Age at onset of obesity, transcription factor 7-like 2 (TCF7L2) rs7903146 polymorphism, adiponectin levels and the risk of type 2 diabetes in obese patients

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Abstract

Introduction: Interaction between obesity and genetic factors involved in the regulatory pathways of glucose homeostasis may play a significant role in diabetes development in the obese. The aim of this study was to investigate the associations between the TCF7L2 rs7903146 polymorphism, adiponectin levels, age at onset of obesity and the occurrence of type 2 diabetes (T2D) in a sample of obese Polish adults.

Material and methods: A total of 474 unrelated obese subjects were included in this study. Real-time PCR was used to detect the TCF7L2 rs7903146 polymorphism. Serum level of adiponectin was determined by the ELISA method. Standard assays were used to measure total cholesterol, HDL cholesterol, triglycerides, glucose and HbA_{1c} concentrations. We used multiple logistic regression to identify factors associated with type 2 diabetes.

Results: We found that the T allele of rs7903146 was significantly associated with T2D risk (odds ratio of 1.59 for T allele, $p = 0.005$). This association persisted after adjusting for confounders in the recessive model (odds ratio of 3.54 for TT genotype, $p = 0.011$). Serum adiponectin levels were significantly lower in diabetic subjects than in nondiabetic individuals (3.6 vs. 5.6 µg/ml, $p < 0.001$). Participants who were obese at age ≥ 20 years had significantly higher odds of having T2D (OR = 4.94) than those with the onset of obesity before 20 years ($p < 0.001$).

Conclusions: Our study highlights the significance of the relationship between the TCF7L2 polymorphism, a person's age at onset of obesity and the prevalence of T2D, and confirms lower adiponectin levels in obese diabetics in comparison to obese nondiabetics.

Key words: obesity, adiponectin, type 2 diabetes, TCF7L2 gene.

Introduction

Type 2 diabetes (T2D) has become a 21st century epidemic. There are now 382 million people living with diabetes, and in 2035 the population of diabetics is projected to reach 592 million. The data gathered by the International Diabetes Federation (IDF) show that about 8.5% of

the European population (i.e., 55 million people) suffer from T2D. Almost half of the adults suffering from diabetes are younger than 60 years of age [1]. Moreover, rising prevalence of overweight and obesity is evident in both men and women, and obesity has been recognized as a major public health problem [2] and as the most important risk factor for T2D [3]. Obesity is associated with increased plasma concentrations of free fatty acids (FFA) which inhibit insulin-stimulated glucose uptake, transport, phosphorylation and oxidation [4]. Lipotoxicity and glucotoxicity may act synergistically and initiate progression from obesity to T2D [3]. A characteristic feature of obesity is a reduction of adiponectin, one of the adipokines produced by adipose tissue [5]. It was recognized that lipid enriched adipocytes by secreting inflammatory factors decrease adiponectin gene transcription and adiponectin production [6]. Adiponectin is involved in insulin sensitivity regulation. It stimulates phosphorylation of insulin receptor and insulin receptor substrates (IRS) binding and enhances insulin signaling transduction, resulting in more effective glucose uptake [7, 8]. In addition, through activation of AMP-activated protein kinase (AMPK) in the liver and skeletal muscle, adiponectin stimulates glucose utilization, and fatty-acid oxidation through the PPAR-α pathway [9]. Moreover, adiponectin lowers hepatic glucose production by reducing the expression of enzymes involved in gluconeogenesis [10]. Therefore, low circulating levels of adiponectin in the obese can cause an elevation in glucose production, induce insulin resistance and disturb glucose utilization, which can result in T2D [7, 10, 11]. However, not all obese individuals are diabetics, indicating that there is considerable variation in responses to metabolic dysregulation, which can be associated with late or early age at onset of obesity and/or presence of other specific factors including genetic variants. Interaction between obesity and genetic factors involved in the regulatory pathways of glucose homeostasis plays a significant role in diabetes development among the obese [12]. Transcription factor 7-like 2 (TCF7L2) is involved in insulin secretion and in the Wnt/β-catenin signaling pathway, which seems to be essential for pancreatic islet development [13]. The *TCF7L2* rs7903146 single nucleotide polymorphism (SNP) constitutes a risk factor for T2D [12, 14–17]. However, there are limited data regarding the influence of this polymorphism on occurrence of T2D in obese individuals. Therefore, the aim of our study was to assess the interaction between the *TCF7L2* rs7903146 polymorphism, a person's age at onset of obesity, adiponectin levels and prevalence of T2D.

