IGF-1 and IGFBP3 as indirect markers of hepatic insulin resistance and their relation to metabolic syndrome parameters in liver steatosis patients

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Objective. The aim of the present study was to assess insulin-like growth factor 1 (IGF-1) and IGF-binding protein 3 (IGFBP3) as markers of insulin resistance in patients with prediabetes and type 2 diabetes mellitus (T2DM).

Patients and Methods. This observational clinical study included 76 obese/overweight patients at the age of 45–75 years with T2DM on oral diabetic medication and ultrasonographically or by a computerized tomography (CT) diagnosed liver steatosis. Correlation analysis was performed between plasma levels of insulin, C-peptide, IGF-1, IGFBP3 and HOMA indexes on the one hand and between plasma levels of ALT, AST, triglyceride, cholesterol, and HDL cholesterol and body mass index (BMI) of patients on the other hand. In case of significant partial correlation coefficients, a multiple linear regression model with IGF-1 and IGFBP3 used as outcome variables adjusted for age and sex groups was calculated. According to these regression models, ROC curves were prepared with HOMA index=3 used as a classifier of insulin resistance.

Results. Significant correlation was found between C-peptide and IGF-1 (r=0.24, p≤0.05), C-peptide and IGFBP3 (r=0.24, p≤0.05), IGFBP3 and cholesterol (r=0.22, p≤0.05) IGFBP3 and ALT (r=0.19, p≤0.05), HOMA index and triglycerides (r=0.22, p≤0.05), and HOMA index and ALT (r=0.23, p≤0.05). Significant correlation adjusted for age and gender was found between C-peptide and IGF-1 plasma levels (R²=0.20, p<0.05), IGFBP3 and cholesterol (R²=0.22, p<0.05) IGFBP3 and ALT (R²=0.19, p<0.05), HOMA index and triglycerides (R²=0.22, p<0.05), and HOMA index and ALT (R²=0.23, p<0.05). Significant correlation adjusted for age and gender was found between C-peptide and IGF-1 plasma levels (R²=0.20, p<0.05) with AUROC 0.685 (p<0.01) and C-peptide and IGFBP3 plasma levels (R²=0.28, p<0.05) with AUROC 0.684 (p<0.01). Significant correlation adjusted for age and gender was found between triglyceride and IGFBP3 plasma levels (R²=0.28, p<0.05) with AUROC 0.616 (p<0.01). After the distribution of patients according to their IGFBP3 levels, we found a difference between the 1st and the 4th quartiles in terms of triglyceride levels.

Conclusion. Our results demonstrate a fundamental role of IGF-1 and IGFBP3 in the pathophysiology of hepatic insulin resistance and suggest them as indirect indicators of the hepatic insulin resistance.

Key words: IGF-1, IGFBP3, insulin resistance, liver steatosis

Insulin resistance in the liver seems to make a significant contribution to the pathophysiology of insulin resistance at a general level. Study of the mechanisms helps to explain the etiology of steatosis and clarify the role of different factors in its pathophysiology. Identification of new predictive factors helps to facilitate the therapeutic approach. The role of insulin-like growth factor 1 (IGF-1) and growth hormone (GH) in the process of saccharide and lipid metabolism is already relatively well described.
However, their importance in the pathomechanism of insulin resistance and metabolic syndrome (MS) is still the topic of research (Lewitt et al. 2014). The role of GH in the pathomechanism of insulin resistance arises from observations when a higher prevalence of diabetes and abdominal obesity was present among patients with acromegaly and patients with the lack of GH (De Ita et al. 2015). On the other hand, IGF-1 seems to act not only as an effector hormone of GH, but due to its similarity to insulin structure it is interconnected with a wide variety of functions (Aguirre et al. 2018).

IGF-1 production in the liver is a result of GH interaction with insulin and it is impaired during the course of insulin resistance development in the liver (Yakar et al. 2004). It is widely accepted that hepatic insulin resistance increases by the central obesity development (Haluzik et al. 2003). In the body mass index (BMI) range of overweight (BMI over 25), there is a negative correlation between IGF-1 production and BMI (Schneider et al. 2006).

