# PROINSULIN AND ADIPONECTIN IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE

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**Abstract**

Liver fat, more than visceral fat is associated with type 2 diabetes (T2D). Although the relationship of nonalcoholic fatty liver disease (NAFLD) was studied in the light of the metabolic syndrome and type 2 diabetes, the relationship of NAFLD with proinsulin and adiponectin levels reflecting the β-cells dysfunction and respectively adipocytes secretion was less studied. In 112 patients (41 men and 71 women) fasting plasma glucose, glycated hemoglobin (HbA1c), total cholesterol, HDL-cholesterol, triglycerides, fasting insulin, proinsulin and adiponectin were determined. Body fat percent and trunk fat content were measured using bioelectrical impedance analysis (TANITA BC-418). Weight, body mass index (BMI), waist circumference, adipose tissue percent, trunk adipose tissue percent, fasting insulin, homeostatic model assessment insulin resistance (HOMA-IR) and visceral fat ratio (VFR) were higher in patients with NAFLD than in patients without NAFLD, the highest value was found in patients with NAFLD and T2D. Proinsulin, proinsulin/insulin and proinsulin/adiponectin ratio were significantly higher in patients with NAFLD and T2D than all the other groups. Adiponectin was significantly lower in patients with than without NAFLD. Proinsulin/insulin ratio can be considered a marker of β-cells dysfunction only in patients with NAFLD and T2D and not in patients with NAFLD and without T2DM, emphasizing that proinsulin is not a marker of liver disease.

**Rezumat**

Adipozitatea intrahepatică mai mult decât cea viscerală este asociată cu diabetul zaharat tip 2 (DZ tip 2). Deşi s-a studiat relaţia steatozei heapatice nonalcoolice (SH) cu sindromul metabolic şi diabetul zaharat tip 2, relaţia cu proinsulina şi adiponectina, reflectînd disfuncţia β-celulară, şi respectiv secreţia adipocitară, a fost mai puţin studiată. La 112 pacienţi (41 barbaţi şi 71 femei) s-au determinat: glicemia *a jeun*, HbA1c, colesterolul total, HDL-colesterolul, trigliceridele, insulinemia *a jeun*, proinsulina şi adiponectina. Procentul de ţesut adipos corporal şi de la nivelul trunchiului s-au determinat prin analiza impedanţei bioelectrice (TANITA BC-418). Greutatea, indicele de masă corporală (IMC), circumferinţa abdominală, procentul de ţesut adipos corporal, cel de la nivelul trunchiului, insulinemia *a jeun*, *homeostatic model assessment insulin resistance* (HOMA-IR) şi *visceral fat ratio* (VFR) au fost semnificativ mai mari la pacienţii cu SH *versus* fără SH, valorile cele mai mari s-au obţinut la pacienţii cu SH şi DZ tip 2.

Proinsulina, raportul proinsulină/insulină şi proinsulină/adiponectină au fost semnificativ mai mari la pacienţii cu SH şi DZ tip 2 faţă de celelalte grupuri. Adiponectina a fost semnificativ mai mică la pacienţii cu SH *versus* fără SH. Raportul proinsulină/insulină poate fi considerat un marker al disfuncţiei β-celulare numai la pacienţii cu SH si DZ tip 2, nu şi la pacienţii cu SH fără DZ tip 2, subliniind că proinsulina nu este un marker al afectării hepatice.

**Keywords:** nonalcoholic fatty liver disease (NAFLD), type 2 diabetes, proinsulin, adiponectin.

## Introduction

Liver fat, more than visceral fat is associated with type 2 diabetes mellitus. Whether the liver fat precedes or follows the disturbance of blood glucose regulation, this is a question that has not been answered yet. Nonalcoholic fatty liver disease (NAFLD) emerged as an entity in the last decades, despite in 1920, in the subchapter "Obesity" from "*Traité de Physiologie Médicale*" [1] it was mentioned that in obesity "liver is voluminous and yellowish and have often its cells infiltrated with lipid granulations". Already in 1912, in the third volume from the "*Traité de Médecine Laceraux-Paulesco*" [2], referring to the role of the liver in the regulation of blood glucose level, the "hepato-pancreas" was described as a binome organ: the internal secretion of the pancreas (not yet discovered at that time) influences the glycogenic function of the liver in maintaining posthepatic blood glucose levels in a normal range. In his previous studies Paulescu observed that after pancreatectomy in dogs, the capacity of liver in storing glycogen decreases. In this way he anticipated that the antidiabetic hormone secreted by the pancreas is essential for the glycogenic function of the liver.