Material and methods

Ethics statement

The study was carried out in accordance with the principles of the Declaration of Helsinki. The whole study protocol as well as the consent procedure were approved by the two Institutional Bioethics Committees (KB/127/2012, at the Medical University of Warsaw; 7/PB/2015, at the Medical Centre of Postgraduate Education). Written informed consent was obtained from each participant after a full explanation of the study.

Patient recruitment

A total of 474 unrelated individuals were enrolled in this study. All subjects were consecutively recruited on the basis of clinical investigation between September 2013 and December 2015 from patients who had been admitted to the Orlowski Hospital in Warsaw due to obesity and/or prior to bariatric surgery. Obesity was classified according to World Health Organization criteria [18] and subjects with body mass index (BMI) ≥ 30 kg/m^2 were considered obese.

A detailed clinical history, including history of obesity and a full physical examination, was obtained for each patient. In all subjects, anthropometric measurements (body weight, and height) were taken and BMI was calculated as the ratio of weight (kilograms) to the square of height (meters). A person's age at onset of obesity was ascertained by a physician prior to classification for bariatric surgery, based on an overview of medical records in which previous body weight measures were reported, and by self-reported data in a questionnaire packet filled out by participants. We grouped participants into two categories: the first consisted of patients with an adult obesity onset (who developed obesity after 20 years of age) and the second with an early onset of obesity (before the age of 20 years).

Diabetes and dyslipidemia diagnosis

Patients were classified as diabetics based on the review of medical records (previous diagnosis of diabetes by a physician, and current use of diabetes medications) and confirmed by current medical examination. The diagnosis was made by using criteria consistent with those proposed by American Diabetes Association [19] (an average fasting plasma glucose concentration ≥ 126 mg/dl on two occasions, and/or 2 h plasma glucose > 200 mg/dl during an oral-glucose-tolerance test, and/or a casual plasma glucose > 200 mg/dl). Determination of dyslipidemia was based on a current or previous medical diagnosis according to the National Cholesterol Education Program-Adult Treatment Panel III [20].

Exclusion criteria

Criteria for exclusion from the study were as follows: diabetes other than type 2, acute endocrine dysfunction, chronic kidney disease, and alcoholism. In addition, individuals with prediabetes were excluded from the study [19].

Analytical procedures

Overnight peripheral fasting blood samples were taken from all subjects with commercially available vacuum tubes. Serum was isolated and used for analyses or stored at –80°C. Standard assays were used to measure total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TGs), glucose and glycated hemoglobin (HbA_{1c}) concentrations. Low-density lipoprotein-cholesterol (LDL-C) levels were calculated using the Friedewald formula [21].

Serum levels of total adiponectin and insulin were determined by the ELISA method using the MEDIAGNOST Adiponectin ELISA E09 and DRG Insulin ELISA (EIA-2935) kits respectively. The method for total adiponectin determination was characterized by sensitivity of < 0.6 ng/ml, an inter-assay coefficient of variation below 6.7% and an intra-assay coefficient of variation below 4.7%. The method for insulin determination was characterized by sensitivity of 1.76 µIU/ml, an inter-assay coefficient of variation of 2.9–6.0% and an intra-assay coefficient of variation of 1.8–2.6%.

Insulin resistance was assessed using the homeostasis model assessment [HOMA-IR index = (fasting glucose in mmol/l × fasting insulin in µIU/ml)/22.5] [22].