During the development of the hepatic insulin resistance in patients with central obesity, certain features of MS can be observed, such as accumulation of free fatty acids (FFA) in the liver causing steatosis and hepatocellular enzyme elevation. These changes are accompanied by the attenuation of IGF-1 synthesis (Fusco et al. 2012). The pathophysiology of steatosis is in this way connected with the hepatic insulin resistance (Haluzik et al. 2013). The severity of steatosis correlates with the severity of insulin resistance and is a good predictor of the MS (De Ita et al. 2015). It is widely agreed that in the course of insulin resistance development in the liver, the synthesis of IGF-1 is also decreased (O’Connell and Clemmons 2002).

Supplementation of IGF-1 improves insulin sensitivity that is confirmed on both in vitro and in vivo levels (Berryman et al. 2013). A protective effect of IGF-1, in terms of MS, had firstly been hypothesized according to the similar mechanism of insulin and IGF-1 action regarding glucose utilization in peripheral tissues. However, the effectivity of IGF-1 is only about 5% of insulin effectivity (Clemmons 2006).

There are two dominant IGF-binding proteins (IGFBP), IGFBP1 and IGFBP3, related to IGF-1 action in the liver (Kotronen et al. 2008). Low IGFBP1 levels are predictors of MS in patients with prediabetes (Pettersson et al. 2009). Fasting serum levels of phosphorylated IGFBP1 have been shown to be noninvasive predictors of liver fat content in non-alcoholic fatty liver disease (NAFLD) (Petaja et al. 2016). The concentration of IGFBP1 is regulated by insulin, which has a suppressive effect on IGFBP1 production resulting in an increase of free IGF-1. However, due to growing hepatic insulin resistance this effect is impaired in the course of diabetes (Reinehr et al. 2011).

The role of IGFBP3, as the main binding protein, which in terms of insulin resistance is responsible for 30% of the binding capacity of IGF-1, is still not exactly elucidated (Kong et al. 2011). The role of insulin resistance in the appearance of the MS features is well recognized. However, the role of IGF-1 and its main binding proteins (IGFBP1, IGFBP3) remains controversial. Little is known about the intensity of IGF1 synthesis in the different stages of insulin resistance accompanied by hyperinsulinemia.

The aim of the present study was to assess the relationship between IGF-1, IGFBP3, and C-peptide (plasma insulin) on the one hand and between clinically most important parameters of MS, such as triglycerides, HDL, and BMI together with ALT as a surrogate marker of steatosis on the other hand in patients with NAFLD.

Concerning the relationships among evaluated parameters, we assessed whether the levels of lipid parameters, hepatocellular enzymes, and BMI are associated with decreased levels of IGF-1, increased levels of IGFBP3 or increased levels of C-peptide (plasma insulin).

Then, we observed the levels of IGF-1 and triglycerides in steatosis, which was formerly sonographically documented; as a measure of steatosis, increased ALT and AST enzyme levels were used. Afterwards, we observed the levels of IGF-1 in obesity, whereas a measure of obesity BMI ≥30 was used.

Patients and Methods

Subjects. Altogether 76 patients (41 females and 35 males) were included into this prospective observational study. The mean age of the sample was 62.8±12.7 years and it was 58.8±12.7 in the male group and 65.8±12.0 in the female group. All patients had Caucasian origin according to self-assigned as well as observer-assigned ethnicity and lived in the Eastern-Slovakian region. Patients were recruited during their regular control examinations performed once in three months and involved in the study after their informed consent. The questionnaire about the participant characteristics was self-administered and regularly updated during each visit in terms of the biometric values of patients. All the evaluated laboratory parameters of every single patient were analyzed during the same blood sampling.
Data were collected from Kosice University Hospital’s medical information system as well as from the output list of laboratory results of patients emitted by RIA Laboratory Kosice. The owner of the data is Kosice University Hospital. Data acquisition and utilization were allowed only for persons authorized by the Ethical Committee of Kosice University Hospital. Data are stored on the server of the 1st Department of Internal Medicine of Kosice University Hospital. The access to data is possible only for persons authorized by the Ethical Committee.

