

**Key words:** adiponectin, leptin, resistin, children, NAFLD, obesity

## INTRODUCTION

The non-alcoholic fatty liver disease (NAFLD) is a common cause of the pathology of the liver in children, however, its prevalence in paediatric population is difficult to estimate. This disease is most often connected with obesity and insulin resistance [1,2] and that is why it can be considered a specific manifestation of metabolic syndrome. The alterations in liver may take the form of steatosis or show progression to steatohepatitis – nonalcoholic steatohepatitis (NASH) and even cirrhosis, liver insufficiency and hepatocellular carcinoma (HCC) [1-5]. A clear diagnosis and staging of NAFLD (in particular NASH) requires a liver biopsy, which, due to its

invasiveness, cannot be used in clinical practice. Therefore, the NAFLD diagnosis is based on an elevated activity of transaminases in blood serum and/or revealing a fatty liver on radiographic studies; the diagnosis also requires the exclusion of other causes of chronic liver disease i.e. viral hepatitis, alpha 1 antitrypsin deficiency, Wilson disease, cystic fibrosis, autoimmune hepatitis and drug-induced liver injury [5].

The adipose tissue has traditionally been considered an energy storage organ, but over the last decade, its new role has emerged, as an endocrine organ. Recently, the significant role of adipokines, peptides derived mainly from adipocytes, in the pathogenesis of obesity, insulin resistance and also NAFLD has been discussed [6].

The aim of the study was to evaluate the serum concentration of selected adipokines: adiponectin, leptin and resistin in obese children with special regard to correlations between their levels and the ultrasound grade of the liver steatosis, anthropometric data and biochemical tests and to find out if the measurements of these adipokines have any clinical applicability as markers of the liver steatosis in the ultrasound examination.

## MATERIAL AND METHODS

### Patients

The study was carried out prospectively with 44 consecutive obese (BMI>97pc) children (mean age 12 years, range 7-17, 25 boys and 19 girls) with a suspected liver disease (hepatomegaly and/or ultrasonographic liver brightness and/or increased ALT activity) who were admitted to 3rd Department of Pediatrics of Medical University of Białystok between December 2005 and November 2006. An informed consent was obtained from all patients' parents and the protocol was approved by the ethics committee of Medical University of Białystok, Poland.

NAFLD was diagnosed in those children with a confirmed steatosis in liver ultrasound and an elevation of serum ALT activity. The viral hepatitis (hepatitis B virus - HBV, hepatitis C virus - HCV, cytomegalovirus - CMV), toxic, autoimmune (AIH) and metabolic liver diseases (Wilson disease,  $\alpha$ 1 antitrypsin deficiency, cystic fibrosis) were excluded in all children. The body mass index (BMI) and waist to hip ratio (WHR) were calculated in all children. The serum level of total cholesterol, lipoproteins: high density (HDL) and low density (LDL), triglycerides as well as standard liver tests, including total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyltranspeptidase (GGT), were measured directly by automated methods. The insulin resistance was assessed by homeostasis model (HOMA-IR) [7].

### Measurement of serum adipokines concentrations

The fasting serum levels of adipokines (adiponectin, leptin and resistin) were measured with EIA technique using R&D Systems Inc., USA commercial kits in all patients and in 24 non-obese controls with comparable age and sex and normal ultrasound. The intra- and inter-assay CVs of adiponectin ranged from 2.5% to 4.7% and 5.8% to 6.9%, respectively. The intra- and inter-assay CVs of resistin ranged from 3.8% to 5.3% and 7.8% to 9.2%, respectively. The intra- and inter-assay CVs of leptin ranged from 3.0% to 3.3% and 3.5% to 5.4%, respectively. The assay sensitivities ranged from 0.079-0.891 (0.246ng/ml) for adiponectin, 0.01-0.055 ng/ml (0.026 ng/ml) for resistin and 7.8 pg/ml for leptin.

### Ultrasonographic examination

The degree of liver steatosis was graded from 0 (no steatosis) to 3 according to Saverymuttu et al. [8] based on ultrasonographic examination (General Electric LOQIQ 500, convex 3-5 MHz).