DNA extraction and rs7903146 genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the Blood Mini genomic DNA purification kit (A&A Biotechnology) according to the manufacturer's instructions. DNA concentration and purity were determined with a Quawell Q5000 micro-volume UV-Vis spectrophotometer as described elsewhere [23]. Genotyping of *TCF7L2* rs7903146 polymorphism was done using a pre-validated TaqMan Assay designed by Life Technologies (Assay ID: C__29347861_10). Probes were labeled with different fluorochromes (VIC or FAM) to identify homozygotes and heterozygotes. Reactions were conducted in 96-well plates, in a total volume of 12 µl using 2 ng of genomic DNA, TaqMan Genotyping Master Mix 1x (Life Technologies) and TaqMan Genotyping Assay 1x. Samples were amplified and fluorescence data were captured using a ViiA7 Real-Time PCR System (Life Technologies). PCR cycling conditions were 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min. Genotype call rates were > 95%, more than 50% of the 474 genotypes were tested twice, and genotyping was 100% concordant.

Statistical analysis

Data were analyzed with the Statistica 12.0 program. Categorical variables are described with the number (percentage) and were analyzed by the χ^2 test. Continuous variables are described with the median (interquartile range) for non-normally distributed data. The Mann-Whitney rank test and Kruskal-Wallis test were used to assess differences between groups. Spearman's correlation was used to assess the degree of the relationship between adiponectin levels and metabolic and anthropometric variables.

Allele frequencies for *TCF7L2* rs7903146 polymorphism were calculated with the gene counting method. Agreement of genotype frequencies with Hardy-Weinberg equilibrium expectations was tested using Pearson's chi-square (χ^2) goodness-of-fit test with one degree of freedom. Pearson's χ^2 test with corresponding odds ratio (OR) and the 95% confidence interval (CI) was also used to evaluate the association between the *TCF7L2* rs7903146 polymorphism and T2D in obese subjects in dominant, recessive, and additive models of inheritance. *P*-values for model fit (p_{fit}) were calculated using the Web-Assotest program (http://www.ekstroem.com). $P_{fit} < 0.05$ indicated that a given model of inheritance should be rejected. A power calculation was performed using a genetic power calculator [24]. Our study could detect an allelic association with power > 80% ($\alpha = 0.05$) conferring an odds ratio of 1.59.

Logistic regression analysis was used to assess the association between age at onset of obesity, male sex, low serum total adiponectin (cut-off value of adiponectin concentration < 50th percentile was 4.87 µg/ml), *TCF7L2* genotype and T2D. Qualitative variables were coded as 0-1 dummy variables as follows: gender: 1 man, 0 woman; age at onset of obesity ≥ 20 years: 1; age at onset of obesity < 20 years: 0. Separately, adjusted logistic regression analysis was used to determine whether the observed association of the *TCF7L2* rs7903146 genotypes with T2D under recessive and dominant models is stronger after adjusting for the effects of age at onset of obesity, male sex, and in the presence of the following confounding factors: dyslipidemia and lower adiponectin level (< 50th percentile). Results from the logistic regression models are presented as odds ratios (OR) with 95% confidence intervals (CI). In all analyses, a *p*-value < 0.05 was considered statistically significant.

Results

Study population

The characteristics of the study population are presented in Table I. Altogether, 129 obese subjects with T2D and 345 obese subjects without T2D, 43.6 ±9.8 years of age, participated in the study. No statistically significant difference in BMI between diabetic and nondiabetic participants was found (Table I). The majority of participants (49.8%) had a BMI above 40 kg/m^2, 25.5% had a BMI in the range of 35–39.9 kg/m^2, and 24.7% of subjects had class I obesity (BMI: 30.0–34.9 kg/m^2). In patients with T2D, 45% of patients received hypoglycemic drugs, 31% of patients received insulin, while 24% of patients received insulin and hypoglycemic drugs. Despite treatment diabetics had higher fasting glucose, insulin and HbA_{1c} concentrations, and higher HOMA-IR, than nondiabetics. In 38% of the obese subjects without T2D, fasting insulin levels exceeded 15 µIU/ml, suggesting hyperinsulinemia [25]. At the same time,

Table I. Clinical and biochemical characteristics of the study participants (*N* = 474)