**Inclusion criteria.** 1) Patients with type 2 diabetes mellitus (T2DM) and ultrasonographically or CT-diagnosed liver steatosis observed in the Diabetes Outpatient Clinic of Kosice University Hospital were treated by the next therapeutic modalities: a) low carbohydrate diet therapy (150 g/day); b) peroral antidiabetic medications from the next groups: metformin, sulphonylurea (gliclazide), DPP-4 (sitagliptine, linagliptine), GLP-1 (liraglutide, dulaglutide), SGLT2 (dapagliflozin, empagliflozin). The diagnosis of T2DM was established by OGTT with 75 g glucose performed by WHO guidelines or by detecting fasting glucose level ≥7.0 mmol/l or randomly detected glucose level ≥11.0 mmol/l. 2) Patients with prediabetic states: impaired fasting glucose (IFG): fasting glucose level ≥6.0 mmol/l and ≤7.0 mmol/l; impaired glucose tolerance (IGT): post-prandial glucose level ≥7.8 mmol/l and ≤11.0 mmol/l. The diagnosis of NAFLD was confirmed also by CT with liver attenuation of less than 40 HU considered as a cut-off value for steatosis.

**Exclusion criteria.** 1) Any insulin or insulin analogue therapy regimen (basal or postprandial) administered either separately or in combination with GLP-1 analogue; 2) any type of autoimmune diabetes (GADA or IAA or ICA positivity); 3) alcohol abuse (alcoholic fatty liver disease) confirmed by CDT analysis or typical parameters detected in the blood count (mean corpuscular volume of erythrocytes ≥96 fl and platelet count ≤150 10⁹/l detected together; any clinical or laboratory signs of liver cirrhosis; 4) any accompanying liver disease different from NAFLD; 5) chronic kidney disease with serum creatinine ≥150 µg/L.

**Laboratory analysis.** Serum glucose and lipid parameters were analyzed routinely using an auto-analyzer (Roche Diagnostics GmbH). Insulin concentrations and the levels of IGF-1 and IGFBP-3 were determined using immunoradiometric assays (Beckman Coulter, Inc.). Glycated hemoglobin (HbA1c) was determined by latex turbidimetric method and is calculated in DCCT measures. The remaining biochemical parameters were detected in Kosice University Hospital Biochemistry Laboratory.

**Statistical analysis.** The normality of the distribution of continuous variables was tested by the Shapiro-Wilk test and the homogeneity of the distribution of continuous values was tested by the F-test. In case of normal distribution, the independent Student T-test was used for the determination of difference between analyzed groups. In case of non-parametric distribution of values, the Mann-Whitney U-test was used. For the assessment of relations between outcome variables and explanatory variables, the Pearson correlation test was applied. In the case of non-parametric distribution, Spearman’s correlation test was used. In case of significant partial correlation coefficients, these were adjusted for age and sex groups. A multiple linear regression model for IGF-1, IGFBP3 and Homeostasis Model Assessment (HOMA) index that were considered as outcome variables was performed. According to these regression models, receiver operating characteristic (ROC) curves were prepared, HOMA index was used as a classifier of insulin resistance. The significance of ROC curves at the determined cut-off values was tested by McNemar’s test. We also performed a distribution of patients according to some predictor variables and examined differences across quartiles in terms of certain outcome variables. Only p values lower than 0.05 were considered statistically significant. All tests were two-tailed and analyses were performed using the SAS statistical package version 9.4 (SAS Institute Inc., Cary, CA).

**Results**

Basic descriptive statistics of continuous variables is presented in Table 1. When comparing parameters between male and female genders, we did not find significant differences in any of the studied parameters.

After baseline characteristics, a correlation matrix to assess linear relationships among predictor variables in order to perform a multiple linear regression model was prepared.

A significant correlation was detected between C-peptide and IGF-1 (r=0.24, p≤0.05), C-peptide and IGFBP3 (r=0.24, p≤0.05). A significant correlation was also found between IGFBP3 and cholesterol (r=0.22, p≤0.05), IGFBP3 and ALT (r=0.19, p≤0.05).

Not significant correlation was found between IGFBP3 and triglycerides (r=0.34, p=0.18) and not significant negative correlation was also found between HDL cholesterol and IGF-1 (r=−0.13,
A borderline negative correlation was found between HDL cholesterol and BMI \( (r=-0.23, p=0.06) \). However, no significant interaction between HOMA index and IGF1 \( (r=0.07, p=0.58) \) or HOMA index and IGFBP3 \( (r=0.09, p=0.39) \) was found.