Proinsulin concentration is low in normoglycemic subjects, having 10-20% of insulin activity regarding glucose lowering [3] and has an increased level in diabetic patients and in subjects with impaired glucose tolerance [4-12]. This is linked with a disturbance of the proinsulin processing inside β-cells [10-13]. Considering both impaired glucose tolerance (IGT) and type 2 diabetes (T2D), associated with overweight/obesity the increasing overload of β-cells induces a disproportionate proinsulin level relative to the insulin level. In the Insulin Resistance Atherosclerosis Study, Hanley et al. demonstrated that the decreasing of acute insulin response and the increasing of proinsulin concentration were significantly associated with the 5-year incidence of diabetes after being adjusted for initial glucose tolerance status and for insulin sensitivity. In a subgroup of subjects with

IGT the high level of proinsulin was associated with an increased risk of diabetes [14].

Adiponectin is the most abundant protein produced by adipose tissue with a higher level in females. Whereas the majority of proteins and free fatty acids secreted by adipose tissue increases with the increasing of visceral adiposity, the adiponectin level decreases [15]. Adiponectin increases the insulin sensitivity, free fatty acids oxidation, glucose uptake in skeletal muscle tissue, increases energy expenditure and reduces liver glucose production. Plasma levels vary from 2 to 10 μg/mL being low in obese and type 2 diabetes patients, and in dyslipidemia. Also it is considered a good marker of cardiovascular risk. In the liver, muscular tissue and adipose tissue adiponectin increases insulin sensitivity [16, 17].

In a study of Kantartzis *et al*, using a state of art technics for determination of visceral fat and liver fat, it was found that the percentage of liver fat increases progressively from 4.43±0.32% in normal glucose to 5.85±1.14% in impaired fasting glucose (IFG), to 7.97±1.14% in impaired glucose tolerance (IGT) and 11.5±1.44 in IFG+IGT. They found that liver fat predicted the degree of glucose deterioration more accurately than total visceral fat. Also this study shows a decrease in adiponectin level from IFG to IGT then IFG and IGT, confirming previous data suggesting that this association depends on obesity [18, 19, 20]. Low levels of adiponectin in patients with liver steatosis are correlated with insulin sensitivity, liver fat content and also with inflammation and fibrosis. Moreover, decreased adiponectin level is considered a better predictor of hepatic steatosis and of the increasing level of ALT and GGT in obese persons without metabolic disorders [21, 22].

Although NAFLD was studied in connection with metabolic syndrome [23, 24, 25], cardiovascular diseases [26], and type 2 diabetes [27], the relationship of NAFLD with proinsulin and adiponectin levels reflecting the β-cells dysfunction and respectively adipocytes function was less studied.

Our study aimed to investigate the relation of proinsulin and adiponectin with nonalcoholic fatty liver disease according to the presence/absence of type 2 diabetes.

## Materials and Methods

Our study included 112 patients (41M/71F) with a mean age of 55.22 ± 8.95 years old recruited from the I. Pavel Diabetes Center belonging to the National Institute of Diabetes, Nutrition and Metabolic Diseases "N. C. Paulescu", Bucharest, Romania, between March and December 2012.