The steatosis grade was assessed in a blinded fashion by a one radiologist without any knowledge of the patients' laboratory or clinical data. In order to determine specificity and sensitivity of the assays we arbitrarily defined an advanced liver steatosis as a score =3 according to Saverymuttu et al. [8].

### Statistical analysis

The serum concentrations in biochemical tests were expressed as mean values  $\pm$  standard deviation (SD). Statistical analysis was performed with the Mann-Whitney two-sample test for nonparametric data. The relationship between the biochemical tests and liver histology scores was analyzed by the Spearman rank-correlation test for non-parametric data and by the Pearson method for parametric data. The tests were considered statistically significant at  $p < 0.05$ . Receiver operating characteristics (ROC) analysis (AccuROC, Montreal, Canada) was used to calculate the power of the assay to detect an advanced liver steatosis. The comparison of the area under curve (AUC) was performed using a two-tailed p-test, which compares the AUC to the diagonal line of no information (AUC 0.5).

## RESULTS

Thirty three children (75 %) showed a liver steatosis in the ultrasound examination (group I); 16 of them also had an increased serum ALT activity (group Ia – hepatopathic obese children – NAFLD children). Eleven children (25%) demonstrated neither liver brightness in the ultrasound nor increased ALT activity (group II – non-hepatopathic obese children). We did not observe children without liver hyperechogenicity in the ultrasound examination and concomitant elevated serum ALT activity. There were no significant differences between the groups in regard to age, BMI, levels of bilirubin, parameters of lipids profile, glucose, leptin and resistin. However, the hepatopathic obese children displayed a significantly higher value of WHR, activity of liver enzymes, HOMA-IR and lower adiponectin level. The characteristics of the examined children are presented in *Tab. 1*.

### Serum concentration of adipokines

The serum leptin level in children with NAFLD (n=16) was significantly higher ( $p=0.000002$ ) and adiponectin - lower ( $p=0.000008$ ) compared to controls (n=24). The resistin level did not differentiate these groups of examined children, however, this adipokine level was higher in children with NAFLD (*Tab. 2*).

There were significant negative correlations of ultrasonographic grade of liver steatosis and adiponectin ( $r=-0.39$ ;  $p=0.0092$ ) and resistin ( $r=-0.34$ ;  $p=0.0413$ ) and positive correlation with WHR ( $r=0.41$ ;  $p=0.011$ ), activity of ALT ( $r=0.66$ ;  $p=0.0001$ ), AST ( $r=0.62$ ;  $p=0.000007$ ), GGT ( $r=0.51$ ;  $p=0.037$ ) as well as triglycerides level ( $r=0.42$ ;  $p=0.0039$ ) and value of HOMA-IR ( $r=0.31$ ;  $p=0.037$ ).

**Table 1. Characteristics of examined obese children.**

Data of the patients	Group I (n = 33) mean ± SD	Group Ia (n = 16) mean ± SD	Group II (n = 11) mean ± SD	p I vs II	p Ia vs II
Age (years)	12.3 ± 2.5	11.9 ± 2.7	11.2 ± 2.7	NS	NS
BMI (kg/m <sup>2</sup> )	28.4 ± 3.5	28.4 ± 3.7	26.6 ± 3.7	NS	NS
WHR	0.99 ± 0.07	1.0 ± 0.06	0.9 ± 0.1	NS	0.0089
ALT (IU/l)	44 ± 28	65 ± 26	21 ± 5	0.0033	0.0001
AST (IU/l)	36 ± 17	46 ± 20	23 ± 5	0.0038	0.0001
GGT (IU/l)	24 ± 13	33 ± 14	17 ± 3	0.0287	0.0003
Bilirubin (mg%)	0.8 ± 0.7	0.7 ± 0.3	0.5 ± 0.1	NS	NS
Cholesterol (mg/dl)	164 ± 40	167 ± 39	153 ± 27	NS	NS
Lipoprotein HDL (mg/dl)	52 ± 11	50 ± 10	47 ± 8	NS	NS
Lipoprotein LDL (mg/dl)	95 ± 30	88 ± 33	87 ± 26	NS	NS
Triglycerides (mg/dl)	120 ± 72	142 ± 87	86 ± 35	NS	NS
Glucose (mg/dl)	86 ± 8	85 ± 8	85 ± 5	NS	NS
Insulin (μIU/ml)	17.4 ± 9.0	19.7 ± 11.0	12.1 ± 4.3	0.0368	0.0317
HOMA-IR	2.2 ± 1.1	2.4 ± 1.3	1.5 ± 0.5	0.0378	0.0314
Adiponectin (μg/ml)	8.7 ± 6.0	6.0 ± 3.1	10.3 ± 3.2	0.065(NS)	0.0007
Leptin (ng/ml)	25.4 ± 13.7	28.3 ± 13.4	31.3 ± 12.2	NS	NS
Resistin (ng/ml)	12.8 ± 3.5	12.0 ± 3.4	13.9 ± 3.0	NS	NS
USG – steatosis grade	1.8 ± 0.8	2.3 ± 0.7	0	-	-