A significant correlation was also observed between HOMA index and triglycerides \( (r=0.22, p\leq0.05) \), HOMA index and ALT \( (r=0.23, p=0.02) \), but also between HOMA index and C-peptide \( (r=0.71, p\leq0.05) \), HOMA index and BMI \( (r=0.45, p\leq0.05) \). A significant correlation was also documented between IGF1 and IGFBP3 \( (r=0.75, p\leq0.05) \), which indicates the role of IGFBP3 as the most important binding protein of IGF1.

A multiple linear regression model demonstrated a significant association between IGF-1 and C-peptide levels adjusted for age and sex \( [\text{IGF1 (ng/ml)} = 183.9+7.31 (\mu g/l) \text{C peptide} – 26.2 \text{ (Male sex)} – 15.7 \text{ Age}; p\leq0.05; F=7.6; R^2 = 0.28; \text{Radj}^2 = 0.25] \) (Figure 2).

A multiple linear regression model demonstrated a significant association between IGFBP3 and triglyceride (Tg) levels adjusted for age and gender \( [\text{IGFBP3 (ng/ml)} = 2342.6+139.7 \text{ (mmol/l) Tg} – 173.8 \text{ (Male sex)} – 11.8 \text{ Age}; p\leq0.05; F=7.35; R^2 = 0.28; \text{Radj}^2 = 0.23] \) (Figure 3).

The ROC curves for specificity and sensitivity of the calculated IGF-1 and IGFBP3, as predictors of insulin resistance, were prepared, where HOMA index=3 was considered as a classifier of insulin resistance.

The area under the ROC curve (AUC) for IGF-1 in the model calculated from C-peptide level was 0.685 (CI 0.54 to 0.82) with a sensitivity of 63.63% and specificity of 64.70% (Figure 1).

The AUC for IGFBP3 in the model calculated from C-peptide level was 0.684 (CI 0.52 to 0.84) with a sensitivity of 59.09% and specificity of 58.82% (Figure 2). The AUC for IGFBP3 in the model calculated from triglyceride level was 0.616 (CI 0.45 to 0.77) with a sensitivity of 54.16% and specificity of 76.47% (Figure 3).
The complete list of values of selected outcome variable parameters across quartiles (1st and 4th quartile) after the distribution according to some predictor variables (insulin, HOMA index, BMI, ALT, IGF-1, and IGFBP3) is listed in Table 2. In the terms of HOMA index, an analysis was performed across the quartiles and the selected cut-off values of HOMA=3. In terms of BMI, an analysis was performed across the selected cut-off value of BMI=30. Values of outcome variables are expressed as Mean±SD (S=significant if \( p \leq 0.05 \); NS=non-significant if \( p \geq 0.05 \); N/A means that the relation was not evaluated).

**Discussion**

The aim of the present study was to assess the interactions between IGF-1, IGFBP3 and three parameters associated with insulin resistance (C-peptide, plasma insulin, HOMA index) considered as outcome variables and between some parameters of MS (triglyceride, HDL cholesterol), BMI as well as ALT and AST levels considered as predictor variables.

In terms of the relations between our outcome variables, a significant correlation between IGF-1 and C-peptide and between IGFBP3 and C-peptide levels was found. In accordance with this association, we prepared significant regression models for these relations adjusted for sex and age. We prepared also ROC curves for IGF-1 and IGFBP3 calculated according to these regression models as predictors of insulin resistance, in which HOMA index was considered as the parameter for classification.

In contrast, we did not prove a significant correlation between IGF-1 levels and other insulin
Table 2
Differences in the selected parameters across quartiles (between the 1st and the 4th quartile) after the distribution according to some predictor variables (Insulin, HOMA index, BMI, ALT, IGF-1, IGFBP3)