Variables	Diabetics (*n* = 129)	Nondiabetics (*n* = 345)	*P*-value*
Age [years]	47.0 (39.0–53.0)	44.0 (38.0–55.0)	0.590
Sex, *n* (%):			
Male	59 (46.0)	89 (26.0)	< 0.001
Female	70 (54.0)	256 (74.0)	
Weight [kg]	110.0 (99.5–130.0)	114.0 (98.4–128.0)	0.743
Height [cm]	168.0 (161.0–178.0)	168.0 (163.0–174.0)	0.523
BMI [kg/m^2]	38.7 (34.9–43.9)	40.2 (35.1–44.5)	0.390
BMI [kg/m^2], *n* (%):			
30–34.9	34 (26.0)	83 (24.0)	
35–39.9	40 (31.0)	81 (23.5)	0.128
≥ 40	55 (43.0)	181 (52.5)	
Age at onset of obesity [years]	30.5 (20.0–43.0)	11.0 (7.0–24.0)	< 0.001
Age at onset of obesity, *n* (%):			
≥ 20 years	98 (76.0)	131 (38.0)	< 0.001
< 20 years	31 (24.0)	214 (62.0)	
Fasting glucose [mmol/l]	7.2 (6.2–9.6)	5.1 (4.7–5.4)	< 0.001
Insulin [µIU/ml]	18.7 (13.6–25.9)	15.7 (11.1–24.2)	< 0.001
HOMA-IR	6.3 (4.2–9.0)	3.5 (2.4–5.7)	< 0.001
HbA_{1c} (%)	7.1 (6.4–8.7)	5.7 (5.4–6.1)	< 0.001
Total cholesterol [mmol/l]	4.6 (3.9–5.4)	4.9 (4.4–5.5)	0.049
LDL cholesterol [mmol/l]	2.6 (1.8–3.3)	3.1 (2.5–3.6)	< 0.001
HDL cholesterol [mmol/l]	1.0 (0.9–1.2)	1.2 (1.0–1.3)	< 0.001
Triglycerides [mmol/l]	1.8 (1.4–2.6)	1.5 (1.1–2.0)	< 0.001
Dyslipidemia, *n* (%):			
Yes	94 (73.0)	153 (44.0)	< 0.001
No	35 (27.0)	192 (56.0)	
Adiponectin [µg/ml]	3.6 (2.6–4.7)	5.6 (3.8–9.3)	< 0.001

*Values in the table are reported as n (%), or median (interquartile range). *Pearson's χ^2 and Mann-Whitney tests were performed where appropriate. BMI – body mass index, HbA_{1c} – glycosylated hemoglobin, HDL cholesterol – high-density lipoprotein cholesterol, LDL cholesterol – low-density lipoprotein cholesterol, HOMA-IR – homeostasis model assessment of insulin resistance.*

insulin resistance was diagnosed in 58% of obese individuals without T2D. In obese subjects with T2D, we observed a high prevalence of dyslipidemia, 73%, as opposed to 44% of the dyslipidemic participants in the nondiabetic group ($p < 0.001$). Patients with dyslipidemia who were taking hypolipidemic drugs received statins (74%), fibrates (16%) or statins plus fibrates (10%).

Association of the *TCF7L2* rs7903146 variants with type 2 diabetes

Distribution of the *TCF7L2* rs7903146 genotypes (Table II) did not deviate from Hardy-Weinberg equilibrium in either diabetics ($p = 0.550$, $\chi^2 = 0.357$, d$f = 1$) or nondiabetics ($p = 0.738$, $\chi^2 = 0.112$, d$f = 1$).

We found a significant difference in *TCF7L2* rs7903146 genotype distribution between diabetics and nondiabetics ($p = 0.014$, $\chi^2 = 8.51$), and an association between T2D and the *TCF7L2* rs7903146 T allele with an odds ratio of 1.59 (95% CI: 1.14–2.21) in the additive genetic model (Table II). Moreover, analysis performed under the recessive genetic model, with two copies of the T allele being required for increased risk, revealed a significant association between the rs7903146 TT genotype and T2D (OR = 2.62, $p = 0.016$). The dominant model (combining TT and CT into one category) conferred an odds ratio of 1.61 (95% CI: 1.07–2.42). In addition, carriers of the TT genotype had a significantly higher risk of T2D compared with the CC homozygotes as the reference genotype, with an OR of 3.02 (95% CI: 1.31–6.93). The logistic regression yielded a significant odds ratio suggesting that the risk allele confers a significant risk for developing T2D in the obese. The genotypic OR from logistic regression under recessive and dominant models also showed a significant association between TT and CT genotypes and T2D (Table II).