NAFLD was diagnosed by ultrasonography. Exclusion criteria: positive serologic markers for viral hepatitis and alcohol consumption > 20g/day. All anthropometric measurements (weight, height, body mass index (BMI), waist circumference (WC) were done by the same physician on the day the blood sample was taken. Venous blood samples were drawn from all subjects after overnight fasting and routine blood tests including fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), total cholesterol (TC), HDL-cholesterol, triglycerides (TG) and fasting insulin (FI) were analyzed. Proinsulin and adiponectin levels were determined by ELISA method using a commercial kit (DRG Instruments GmbH, Germany, EIA 1560 and EIA 4177). Total analytical variability, expressed as coefficient of variation (CV) was 4.3% and 6.8% respectively. BMI was calculated as the weight (kg) divided by square of the height (m). LDL–cholesterol was calculated with Friedewald's formula: TC (total cholesterol) – HDL–cholesterol – triglycerides/5 (for TG < 400 mg/dL). We also calculated: Weight/Height (W/H) ratio, Proinsulin/Insulin ratio (P/I) and Proinsulin/Adiponectin ratio (P/A). Insulin sensitivity was calculated by HOMA-IR index, (*Homeostasis Model Asessment for Insulin Resistance*): [fasting plasma glucose (mmol/L) x fasting insulin (μU/mL)]:22.5 [28]. Individuals with HOMA-IR > 2.7 were considered as insulin resistant. Body fat percent and trunk fat content were measured using bioelectrical impedance analysis (TANITA BC-418).

The control group included 20 healthy subjects in which both NAFLD and T2D were excluded. The study protocol was approved by The Ethical Committee of "N.C. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Disease, Bucharest, Romania. A written informed consent was obtained from each study participant.

*Statistical analysis*: Statistical Package for Social Sciences (SPSS 21.0) software (IBM). Kolmogorov-Smirnov test was used to analyze continuous data distribution, according to which appropriate tests were further used in analysis: ANOVA or Kruskal-Wallis test for differences between means of 4 independent groups. Spearman's correlation coefficients were calculated in order to test the association between variables. p-values < 0.05 were considered as statistically significant**.**

## Results and Discussion

The anthropometric and metabolic characteristics of the studied groups are shown in Table I. According to the presence or absence of NAFLD and T2D the cohort was divided into four groups: **group 1** – patients with T2D and without NAFLD (10.71 %); **group 2** – patients with

NAFLD and without T2D (17.86 %); **group 3** – patients with NAFLD and T2D (53.57 %) and the **control group** - subjects without NAFLD and T2D (17.86 %).

**Table I**

Anthropometric and metabolic characteristics of the studied groups

| | Control (n=20) | T2D (n=12) | NAFLD ( n=20) | NAFLD+T2D (n=60) | p-value |
|---|---|---|---|---|---|
| **Age (years)** | 42.80±10.87 | 59.42±0.63 | 49.70±8.42 | 56.73±7.59 | <0.001* |
| **Weight (kg)** | 66.06±13.14 | 66.93±11.00 | 83.11±13.96 | 88.65±15.34 | <0.001* |
| **BMI (kg/m$^2$)** | 24.33±3.95 | 24.84±1.91 | 29.89±4.57 | 32.72±4.89 | <0.001* |
| **WC (cm)** | 83.15±11.69 | 93.75±6.68 | 101.55±10.96 | 108.73±9.97 | <0.001* |
| **FPG (mg/dL)** | 91.51±8.31 | 137.19±48.23 | 102.13±12.20 | 146.71±38.65 | <0.001* |
| **HbA1c (%)** | 5.28±0.42 | 6.54±0.56 | 5.62±0.31 | 7.09±1.36 | <0.001** |
| **TC (mg/dL)** | 204.54±52.52 | 208.24±43.44 | 235.44±41.74 | 206.38±45.26 | 0.099* |
| **HDL-c (mg/dL)** | 56.81±10.99 | 55.30±16.47 | 49.39±13.40 | 46.38±12.49 | 0.008* |
| **LDL- c (mg/dL)** | 129.87±52.54 | 125.65±39.60 | 151.80±47.82 | 126.22±41.84 | <0.001* |
| **Triglycerides (mg/dL)** | 89.57±35.97 | 150.45±81.53 | 189.33±133.83 | 183.47±105.89 | <0.001* |
| **Fasting insulin (µU/mL)** | 7.99±3.31 | 8.61±2.13 | 11.68±6.86 | 16.62±9.91 | <0.001** |
| **HOMA-IR index** | 1.83±0.85 | 3.10±1.22 | 3.05±2.08 | 5.99±3.64 | <0.001** |
| **Proinsulin (µU/mL)** | 1.68±1.40 | 3.62±2.58 | 2.45±1.96 | 11.33±13.99 | <0.001** |
| **PI/I ratio** | 0.20±0.19 | 0.39±0.24 | 0.22±0.23 | 0.69±0.62 | <0.001** |
| **Adiponectin (ng/mL)** | 12.31±6.92 | 13.90±7.63 | 9.75±8.84 | 7.33±4.86 | 0.005* |
| **PI/A ratio** | 0.26±0.42 | 0.38±0.41 | 0.48±0.45 | 5.58±12.90 | <0.001** |
| **% Adipose tissue** | 27.50±9.34 | 26.37±7.77 | 35.28±8.22 | 37.97+8.41 | <0.001* |
| **% Trunk adipose tissue** | 25.40±9.87 | 23.67±7.90 | 34.59±7.31 | 35.67±7.18 | <0.001* |
| **VFR** | 5.41±2.85 | 8.62±2.92 | 10.66±4.25 | 12.55±3.17 | <0.001* |