<table>
<thead>
<tr>
<th>Distribution according to</th>
<th>ALT 1st vs. 4th quartile (µkat/l)</th>
<th>BMI 1st vs. 4th quartile (kg/m²)</th>
<th>Tg 1st vs. 4th quartile (mmol/l)</th>
<th>AST 1st vs. 4th quartile (µkat/l)</th>
<th>C-peptid 1st vs. 4th quartile (ng/ml)</th>
<th>HOMA index 1st vs. 4th quartile</th>
<th>IGF-1 1st vs. 4th quartile (ng/ml)</th>
<th>IGFBP3 1-st vs. 4-th quartile ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (mIU/l)</td>
<td>0.42±0.30</td>
<td>26.9±5.3</td>
<td>1.47±1.00</td>
<td>0.41±0.20</td>
<td>N/A</td>
<td>N/A</td>
<td>125.8±48.8</td>
<td>1532.9±470.9</td>
</tr>
<tr>
<td></td>
<td>0.57±0.30</td>
<td>34.6±7.6</td>
<td>1.57±0.80</td>
<td>0.49±0.30</td>
<td>N/A</td>
<td>N/A</td>
<td>129.5±55.9</td>
<td>1656.7±334.6</td>
</tr>
<tr>
<td></td>
<td>P=0.30</td>
<td>P=0.02</td>
<td>P=0.82</td>
<td>P=0.36</td>
<td></td>
<td></td>
<td>P=0.87</td>
<td>p=0.57</td>
</tr>
<tr>
<td>HOMA index (quartile)</td>
<td>0.35±0.20</td>
<td>26.7±5.6</td>
<td>1.11±0.40</td>
<td>0.37±0.20</td>
<td>N/A</td>
<td>N/A</td>
<td>121.1±456</td>
<td>1399.3±434.6</td>
</tr>
<tr>
<td></td>
<td>0.61±0.30</td>
<td>32.9±7.8</td>
<td>1.65±1.00</td>
<td>0.51±0.30</td>
<td>N/A</td>
<td>N/A</td>
<td>132.1±57.2</td>
<td>1714.7±512.8</td>
</tr>
<tr>
<td></td>
<td>p=0.005</td>
<td>p=0.02</td>
<td>p=0.07</td>
<td>p=0.08</td>
<td></td>
<td></td>
<td>p=0.56</td>
<td>p=0.08</td>
</tr>
<tr>
<td>HOMA=3.0 cutoff value</td>
<td>0.41±0.30</td>
<td>26.8±5.2</td>
<td>1.24±0.60</td>
<td>0.39±0.20</td>
<td>N/A</td>
<td>N/A</td>
<td>125.1±46.9</td>
<td>1447.6±480.0</td>
</tr>
<tr>
<td></td>
<td>0.51±0.30</td>
<td>32.7±6.0</td>
<td>1.87±0.90</td>
<td>0.45±0.20</td>
<td>N/A</td>
<td>N/A</td>
<td>124.8±50.7</td>
<td>1590.5±501.7</td>
</tr>
<tr>
<td></td>
<td>P=0.26</td>
<td>p=0.02</td>
<td>p=0.003</td>
<td>p=0.24</td>
<td></td>
<td></td>
<td>p=0.98</td>
<td>p=0.31</td>
</tr>
<tr>
<td>BMI=30 cutoff value</td>
<td>0.46±0.30</td>
<td>N/A</td>
<td>1.55±0.90</td>
<td>0.44±0.20</td>
<td>2.94±1.00</td>
<td>3.51±2.60</td>
<td>132.7±48.2</td>
<td>1628.9±479.0</td>
</tr>
<tr>
<td></td>
<td>0.51±0.20</td>
<td>N/A</td>
<td>1.78±0.90</td>
<td>0.42±0.20</td>
<td>4.18±1.70</td>
<td>6.12±4.30</td>
<td>117.3±51.6</td>
<td>1508.4±489.8</td>
</tr>
<tr>
<td></td>
<td>p=0.57</td>
<td>N/A</td>
<td>p=0.36</td>
<td>p=0.46</td>
<td>P=0.01</td>
<td>N/A</td>
<td>p=0.88</td>
<td>p=0.95</td>
</tr>
<tr>
<td>ALT (µkat/l)</td>
<td>N/A</td>
<td>28.3±4.2</td>
<td>1.20±0.50</td>
<td>0.30±0.10</td>
<td>3.40±2.00</td>
<td>3.64±2.40</td>
<td>120.4±58.9</td>
<td>1436.5±549.6</td>
</tr>
<tr>
<td></td>
<td>32.1±4.7</td>
<td>2.02±1.10</td>
<td>0.63±0.20</td>
<td>3.97±1.40</td>
<td>7.24±5.20</td>
<td>7.24±5.20</td>
<td>122.8±51.0</td>
<td>1628.2±486.2</td>
</tr>
<tr>
<td></td>
<td>P=0.02</td>
<td>p=0.01</td>
<td>p=0.01</td>
<td>P=0.48</td>
<td>P=0.03</td>
<td>N/A</td>
<td>p=0.92</td>
<td>p=0.32</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>0.47±0.30</td>
<td>31.4±5.4</td>
<td>1.52±0.70</td>
<td>0.47±0.30</td>
<td>3.36±1.10</td>
<td>4.7±2.9</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>0.53±0.40</td>
<td>31.5±7.7</td>
<td>1.85±1.20</td>
<td>0.43±0.20</td>
<td>3.91±2.30</td>
<td>4.9±3.1</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>p=0.60</td>
<td>P=0.78</td>
<td>P=0.33</td>
<td>P=0.52</td>
<td>P=0.83</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>IGFBP3 (ng/ml)</td>
<td>0.38±0.20</td>
<td>30.2±4.1</td>
<td>1.36±0.20</td>
<td>0.37±0.10</td>
<td>3.44±1.00</td>
<td>4.6±2.8</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>0.55±0.40</td>
<td>31.3±7.9</td>
<td>2.14±1.40</td>
<td>0.44±0.20</td>
<td>4.20±2.30</td>
<td>5.4±3.2</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>p=0.09</td>
<td>P=0.74</td>
<td>p=0.02</td>
<td>p=0.27</td>
<td>P=0.24</td>
<td>N/A</td>
<td>p=0.46</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Abbreviations: BMI – body mass index; HOMA – Homeostatic Model Assessment; IGF-1 – insulin-like growth factor 1; IGFBP3 – IGF binding protein 3; Tg – triglycerides. In terms of HOMA index, the analysis was performed across the quartiles and the selected cutoff values of HOMA=3. In terms of BMI, the analysis was performed across BMI=30 value. Data of outcome values are expressed as Mean±SD. (S = significant – p≤0.05; NS = not significant – p≥0.05; N/A = not evaluated).

