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Original Article

# Higher levels of thioredoxin interacting protein (TXNIP) in patients with prediabetes compared to obese normoglycemic subjects

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ABSTRACT

*Background:* Thioredoxin interacting protein (TXNIP) is one of the mediators of oxidative stress induced beta-cell glucotoxicity. TXNIP might play a key role in impaired glucose homeostasis preceding overt T2DM. The **aim** of the present study was to compare TXNIP levels between patients with prediabetes and obese normoglycemic controls and to evaluate the link between TXNIP and metabolic risk factors.
*Patients and methods:* In the present study we included 79 patients with mean age 50.3 ± 10.6 years, divided into two age and BMI matched groups –control group with obesity without glycemic disturbances (NGT) (n = 40) and prediabetes (n = 39).
*Results:* We found significantly higher levels of TXNIP in patients with prediabetes compared to normoglycemic obese controls (54.2 ± 69.9 vs. 23.9 ± 47.1 pg/ml; p = 0.03). The levels of TXNIP gradually increased from normal glucose tolerance trough IFG/IGT only to IFG + IGT (27,1; 44.0; 49.9 and 95.7 pg/ml respectively; p = 0.025 between NGT and IFG + IGT). TXNIP levels correlated weakly only with fasting blood glucose (r = 0.235; p = 0.04) but not with glucose during OGTT or the markers of insulin resistance.
*Conclusions:* The levels of TXNIP are higher in patients with prediabetes compared to normoglycemic controls as they increase gradually from NGT trough IFG/IGT only to IFG + IGT.


## 1. Introduction

Prediabetes usually precedes overt diabetes and includes impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). Although insulin resistance is more prominent pathogenic factor than impaired insulin secretion during the prediabetic state, diabetes and prediabetes are similar in the context of disglycemia and hyperglycemic glucotoxicity [1,2].

Impairment of beta-cell function and reduced beta-cell mass have a role in development of type 2 diabetes and reduced insulin secretion. Glucotoxicity induces pancreatic beta-cell apoptosis, dedifferentiation or transdifferentiation, diabetes and diabetic complication progression. The possible mechanisms include beta-cell overstimulation, advanced glycation end-products formation, hexosamine pathway activation, proteinkinase C activation, inflammation and hypoxia [3,4].

Thioredoxin interacting protein (TXNIP) is one of the mediators of oxidative stress induced beta-cell glucotoxicity [5,6]. Recently it has been found that it regulates beta-cell micro-RNA expression, beta-cell function and insulin production [7]. High glucose levels activate TXNIP expression [8], while insulin suppresses TXNIP production [9]. This represents a negative feedback loop that restrains the stimulation of TXNIP by chronic hyperglycaemia. Suppression of TXNIP by insulin is probably an important compensatory mechanism protecting beta cells from oxidative damage and apoptosis. In patients with diabetes TXNIP levels are elevated in various tissues, including the retina, where it plays a key role for oxidative stress and inflammation [10–13].

There is plenty of data that TXNIP plays a key role in diabetes progression [14]. TXNIP induces beta-cell apoptosis, while its deficiency is protective against type 1 and type 2 diabetes. TXNIP also modulates the activity of the angiogenic cytokine VEGF and impairs endothelial cell function, which makes TXNIP a key factor for diabetes related impairment of ischemia mediated angiogenesis [15]. On the other hand TXNIP regulates both insulin-dependent and insulin-independent glucose uptake in human skeletal muscle [16]. These data suggest that TXNIP might play a key role in impaired glucose homeostasis preceding overt T2DM.

There is scarce data however on the changes in TXNIP levels

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during prediabetic stage and the link with elevated glucose levels and other metabolic risk factors. The aim of the present study was to compare TXNIP levels between patients with prediabetes and obese normoglycemic controls and to evaluate the link between TXNIP and metabolic risk factors.

## 2. Patients and methods

### 2.1. Study population

A total of 79 Caucasian subjects, recruited from 2014 to 2016 in a university hospital setting participated in the study. Inclusion criteria were: impaired glucose tolerance (glucose on 120 min of OGTT between 7.8 and 11.0 mmol/l) and/or impaired fasting glucose (fasting glucose between 6.1 and 6.9 mmol/l) OR BMI≥ 30kg/m2 and age between 35 and 74 years. Study participants were not included if any of the following were present: liver dysfunction (any hepatic enzyme > 3 times the upper-limit of normal); chronic kidney disease (eGFR estimated by CKD-EPI calculation < 60 ml/min/1.73m2); neoplastic disease; intake of any oral antidiabetic drug 3 months prior inclusion.

The project was approved by the University ethics committee for clinical studies and all patients included in the study signed an informed consent for participation in the project.