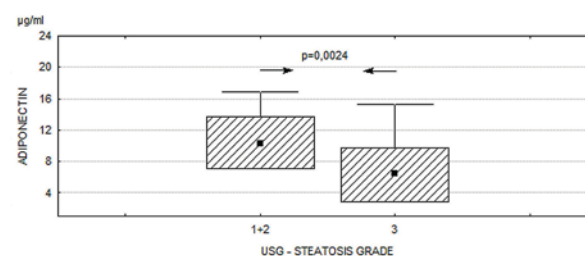
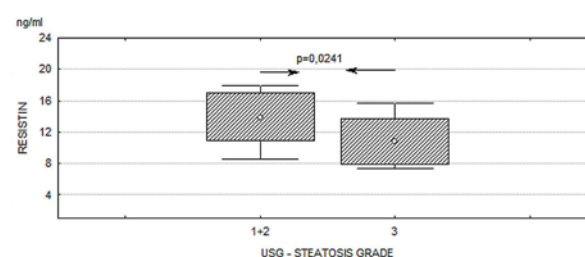
**Table 2. Mean concentration of adipokines in hepatopatic obese children (n=16) and in controls (n=24).**

Adipokines	Group Ia (n=16)	Control group (n=24)	P
Adiponectin (μg/ml)	6.1 ± 3.1	12.1 ± 3.2	0.000002
Leptin (ng/ml)	28.3 ± 13.4	5.7 ± 3.4	0.000008
Resistin (ng/ml)	12.1 ± 3.4	11.0 ± 2.4	NS

We also found a significant negative correlation of adiponectin and insulin level ( $r=-0.41$ ;  $p=0.018$ ), HOMA-IR ( $r=-0.39$ ;  $p=0.022$ ) and GPT ( $r=-0.33$ ;  $p=0.05$ ) and positive correlation with level of lipoprotein HDL ( $r=0.42$ ,  $p=0.016$ ). Leptin correlated with BMI value ( $r=0.45$ ,  $p=0.008$ ).

### Diagnostic value of adipokines for identification of patients with advanced liver steatosis

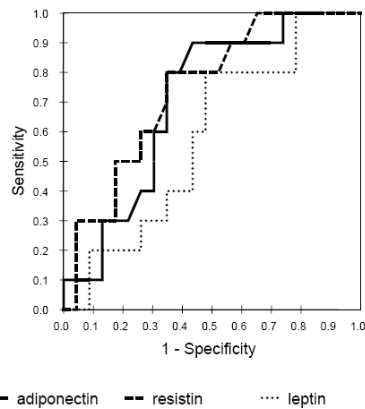
Twenty three children (70%) had a mild liver steatosis: 14 of them – score 2, 9 – score 1 and 10 children (30%) had an advanced steatosis (score = 3 according to Saverymuttu et al). The concentration of adiponectin and resistin was significantly lower in children with advanced liver steatosis ( $p=0.0024$ ;  $p=0.0241$  respectively) (Fig. 1, 2). The children with the advanced liver steatosis according to Saverymuttu et al. [8] had also a significantly higher ALT and GGT activity and triglycerides level ( $58 ± 29$  IU/l,  $31 ± 13$  IU/l,  $177 ± 95$  mg/dl respectively) than patients with a mild steatosis ( $37 ± 26$  IU/l,  $p=0.0229$ ;  $21 ± 12$  IU/l,  $p=0.0216$ ;  $96 ± 43$  mg/dl,  $p=0.0177$  respectively).