*ANOVA; **Kruskal-Wallis; BMI: body mass index; WC: waist circumference; TC: total cholesterol; FPG: fasting plasma glucose; FI: fasting insulin. VFR: visceral fat ratio; PI/I:Proinsulin/Insulin; PI/A: Proinsulin/Adiponectin

Weight, BMI, waist circumference, adipose tissue percent, trunk adipose tissue percent, HOMA-IR and VFR were higher in patients with NAFLD than in patients without NAFLD, the highest value was found in patients with NAFLD and T2D. Moreover, in patients with NAFLD, fasting insulin was significantly higher than in patients without NAFLD, while HDL-cholesterol was significantly lower in patients with NAFLD comparing with control group.

As shown in Figure 1**,** proinsulin was significantly higher in patients with NAFLD and T2D (11.33 µg/dL) than all other groups included in our study (1.68 µg/dL – control group; 3.62 µg/dL) – group 1 and 2.45 µg/dL – group 2 respectively). Adiponectin was significantly lower in patients with (7.3 ng/mL) than without NAFLD (12.31 ng/mL; 13.90 ng/mL), (Figure 2). For proinsulin/insulin ratio (Figure 3) and proinsulin/adiponectin ratio (Figure 4) we found higher level in patients which associated NAFLD and T2D.