The following study methods were used:

1. **Anthropometric measurements** included WC, weight, height, NC, BMI, arterial blood pressure. BMI was calculated as weight in kilograms divided by height squared in squared meters as a measure of general obesity. Waist circumference was measured at the midpoint between the inferior costal margin and the superior border of the iliac crest on the mid-axillary line. Hip circumference was measured at the level of the greater trochanter. Waist-to-hip ratio (WHR) and waist-to-stature ratio (WSR) were calculated. Calculation of VAI (visceral adiposity index) was performed using the following formula: VAI = (WC/(36.58 + (1.89 xBMI))) x (TG/0,81) x (1,52/HDL). Percentage (Body Fat%) was measured by means of Body Impedance (BIA) by a TANITA™ TBF-215 GS Body Composition Analyzer in the fasting state.
2. **Investigation of carbohydrate metabolism** - An oral glucose tolerance test (OGTT) with measurement of glucose and immunoreactive insulin (IRI) (ECLIA – Roche Diagnostics™) on 0 min, 60 min and 120 min. HOMA index (fasting glucose X fasting immunoreactive insulin/22.5) was calculated.
   Insulin resistance was assumed at IRI 0 min > 17 mU/l, IRI 60 min > 130 mU/l, IRI 120 min > 80 mU/l, HOMA index> 2,6.
3. The presence of **metabolic syndrome (MS)** was determined based on the IDF criteria. Patients were considered to have MS if they had 3 of the following disturbances: 1) abdominal obesity, defined as waist circumference ≥94 cm for men, and ≥80 cm for women. 2) elevated blood pressure, defined as systolic blood pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (DBP) ≥ 85 mmHg, or current antihypertensive drug treatment; 3) elevated fasting blood glucose level ≥ 5,6 mmol/l or current use of blood glucose lowering agents or history/diagnosis of type 2 diabetes; 4) decreased HDL cholesterol level (<1,03 mmol/l in men or < 1,30 mmol/l in women) or drug treatment aimed to increase HDL cholesterol; and 5) hypertriglyceridaemia (triglyceride level ≥ 1,70 mmol/l) or drug treatment for elevated triglycerides.
4. **Measurement of serum TXNIP levels** was performed by enzyme-linked immunosorbent assay (ELISA) (BYOVENDOR). The blood was taken after overnight fasting, was immediately centrifuged for 15 min on 4000 rpm and the serum was stored at (−80°C) until the test was performed

### 2.2. Statistical methods

The data were processed using the statistical package SPSS 16.0 (IMB™). The level of significance for rejecting the null hypothesis was $p < 0.05$. The following statistical methods were applied: descriptive analysis, variation analysis, Kolmogorov– Smirnov's one sample non-parametric test, Student's t-test for two independent samples, Mann–Whitney's non-parametric test for two independent samples, one-way analysis of variance between-groups ANOVA, correlation analysis. Data are presented as mean ± SD.

## 3. Results

In the present study we included 79 patients with mean age 50.3 ± 10.6 years, divided into two groups – *group 1* (control group) with obesity without glycemic disturbances (NGT) (n = 40) and *group 2* with prediabetes (n = 39). The characteristics of the two groups are presented on Table 1. The two groups were similar in age, body weight, fat%, BMI, WHR, WSR and VAI.

There was no difference in the prevalence of hypertension and dyslipidemia between the groups.

Patients with prediabetes had higher levels of IRI on 0 and 120 min of OGTT and higher HOMA index although similar rates of hyperinsulinemia/insulin resistance were observed between the two groups (Table 2). The patients with prediabetes also had higher prevalence of metabolic syndrome and a higher number of components of MetS (3.3 ± 1.19 vs. 2.8 ± 1.07; p = 0.04) compared to the patients without carbohydrate disturbances.

We found higher levels of TXNIP in patients with prediabetes compared to normoglycemic obese controls (54.2 ± 69.9 vs. 23.9 ± 47.1 pg/ml; p = 0.03) with borderline statistical significance. When the patients with prediabetes were divided into those with IFG only (n = 6), IGT only (n = 26) and IFG + IGT (n = 7) we found gradually increasing levels of TXNIP form normal glucose tolerance trough IFG/IGT only to IFG + IGT (p = 0.025 between NGT and IFG + IGT) (Fig. 1).

TXNIP levels correlated weakly only with fasting blood glucose (r = 0.235; p = 0.04) but not with glucose during OGTT or the markers of insulin resistance. There was no difference in TXNIP levels between patients with and without metabolic syndrome.