**Figure 1. Mean adiponectin concentration in children with mild (n=23) and advanced liver steatosis (n=10) in ultrasonographic examination.****Figure 2. Mean resistin concentration in children with mild (n=23) and advanced liver steatosis (n=10) in ultrasonographic examination.**

The ability of resistin and adiponectin to differentiate the children with an advanced liver steatosis from those with mild steatosis was significant (AUC= $0.7413 ± 0.0897$ ,  $p=0.029$ ;  $0.7043 ± 0.094$ ,  $p=0.03$ , respectively). A serum resistin level (cut-off) above 12.5 ng/ml had sensitivity of 80% and specificity of 65.2%, and serum adiponectin level above 7.2 μg/ml (cut-

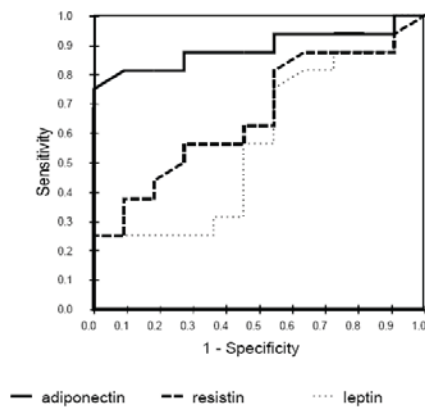
**Figure 3.** ROC curves of ability of examined adipokines to detect advanced liver steatosis according to Saverymuttu et al. [8] in children with fatty liver.

#### Receiver Operating Characteristic Analysis



**Figure 4.** ROC curves of ability of examined adipokines to detect NAFLD in children with obesity.

#### Receiver Operating Characteristic Analysis



off) had a sensitivity of 90% and specificity of 56.5%. Leptin was not a good predictor of the ultrasonographically diagnosed liver steatosis (Fig. 3).

We analysed also the results of adipokines in the groups of hepatopathic (Ia) and non-hepatopathic obese patients (II). The ability of adiponectin to differentiate hepatopathic children from non-hepatopathic obese children was significant ( $AUC=0.8892 \pm 0.0678$ ;  $p=0.0007$ ). A serum adiponectin level (cut-off) above  $6.5 \mu\text{g/ml}$  had a sensitivity of 75% and specificity of 100%. Leptin and resistin did not differentiate these groups of children (Fig. 4).

## DISCUSSION

So far few studies analyzed serum concentration of adiponectin, leptin and resistin in children with the fatty liver [9-13].

We confirmed lower adiponectin level in children with NAFLD than in controls and non-hepatopathic obese children and these results are in agreement with data presented by

other authors [9,10,12]. We also found higher leptin level in NAFLD patients than in controls. The study of Mandato et al. [11] is not consistent with ours, because they did not find differences in the leptin levels between the group of children with NAFLD and controls. The interpretation of this fact is closely related to BMI, a surrogate marker of fat mass in the examined children. In our study the controls were lean and all children with NAFLD were obese but in study of Mandato et al. [11] all patients were obese and there were no differences in the mean BMI values in the examined groups. Many authors found correlation between BMI and serum leptin level [14-16] which is consistent with our findings. However, Iorio et al. [13] confirmed correlation between leptin and BMI only in obese controls and not in children with obesity related liver disease. Our data regarded resistin level are consistent with the study of Zou et al. [10], because they also did not find differences between patients with NAFLD and controls.

The main aim of this study was to examine the correlation between adipokines level and the grade of liver steatosis in ultrasonographic evaluation. A liver biopsy was not performed in our patients because in children the role of this procedure for diagnosis and staging of NAFLD has yet to be established. Furthermore, a histological diagnosis of NAFLD does not affect the treatment of an obesity-related liver disease in paediatric patients [2,13]. For that reason we used the ultrasound examination to identify and grade the fatty liver due to its non-invasiveness, availability and sufficient specificity and sensitivity [17]. It has to be stressed that the liver ultrasound in our study was performed by the same radiologist and using the same equipment, increasing the reliability of the procedure.