Association between male sex, age at onset of obesity, adiponectin levels and type 2 diabetes

Among the diabetic participants, 46% were male, as opposed to 26% of the male participants in the nondiabetic group ($p < 0.001$, Table I), and the obese males studied, compared to females, had a 2.42-fold higher risk (OR = 2.42; 95% CI: 1.59–3.70) of having T2D (Table III). The age at onset of obesity was significantly different between the groups with and without T2D (30.5 years vs. 11.0 years, $p < 0.001$). Development of obesity at older age was associated with increased prevalence of diabetes (76%, Table I). Subjects who were obese at age ≥ 20 years had a 5.16 higher odds (95% CI: 3.26–8.17; Table III) of having T2D than those with the onset of obesity before the age of 20 years. In further statistical analyses no

Table II. Genotype distribution and allele frequency of the *TCF7L2* rs7903146 polymorphism in diabetic and nondiabetic subjects

Variable	CC	CT	TT	Total
Genotype distribution:				
Diabetics	67 (52%)	50 (39%)	12 (9%)	**129**
Nondiabetic subjects	219 (63%)	113 (33%)	13 (4%)	345
Total	286	163	25	474
Allelic distribution	**C**	**T**		**Total**
Diabetics	184 (71%)	74 (29%)		258
Nondiabetic subjects	551 (80%)	139 (20%)		690
Total	735	213		948
Genetic models and statistics				
Genetic model	**Unadjusted OR (95% CI)**	χ^2	**D*f***	***P*-value**
Association test (genotypes)	1	8.51	2	0.014
Homozygote (TT vs. CC)	3.02 (1.31–6.93)	7.33	1	0.007
Heterozygote (CT vs. CC)	1.45 (0.94–2.23)	2.83	1	0.092
Recessive TT vs. (CT + CC)	2.62 (1.16–5.90)	5.76	1	0.016
Dominant (TT + CT) vs. CC	1.61 (1.07–2.42)	5.22	1	0.022
Additive model (alleles T vs. C)	1.59 (1.14–2.21)	7.86	1	0.005

OR – odds ratio, CI – 95% confidence interval for the odds ratio, Df – degrees of freedom.

Table III. Crude and adjusted estimations for type 2 diabetes

Variable	Unadjusted OR (95% CI)	*P*-value[a]	Adjusted OR (95% CI)	*P*-value[b]
Age at onset of obesity ≥ 20 years	5.16 (3.26–8.17)	< 0.001	4.94 (2.70–9.06)	< 0.001
Male sex	2.42 (1.59–3.70)	< 0.001	2.05 (1.27–3.31)	0.0031
Serum adiponectin < 50th percentile	5.93 (3.81–9.21)	< 0.001	4.81 (2.48–9.31)	< 0.001
TCF7L2 TT genotype (recessive model)	2.62 (1.16–5.90)	0.016	3.54 (1.33–9.43)	0.011
TCF7L2 TT + CT (dominant model)	1.61 (1.07–2.42)	0.022	1.49 (0.91–2.44)	0.106

OR – odds ratio, CI – confidence interval. [a]Crude logistic regression model. [b]Adjusted for TCF7L2 rs7903146 genotypes (recessive and dominant models), age at onset of obesity above 20 years, male sex, dyslipidemia, and serum total adiponectin level < 50th percentile (the cut-off value of adiponectin concentration < 50th percentile was 4.87 µg/ml).

significant difference in *TCF7L2* rs7903146 genotype distribution was observed between patients with an adult obesity onset (≥ 20 years) and patients with an early onset of obesity (< 20 years) ($p = 0.346$, $\chi^2 = 2.12$).