resistance parameters (HOMA and plasma insulin levels), which may be explained by a high compensatory ability of GH receptors in the liver regarding IGF-1 synthesis (Lee and Gorospe 2010). This observation demonstrates that IGF-1 production is maintained for the long term in spite of increasing insulin resistance. These findings are also supported by studies performed on patients with a high grade of central obesity (Savastano et al. 2012).

Regarding the interactions between our predictor and outcome variables, we found a significant linear correlation between IGFBP3 and triglyceride levels. In accordance with this association, we prepared a significant regression model for this relation adjusted for gender groups and age groups. We also prepared a ROC curve for IGFBP3 calculated according to this regression model as a predictor of insulin resistance, in which HOMA index was considered as the parameter of classification.

IGF-1 production contributes to hepatic insulin sensitivity in an important way and vice versa, deterioration of insulin sensitivity attenuates IGF-1 production in the liver (Clemmons 2004). The most important effect of IGF-1 on glycide metabolism is the stimulation of glucose utilization in the peripheral muscle. Consequently, in patients with inborn IGF-1 deficit as a result of GH receptor resistance in the liver (Laron syndrome), some features of MS are already observed at a young age (Ginsberg et al. 2009). An epidemiologic study, which has reported the prevalence of the promotor gene of IGF-1 production in the Dutch population, found that persons with a specific genotype had decreased IGF-1 levels by 40% and the prevalence of T2DM was 2.2 times higher (Vaessen et al. 2001). Another study, which included 7665 patients and assessed the risk factors of T2DM, concluded that
there is also a higher risk for the development of T2DM in patients with extremely low as well as extremely high IGF-1 levels (Friedrich et al. 2012). Moreover, the distribution of IGF-1 values in patients with T2DM is wide and may be influenced by a large number of factors, such as differences in cytokine production related to the proportion of central obesity, degree of insulin resistance in the liver, and corresponding levels of IGFBP3 and IGFBP1 (Aguirre et al. 2016).

Concerning this issue, an important finding is that persons with a low gestational weight related to their age (small for their age) and consequently with low IGF-1 levels have a higher risk for the development of T2DM than persons with normal gestational weight (Lewitt et al. 2008). In some studies with diabetic patients, a negative correlation has been observed between IGF-1 levels and some parameters of MS, such as triglycerides, HDL cholesterol, waist-hip ratio or BMI (Gleeson et al. 2005). Moreover, a higher degree of insulin resistance has been observed in relation to lower IGF-1 levels, which was associated with an interaction measured between IGF-1 and adiponectin levels (Oh et al. 2012; Granata 2016). However, this correlation was proven to be significant only in patients under 65 years of age (Kreitschmann-Andermahr et al. 2010). The reason for this phenomenon may be a physiologic decrease in IGF-1 production at a higher age (Teppala and Shankar 2010).