In our study only adiponectin and resistin negatively correlated with the ultrasonographic grade of liver steatosis. In adults Baranova et al. [18] found that a low serum adiponectin level predispose to the development of NAFLD and even progression to NASH. Louthan et al. [9] suggested that in pediatric population depressed adiponectin plays a more proximal role than elevated levels of circulating pro-inflammatory cytokines in the development of fatty liver. Therefore adiponectin could have a protective effect and a high serum level of this adipokine suggests lack or low grade of liver steatosis. Hypoadiponectinemia was recently shown to be responsible for the accumulation of hepatic fat as well as the development of insulin resistance [18-20]. This finding is consistent with our study because we also confirmed a negative correlation between adiponectin and insulin resistance (HOMA-IR). On the other hand, adiponectin may protect against the progression of NAFLD to NASH through its anti-inflammatory action because it inhibits liver's TNF alpha expression and other cytokines in hepatic stellate cells [21].

The role of resistin in the pathogenesis of NAFLD seems to be unclear. Our study, in which we found negative correlation of resistin with ultrasonographic grade of liver steatosis, is not consistent with the findings of Aller et al. [22] because they confirmed a positive relationship between that

adipokine level and the grade of the steatosis in liver biopsy. On the other hand, they found a lack of association between resistin and the grade of the steatosis in multivariate logistic analysis, when HOMA was included. Perseghin et al. [23], using <sup>1</sup>H magnetic resonance spectroscopy, demonstrated that excessive ectopic fat in the liver of insulin resistance subjects is associated with a lower serum resistin concentration and not with hyperresistinemia. The resistin may circulate in different molecular isoforms in human peripheral blood and this phenomenon may raise problems for the determination of its serum level and especially, for the comparison of levels measured with different assay systems [24].

Recently ROC analysis has been recommended to calculate the power of serum assays to detect advanced liver pathology, mainly fibrosis [25,26]. There is no data concerning the predictive values of examined serum adipokines in liver steatosis in children with NAFLD. In this study we found lower serum adiponectin and resistin concentrations in children with an advanced liver steatosis. The ability of the examined adipokines to differentiate hepatopathic with non-hepatopathic obese children was not significant except adiponectin, while adiponectin and resistin differentiated children with advanced liver steatosis from those with mild steatosis. Therefore, adiponectin and resistin might be regarded as suitable biomarkers of liver steatosis, better than leptin. Recently, Manco et al. [27], using ROC analysis, tested the power of tumor necrosis factor alpha and leptin in predicting the degree of liver involvement in children with biopsy proven NAFLD and found that this cytokine seemed to be a specific laboratory marker of NASH.

We also found that insulin resistance determined as HOMA-IR correlated with the grade of liver steatosis in the group of children with NAFLD. Other authors also detected this relation using either HOMA or the euglycemic-hyperinsulinemic clamp [21,28-31]. Insulin resistance is common in obese children and adults and it plays a major role in the pathogenesis of NAFLD [32,33]. However, it has to be stressed that studies performed in children have special value because children could be regarded as an ideal model for the study of natural history and pathogenesis of obesity-related liver disease for the earlier stages of the disease, absence of major confounding factor of liver pathology such as alcohol consumption and other environmental influences, often seen in adults. Insulin resistance may play a role not only in the development of steatosis but also in the development of liver fibrosis by increasing hepatic fatty acid beta-oxidation and oxidative stress [34].

In this study a negative correlation of serum adiponectin level and ALT activity was also found. The highest ALT activity and lowest adiponectin level were observed in the group of hepatopathic obese children. Moreover, these children also had the highest value of HOMA-IR and ultrasonographic grade of liver steatosis. Therefore, we suggest that this group of patients with NAFLD needs special attention due to the risk of NASH developing.

## CONCLUSIONS

In conclusion, these data suggest a role of both adiponectin and resistin in the pathogenesis of NAFLD in obese children and confirm the association between adiponectin and insulin resistance. Adiponectin and resistin may be suitable serum markers in predicting significant liver steatosis in children with NAFLD but these findings need to be confirmed in larger studies.

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