Serum total adiponectin levels were significantly lower in diabetic subjects ($n = 129$) than in nondiabetic individuals ($n = 345$), (3.6 vs. 5.6 µg/ml, $p < 0.001$; Table I), and occurrence of low serum total adiponectin (< 50th percentile) was associated with almost 6 times higher chance of having T2D (OR = 5.93, 95% CI: 3.81–9.21; Table III). Having in mind that *TCF7L2* rs7903146 polymorphism was significantly related to T2D risk (Table II), we carried out further analyses and found that serum total adiponectin levels did not differ significantly according to *TCF7L2* rs7903146 genotypes in the whole sample ($p = 0.3814$; Kruskal-Wallis test), in diabetics ($p = 0.1252$; Kruskal-Wallis test), or in nondiabetics ($p = 0.2214$; Kruskal-Wallis test). Moreover, serum total adiponectin levels did not differ between patients with an adult obesity onset (≥ 20 years) and patients with an early onset of obesity (< 20 years) ($p = 0.646$; Mann-Whitney test).

Multivariable logistic regression model

Variables that were significantly associated with T2D susceptibility in univariable models were then combined in the multivariable logistic regression model. The results presented in Table III showed that all these variables (i.e. age at onset of obesity over 20 years, serum total adiponectin concentration < 50th percentile, male sex, and *TCF7L2* rs7903146 TT genotype) were still significantly associated with T2D; and age at onset of obesity above 20 years was the strongest risk factor, the second one was serum total adiponectin concentration < 50th percentile, and the third one was *TCF7L2* TT genotype.

Adiponectin levels and anthropometric/biochemical characteristics

Given the association between low serum total adiponectin and T2D, correlations between adiponectin and anthropometric/biochemical characteristics were performed (Table IV). Among nondiabetics, serum adiponectin was inversely correlated with weight, BMI, fasting glucose, insulin, and HOMA-IR, as presented in Table IV.

Table IV. Correlations between adiponectin concentrations and metabolic and anthropometric variables in obese individuals with and without type 2 diabetes

Parameter	Diabetics		Nondiabetics	
	Adiponectin		Adiponectin	
	R	*P*-value	*R*	*P*-value
Weight [kg]	0.005	0.964	–0.565	< 0.001
BMI [kg/m^2]	0.138	0.234	–0.495	< 0.001
Glucose [mg/dl]	–0.147	0.205	–0.189	0.009
Insulin [µIU/ml]	–0.166	0.153	–0.497	< 0.001
HOMA-IR	–0.211	0.067	–0.490	< 0.001
HbA_{1c} (%)	–0.217	0.065	–0.114	0.188

R – Spearman's correlation coefficient. BMI – body mass index, HbA_{1c} – glycosylated hemoglobin, HOMA-IR – homeostasis model assessment of insulin resistance.

In contrast, in diabetics no significant relationships between studied anthropometric and biochemical parameters and total adiponectin were identified, indicating that other factors associated with T2D but not obesity *per se* disturb these relationships.

Discussion

TCF7L2 is among the most common associated loci reported in genome-wide appraisals of type 2 diabetes, and the *TCF7L2* rs7903146 SNP particularly constitutes a risk factor for type 2 diabetes in many separate studies [14–17, 26]. However, the mechanism underlying an increased risk of T2D in the presence of a specific allele still remains unclear. Previous studies suggest that the T allele of *TCF7L2* rs7903146 polymorphism impairs β-cell function and insulin secretion [27, 28]. In our study, an association was found between the rs7903146 polymorphism in the *TCF7L2* gene and type 2 diabetes risk in obese adults. The odds ratio for the T allele was similar to that reported in previous studies which employed both obese and normal-weight participants [14–17, 26]. As revealed by statistical analysis, obese carriers of the *TCF7L2* rs7903146 TT genotype had 2.62 times higher odds of T2D compared to those with other genotypes, and the latter relationship appeared even stronger after adjusting for the effects of age at onset of obesity, male sex, dyslipidemia and lower serum total adiponectin. Our observation is consistent with a study by Yan *et al.* [29] which suggests that the risk of developing impaired fasting glucose that is associated with the TT genotype is stronger in obese than in nonobese Caucasians. It indicates that in the case of obesity, which itself is a strong risk factor for T2D, occurrence of certain genetic variants can further increase the risk of this disease.