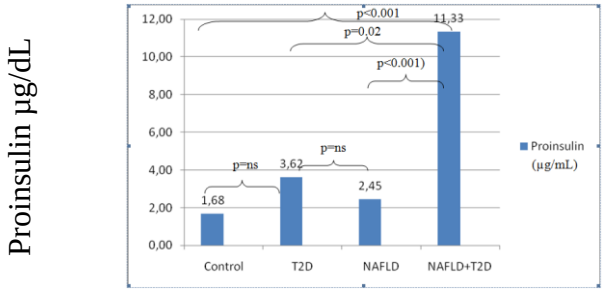


**Figure 1.**
The measured values of proinsulin in the studied groups

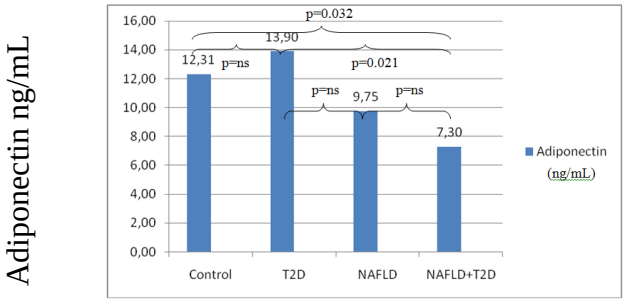


**Figure 2.**
The measured values of adiponectin in the studied groups

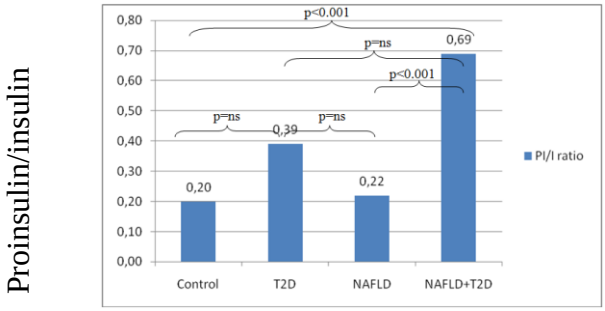


**Figure 3.**
The proinsulin/insulin ratio in the studied groups

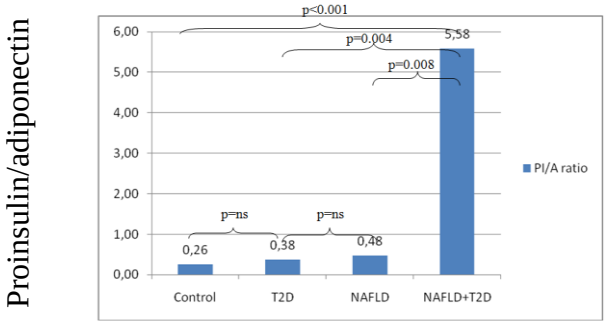


**Figure 4.**
The proinsulin/adiponectin ratio in the studied groups

In patients with NAFLD and T2D **proinsulin** was correlated with weight, BMI, waist circumference, VFR, fasting plasma glucose, HbA1c, HOMA-IR and triglycerides; **adiponectin** was positively correlated with HDL-cholesterol and negatively with waist circumference and fasting insulin; **proinsulin to insulin ratio** was positively correlated with VFR, FPG, HbA1c; **proinsulin to adiponectin** ratio was positively correlated with weight BMI, waist circumference, VFR, fasting insulin, HOMA-IR and triglycerides (Tabel II). After adjusting for BMI and waist circumference the correlations between P/A ratio with FI and HOMA-IR remained significantly statistic.

**Table II**
The correlations coeficients between the studied parameters in patients with NAFLD and T2D

| | **Proinsulin** | **Adiponectin** | **Proinsulin/ Insulin** | **Proinsulin/ Adiponectin** |
|---|---|---|---|---|
| **Weight** | 0.397* | -0.273 | 0.233 | 0.379* |
| **BMI** | 0.348* | -0.249 | 0,090 | 0.463** |
| **WC** | 0.359* | -0.335 | 0,089 | 0.452* |
| **VFR** | 0.471** | -0.211 | 0.451** | 0.394* |
| **FPG** | 0.346* | 0.016 | 0.387* | 0.134 |
| **HbA1c** | 0.514** | 0.036 | 0.480** | 0.347 |
| **FI** | 0.295 | -0.343* | -0.241 | 0.387* |
| **HOMA-IR** | 0.412** | -0.310* | -0.072 | 0.418* |
| **Triglycerides** | 0.522** | -0.185 | 0.287 | 0.382* |
| **HDL-c** | -0.242 | 0.381** | -0.151 | -0.143 |

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

In patients with NAFLD and without T2D we found a positive correlation for **proinsulin** with FPG (rho= 0.496, p<0.05) and triglycerides

(rho=0.484, p<0.05) and for **adiponectin** with HDL-cholesterol (r=0.587, p<0.01).