Decrease in IGF-1 production in response to inflammation process triggered by some cytokines has also an impact on the development of NAFLD (Hribal et al. 2013). The degree of NAFLD progression is also related to the decline in IGF-1 production in the liver. There is a higher prevalence of NAFLD in patients with attenuated IGF-1 production as a result of GH deficiency. It is supposed that low GH levels contribute to the development of steatosis by promoting triglyceride secretion by hepatocytes (Thankamony et al. 2014). Decreased IGF-1 levels in NAFLD patients have also been demonstrated by the observational study conducted by Arturi et al. (2011). They have concluded that impairment of IGF-1 production is a result of hepatic insulin resistance (Arturi et al. 2011). In the study of Ferrari et al. (2021), the relation between IGF-1 levels and aminotransferase levels in patients with NAFLD maintained statistical power after adjustment for age, gender, HOMA-index, and obesity.

IGFBP3/IGF-1 ratio seems to be the best parameter reflecting the proportion of the free form of IGF-1 (Sierra-Johnson et al. 2009). In publications evaluating the relationship between IGF-1/IGFBP3 ratio and parameters of MS, three times higher risk of meeting the ATPIII criteria of MS has been observed in patients with IGF-1 levels in the lowest quartile of IGF-1/IGFBP3 values (Saydah et al. 2009). Because of this finding, low IGF-1 level or low IGF-1/IGFBP3 ratio is considered to be the predictor of impaired glucose tolerance and consequently that of T2DM (Ichikawa et al. 2007). IGF/1IGFBP3 ratio has also been shown to be decreased in patients with NAFLD confirmed either by USG or by increased aminotransferase levels as well as the subgroup of patients with portal fibrosis (Clemmons et al. 2005).

In an observation study assessing ultrasonographically diagnosed NAFLD patients, there was a difference in IGF-1 levels and HOMA indexes between the groups of patients assorted by different ultrasonographically recognized degrees of NAFLD. Moreover, BMI was negatively correlated with IGF-1 levels (Mallea-Gil et al. 2012). Significantly lower levels of IGF-1 mRNA, IGFBP3 and IGF-1 in immortalized human hepatocyte models have also been observed in the experimental study conducted by Zhang et al. (2012). On the contrary, in a logistic regression analysis performed in a cross-sectional analysis of data from a representative sample of the US adult population, data from the highest IGF-1 and IGFI/IGFBP3 quartiles have been associated with a lower likelihood of NAFLD and a lower grade of steatosis, but after adjustment for BMI and waist circumference these associations became less significant (Runchey et al. 2014). Based on these observations authors have concluded that the role of IGF1/IGFBP3 in the pathophysiology of NAFLD is probably not direct. On the other hand, according to some experimental studies the fact that the grade of hepatocellular function and the degree of insulin resistance is related to IGF-1 levels indicates an important role of IGF-1 in the regulation of insulin sensitivity on the general level (Sesti et al. 2013).

After the distribution of patients according to their IGFBP3 levels, but not IGF-1 levels, we found a difference between the 1st and the 4th quartiles in terms of triglyceride levels. This finding is in accordance with the linear relation found in our regression model. We could explain the discrepancy in terms of the two relations by the fact that IGFBP3 is a better marker of hepatic insulin resistance than IGF-1 in the later period of the process when insulin is – despite its high level due to hyperinsulinemia – already not able to suppress IGFBP levels (Sesti et al. 2013). This may correspond with the high level of hepatic insulin resistance causing triglyceride accumulation in the liver. After the distribution of patients according to their HOMA index, we observed a
significant difference between the highest and the lowest quartile of triglyceride and ALT values. Vice versa, after the distribution of ALT values, there was an interquartile difference in HOMA values. In addition, AST, BMI, and triglyceride levels differed across quartiles of ALT levels. These differences also underline the importance of insulin resistance in the development of NAFLD.