In fact, we demonstrated that the risk of developing T2D which is associated with *TCF7L2* rs7903146 polymorphism is substantially greater in the context of other factors such as male sex and the age at onset of obesity. Indeed, among 245 participants who were obese before the age of 20, only 13% developed T2D, while among subjects who developed obesity at an older age, 43% had T2D. It can be hypothesized that individuals who have early onset of obesity have a different degree of abnormalities in glucose homeostasis and a lower probability of developing T2D than those who became obese as adults. It may be related to age-associated changes in adipose tissue distribution [30] and variation in responses to metabolic dysregulation associated with an early onset of obesity. The greater risk of T2D in males versus females, which we observed, underlines this suggestion, since men typically have higher abdominal adipose tissue accumulation than women, and gender differences in insulin resistance were also noted [31].

Adiponectin has been reported to be lower in subjects with T2D [32, 33]. The present study confirms and extends these associations by demonstrating that adiponectin levels are significantly lower in obese individuals with T2D than in obese nondiabetic individuals matched for age and BMI. Among obese nondiabetics, significant inverse correlations were observed between serum adiponectin and weight, BMI, fasting glucose, insulin, and HOMA-IR, while no significant correlations were observed in obese diabetics. Adiponectin is secreted by adipose tissue, increases insulin sensitivity, and improves glucose homeostasis [11], but the present study confirms that occurrence of T2D and obesity impairs the physiological role of adiponectin. Recently, it was suggested that a fall in adiponectin concentrations in T2D may be associated with other factors such as decreased adiponectin serum half-life. Significantly lower serum total adiponectin in nonobese subjects with T2D than in matched healthy controls was reported [34], and it was not accompanied by decreased adiponectin production in adipocytes. The adiponectin half-life was found to be about 20% lower in T2D patients than in healthy controls, and this difference may be biologically relevant, since adiponectin is an abundant serum protein.

Obesity is strongly associated with a high prevalence of insulin resistance. The situation in which the hyperinsulinemia that occurs in obesity is able to compensate for insulin resistance is often accompanied by normal glucose levels [35, 36] and can last for many years, and the dysregulation of adiponectin secretion and action probably plays a fundamental role in the development of type 2 diabetes in obese subjects. Moreover, our results show that about 70% of patients with type 2 diabetes who had lower adiponectin concentrations than nondiabetics met dyslipidemia criteria, which is in accordance with literature data suggesting that adiponectin has a direct effect on the regulation of lipid metabolism [11] and is negatively correlated with the visceral adiposity index (VAI) in obese females [37].

In the studied group, 73% of the diabetics and 44% of the nondiabetics had dyslipidemia, and these patients receive lipid-lowering drugs, which may affect insulin sensitivity and pancreatic β-cell function, and enhance PPAR-γ activation and adipokine secretion [38, 39]. However, despite the fact that the majority of diabetics had dyslipidemia and received hypolipemic treatment, which may enhance adiponectin production, serum adiponectin concentrations in diabetics were significantly lower than in nondiabetics. In the

meta-analysis by Chrusciel *et al.* no significant effect of statin therapy on adiponectin concentration was found in diabetics [39]. The reduced adiponectin half-life in T2D reported by Andersson *et al.* [34] may be responsible for observed different relations between adiponectin and metabolic parameters as well as different effect of statins on adiponectin concentrations in diabetics compared to nondiabetics.

The study has several limitations that should be noted. The prevalence of insulin resistance was measured by the HOMA algorithm in the fasting state rather than by euglycaemic clamp-assessed insulin sensitivity [40]. In addition, the genetic clock of T2D may start ticking long before the onset of overt diabetic hyperglycemia and its impact may depend on other genetic and environmental factors, which in turn can limit our ability to recognize a causal relationship between genes and T2D in obesity. Finally, lifestyle, diet and medications can to some extent modify the observed effect.

In conclusion, our study highlights the importance of the relationship between adult obesity, the *TCF7L2* polymorphism and the prevalence of T2D, and confirms lower adiponectin levels in obese diabetics in comparison with obese nondiabetics. However, the impact of an early age at obesity onset on the likelihood of development and severity of T2D needs to be clarified in future studies.

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Conflict of interest

The authors declare no conflict of interest.

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