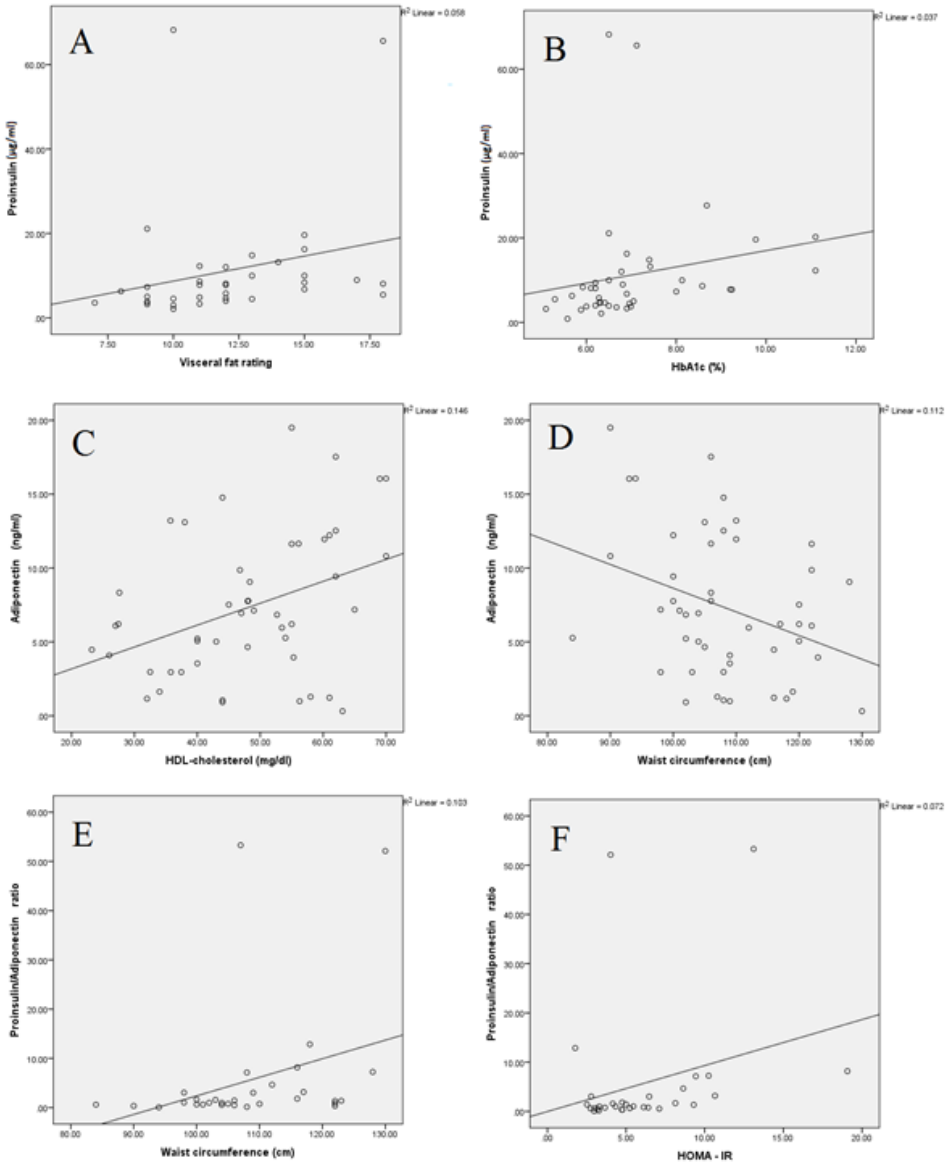


**Figure 5.**

The correlations between the studied parameters: proinsulin and visceral fat rating **(A)**, proinsulin and $HbA_{1c}$ **(B)**, adiponectin and HDL-cholesterol **(C)**, adiponectin and waist circumference **(D)**, proinsulin/adiponectin ratio and waist circumference **(E)**, proinsulin/adiponectin ratio and HOMA-IR **(F)**

Liver is an important organ involved in the regulation of energy metabolism especially in the energy overflow induced by a high caloric/fat intake and the decrease of the physical activity. Placed between the pancreas and the adipose tissue, the liver is influenced on the one site by the insulin secretion (the portal vein contains the highest level of insulin) and on the other site by the high flow of the fatty acids released from lipolysis in the adipose tissue. Also, the liver receives not only adiponectin (protective hormone) but the proinflammatory cytokines as well, produced either by "stressed and aggressive" adipocytes [29, 30] or by the macrophages (TNF-alpha, IL-6) which invade the adipose tissue when the diameter of adipocytes overpass a limit (more than 110μm diameter) [31, 32, 33].

The most common conditions associated with NAFLD are obesity, T2D and dyslipidemia, liver fat being correlated with waist circumference and metabolic syndrome features [34]. NAFLD precedes and predicts T2D and cardiovascular diseases, independent of obesity [35]. Fasting insulin and HOMA-IR index in patients which associated T2D and fatty liver, are better correlated with total body adipose tissue than with trunk adiposity [36].

In our study the association of NAFLD and T2D was characterized by high values of BMI, WC, % body adipose tissue, % trunk adipose, VFR, insulin and HOMA-IR values.