## 4. Discussion

Prediabetes represents a metabolic condition that stands in between normal glucose homeostasis and type 2 diabetes. Insulin resistance and impaired insulin secretion are the main components in type 2 diabetes pathogenesis although they play a different role in the different stages of impaired glucose homeostasis form normal glucose tolerance to overt type 2 diabetes. Insulin resistance is usually the earliest presentation of diabetes development and can be detected as early as 5–10 years before postprandial hyperglycemia reaches diabetes threshold (11.1 mmol/l). Normal glucose tolerance can be maintained until beta-cells are able to compensate for insulin resistance with increased insulin secretion. Direct evidence for this balance are obtained from a prospective study exploring the link between insulin sensitivity, beta-cell function and glucose tolerance deterioration that precedes type 2 diabetes diagnosis in patients that progress or do not progress to overt diabetes [17]. The subjects in this study have 29% lower HOMA-S but 13% higher HOMA-B 13 years before diabetes diagnosis. HOMA-B decreases linearly up to 5 years before the diagnosis

**Table 1**
Anthropometric characteristics of the study groups.

| | Group 1 Obesity + NGT | Group 2 Obesity + prediabetes (IGT and/or IFG) |
|---|---|---|
| Age (y) | 50.4 ± 9.7 | 50.3 ± 11.5 |
| Weight (kg) | 97.5 ± 18.1 | 99.6 ± 20.3 |
| BMI (kg/m$^2$) | 36.7 ± 5.2 | 37.7 ± 6.1 |
| % fat tissue | 44.2 ± 4.7 | 44.6 ± 6.5 |
| WHR | 0.90 ± 0.08 | 0.92 ± 0.07 |
| WSR | 0.66 ± 0.07 | 0.68 ± 0.08 |
| VAI | 3.3 ± 4.0 | 3.4 ± 2.0 |

All differences NS.

**Table 2**
Markers of insulin resistance.

| | Group 1 Obesity | Group 2 Prediabetes |
|---|---|---|
| IRI 0 min (mU/l) | 16.6 ± 7.4 | 21.9 ± 13.4* |
| IRI 60 min (mU/l) | 125.8 ± 81.3 | 123.8 ± 69.8 |
| IRI 120 min (mU/l) | 65.6 ± 64.7 | 121.2 ± 83.3** |
| HOMA index | 3.7 ± 1.7 | 5.5 ± 3.4** |
| Prevalence of insulin resistance (%) | 66.7 | 78.4 |

* p<0.05; ** p<0.01.

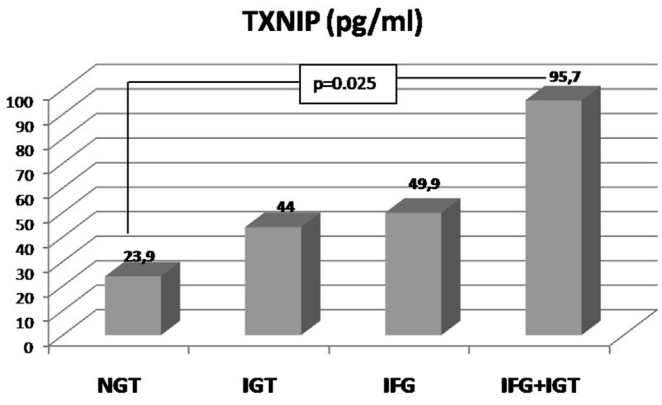


**Fig. 1.** TXNIP levels increasing form NGT through IFG/IGT only to IFG + IGT.

and much faster in the last 5 years. This shows that insulin resistance is an early defect that is compensated to a certain point with increased insulin secretion long before the manifestation of overt carbohydrate disturbances. Normal glucose tolerance subjects that later progress to IGT or diabetes have decreased first phase insulin secretion compared to nonprogressors [18]. It is not yet clear however if the reduced insulin secretion in the early phases of impaired glucose homeostasis is a primary defect of a result of glucotoxicity and beta-cell overstimulation.

TXNIP is a glucose and insulin regulated factor that can promote oxidative stress and beta-cell apoptosis [19] thus having a role in progression of carbohydrate disturbances. Higher levels of TXNIP were found in patients with impaired glucose regulation and hypertriglyceridemia [20]. We also found higher levels of TXNIP in prediabetes but the most interesting finding was that TXNIP levels increased from NGT trough IFG/IGT and were much higher when both IFG and IGT were present.

In our study we included obese patients with IFG/IGT and patients with obesity without glycemic disturbances as control group in order to investigate the effect of TXNIP on early glycemic disturbances that is independent of body weight and other vascular risk factors. We deliberately did not include patients with diabetes, because the role of TXNIP in diabetes and diabetes complications is well established. A weakness of our study are the small number of the patients and the imbalance between IFG, IGT and IFG + IGT patients in prediabetes group.

## 5. Conclusions

The levels of TXNIP are higher in patients with prediabetes compared to normoglycemic controls as they increase gradually from NGT trough IFG/IGT only to IFG + IGT.

## Disclosure

Authors declare no conflicts of interest.

## Contributions

Antoaneta Gateva performed the study, collected the data and wrote the paper.

Yavor Assyov performed the study and collected the data.

Tsvetelina Velikova performed the laboratory tests.

Zdravko Kamenov designed the study and reviewed the final paper.

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