In an experimental study conducted on spontaneous dwarf rats (SDR), a decrease in the triglyceride content of the liver as well as a decline in serum ALT and AST levels was observed after treatment with GH and IGF-1 (Nishizawa et al. 2012). Similarly, damage to mitochondrial morphology and consequently a decrease in their functional capacity enhanced the intensity of oxidative stress observed in SDR. However, after GH or IGF-1 application, these changes were reversible (Nishizawa et al. 2012). IGF-1 application has been shown to be effective in the process of the restoration of insulin sensitivity in the liver, the reduction of cholesterol and triglyceride levels and consequently in the increase of FFA levels in experimental conditions (Garcia-Fernandez et al. 2008). An experimental study reported by Xu et al. (2018) has suggested an important role of miR-190b inhibition in the attenuation of lipid accumulation in hepatocytes.

In the evaluation of associations of IGF-1 and IGFBP3 with our predictor variables, we found more significant correlations between predictor variables in relation to IGFBP3 than to IGF-1. We could explain this by the fact that IGFBP3 is supposed to be a better marker of hepatic insulin resistance than IGF-1 in the later periods of the process, when insulin is despite its high levels already not able to suppress IGFBP production (Yuen and Dungar 2007). This finding corresponds with higher triglyceride accumulation in the liver, which occurs in response to hepatic insulin resistance.

After the distribution of patients according to their BMI values corresponding to cut-off values of obesity (BMI 30), we registered an interquartile difference only in the relation of HOMA index and C-peptide levels, but not in relation to IGF-1 or IGFBP3 levels. Although the differences regarding IGF-1 and IGFBP3 were not significant, the tendency according to that higher BMI values had lower IGF-1 and IGFBP3 values underlines the data observed by other authors (Saukkonen et al. 2006). Ambiguity in the relation between BMI and IGF-1 arises from the finding that the distribution curve of IGF-1 is similar to the shape of Gauss-curve (Saukkonen et al. 2006). Within the normal BMI range, IGF-1 production has a tendency to raise, but within the range of overweight, it starts to fall. The exact cut-off value between the range of its increase and decrease is interindividually different and depends on the proportion of centrally distributed fat tissue. Moreover, the negative correlation between the centrally distributed fat mass and free IGF-1 levels, observed by other authors, corresponds with the decreasing arm of the curve demonstrating the relation between IGF-1 and BMI, which is similar to Gauss distribution (Saukkonen et al. 2006). The same relationship has also been confirmed in Framingham and NHANES III studies (Sierra-Johnson et al. 2009; Lam et al. 2010).

**Study limitations.** 1) There was no possibility for direct analysis of GH levels. It is widely accepted that with respect of their diurnal variations it is difficult to directly detect GH levels under normal conditions. It is feasible only by provocative tests performed by glucagon application or hypoglycemia in response to insulin application. Nevertheless, ex iuvantibus GH application in different therapeutic regimens has several insulin resistance-decreasing effects on several levels of function and in different ways (Saukkonen et al. 2006). These effects represented by the stimulation of IGF-1 synthesis with all benefits arising from that are probably also related to the promotion of FFA utilization in the muscles, to stimulation of lipolysis in the fat tissue and to the prevention of FFA accumulation in the liver because of negative feedback mechanism on GH synthesis (Frystyk 2004). 2) The other problem concerning the evaluation of the interaction between IGF-1 levels and insulin sensitivity is related to the fact that so far no generally accepted cut-off values of IGF-1 have been established.

**Summary.** The arising issues in terms of these complex relations could be addressed by further evaluation of feedback mechanisms between insulin, GH, and IGF-1. In terms of these mechanisms, it is widely accepted that hyperinsulinemia and peripheral insulin resistance causes inhibition of GH production. One of the possible mechanisms of this phenomenon is the suppression of IGFBP-1 secretion by hyperinsulinemia in the liver and consequently the increase of free IGF-1 levels, which inhibits GH secretion by a negative feedback mechanism (Gomez et al. 2004). However, the capacity of IGFBP-1 suppression and IGF-1 synthesis declines with the raise of hepatic insulin resistance (Cornford et al. 2011). Another conflicting piece of evidence for this theory is that in some in vitro studies the fall of GH levels has been observed earlier than the rise of free
IGF-1 levels (Schneider et al. 2006). The other possible explanation may be that insulin acts independently in relation to IGF-1 and GH synthesis on a local level corresponding to its paracrine activity exerted directly in the liver and the hypophysis in a different time manner (Franco et al. 2006). Further analyses are needed to clarify the exact role of IGF-1 and IGFBP3 in the pathophysiology of hepatic insulin resistance.

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References