Increased proinsulin and proinsulin/insulin ratio (marker of disproportionate release of proinsulin from β-cells), are correlated with insulin response being markers of β-cells secretory dysfunction [37, 38]. In patients with NAFLD and T2D proinsulin level was increased and correlated with anthropometric measures (weight, BMI, WC, VFR) and also with metabolic parameters (FPG, $HbA_{1c}$, TG and HOMA-IR). Although NAFLD is considered to be a slowly progressive disease, the progression to various degrees of fibrosis is unexpectedly high. In a study of 103 patients who underwent serial liver biopsies (medium-interval of 3.2 years) fibrosis progressed in 37%, remained stable in 34% and regressed 29% in of the cases. The higher rate of progression has been noted in T2D patients or in patients with an initial even low degree of fibrosis [39]. In our study patients with NAFLD and without T2D had higher values of fasting insulinemia and HOMA-IR index compared with the control group, while proinsulin and proinsulin/insulin ratio were not found to be significantly changed. These data might be related to a decrease in the clearance of insulin related to the fat deposit within this organ. In this group, proinsulin was significantly correlated with FPG and TG. These data are consistent with those in a study performed on 64 Japanese type 2 diabetic subjects that have found a

significant positive correlation of proinsulin with BMI, hypertension, glucose, HbA1c, insulin and HOMA-IR. Proinsulin/Insulin ratio showed a significant positive correlation with glucose, HbA1c and with advanced glycation endproducts, as a consequences of glucotoxicity and causative for beta cells dysfunction and a significant negative correlation with insulin and HOMA-IR [13].

Mykkanen *et al* [40] showed that fasting insulin and intact proinsulin were inversely correlated and proinsulin-to-insulin ratio positively correlated with insulin sensitivity. Proinsulin/insulin ratio was inversely correlated with acute insulin response. The associations were independent of age, sex, BMI. The relation of acute insulin response with proinsulin-to-insulin ratio was independent of insulin sensitivity. Normoglycemic subjects with a low acute insulin response had a high level of proinsulin-to-insulin ratio compared with the patients with high acute insulin response and low proinsulin-to-insulin ratio. This can be explained by intensive processing of proinsulin in subjects with normal glucose. In normoglycemic subjects with insulin resistance proinsulin related to insulin level are not increased [40].

In a cross-sectional study on subjects without diabetes it was observed that proinsulin levels and proinsulin/insulin ratio increased with age both in men and women after adjusting for glucose and insulin sensitivity suggesting that β-cells' function decreases with age [41]. In our study, proinsulin and proinsulin to insulin ratio are significantly higher in patients with NAFLD only in association with T2DM, before and after adjusting for age, suggesting that the β-cells secretory dysfunction is not present in patients with NAFLD and without T2D.

It is well known that between plasma levels of adiponectin and the risk of obesity, diabetes and cardiovascular diseases exists a negative correlation, adiponectin concentration being negatively correlated with adipose tissue mass [16, 17].

Adiponectin levels can predict the presence of metabolic syndrome. The level of adiponectin was positively correlated with age, female gender, HDL-cholesterol and negatively with WC, BMI, insulin resistance, triglycerides and aminotransferase levels. In patients with NAFLD the plasma level of adiponectin is low and is correlated with increased age, female gender and triglycerides and inversely correlated with the percentage of hepatic fat content [42]. Bugianesi and Pagano showed that the adiponectin level in patients with NAFLD is 20-60% lower than in normal subjects [42, 43]. Moreover, Speliotes *et al* [44] demonstrated that NAFLD is associated with metabolic syndrome and with a low level of adiponectin independent of BMI, waist to hip ratio and visceral obesity. Our data

support those observations: adiponectin had the lowest value in NAFLD independent of the presence of T2D.

Proinsulin/adiponectin ratio as a marker of insulin resistance [45] was correlated in patients with NAFLD and T2D with anthropometric measures: weight, BMI, WC, VFR and also with insulin level, HOMA–IR index and triglycerides.

## Conclusions

The relationship between T2D and NAFLD could be bilateral, metabolic disorders may cause NAFLD and NAFLD may enhance metabolic disorders of diabetes.

Proinsulin had the highest values in patients with NAFLD and T2D. Proinsulin/insulin ratio can be considered a marker of β-cells dysfunction in patients with NAFLD and T2D but not in patients with NAFLD and without T2D, emphasizing that proinsulin is a marker of β-cells dysfunction and not a marker of liver disease.

The studied subjects presented decreased levels of adiponectin in NAFLD patients independent of the presence of T2D. Proinsulin /adiponectin ratio had the highest values in patients with NAFLD and T2D which may have a disturbance of β-cells function related to the excess of adipose tissue.

The early diagnosis and appropriate treatment of diabetes might represent the best way to prevent the progression of NAFLD.

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