Diabetes & Metabolic Syndrome: Clinical Research & Reviews 8 (2014) 133–137

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Original Article

Studying progression from glucose intolerance to type 2 diabetes in obese children

Irina A. Dubinina [a], Dimitry A. Chistiakov [b,*], Irina A. Eremina [a], Alexei N. Brovkin [c], Lyubov I. Zilberman [a], Alexei G. Nikitin [c], Tamara L. Kuraeva [a], Valery V. Nosikov [c], Valentina A. Peterkova [a], Ivan I. Dedov [a]

[a] Department of Pediatrics, Endocrinology Research Center, Moscow, Russia
[b] Department of Medical Bionanotechnology, Pirogov Russian State Medical University, Moscow, Russia
[c] Federal Research Clinical Center of Federal Medical and Biological Agency of Russia, Moscow, Russia

ARTICLE INFO

Keywords:
Obesity
Glucose tolerance
Insulin resistance
Insulin sensitivity
PPARG

ABSTRACT

Aim: Identification of metabolic and genetic factors capable to mediate progression from normal glucose tolerance (NGT) through impaired glucose tolerance (IGT) to type 2 diabetes (T2D) in childhood obesity.
Patients and methods: Three groups of obese children with NGT ($n = 54$), IGT ($n = 35$), and T2D ($n = 62$) were evaluated. A control group of non-obese normal children ($n = 210$) was also studied. In obese patients, an oral glucose tolerance test (OGTT) was performed. Insulin resistance (IR) was assessed using HOMA-IR index. Insulin sensitivity (IS) was assessed according to the Matsuda formula. Genomic DNA from obese and control children was genotyped for genetic variants of PPARG, ADIPOQ, ADIPOR1, FTO, TCF7L2, and KCNJ11 using a real-time PCR strategy. The unpaired Student's *t*-test and Kruskal–Wallis one-way test were used to compare quantitative data in two and more groups. To assess the extent to which the various genetic variants were associated with pathology, ORs (odds ratios) and 95% CI (confidence interval) were estimated.
Results: In T2D children, HOMA-IR value (7.5 ± 3.1) was significantly ($P < 0.001$) higher than that in IGT (4.21 ± 2.25) and NGT (4.1 ± 2.4) subjects. The Matsuda IS index was significantly increased in normoglycemic patients compared to IGT individuals (2.8 ± 1.75 vs. 2.33 ± 1.2, $P < 0.05$). The Pro12Ala polymorphism of PPARG was significantly associated with obesity (OR = 1.74, 95% CI = 1.19–2.55, $P = 0.004$) and T2D in obesity (OR = 2.01, 95% CI = 1.24–3.26, $P = 0.004$).
Conclusion: IR is a major risk factor that mediates progression from NGT to clinical T2D in Russian obese children. This progression may be genetically influenced by the Pro12Ala variant of PPARG.

1. Introduction

Within the last three decades, significant increase in childhood overweight/obesity was observed worldwide. In many industrialized countries including USA, UK, Japan, Germany, France, Australia, and Canada, the prevalence of pediatric overweight/obesity doubled or even trebled since late 1970s [1]. In Russia, according to data of the Russian Longitudinal Monitoring Survey, the prevalence of overweight among older children and adolescents dropped from 15.6% to 9.0% since 1992 till 1998, a period of serious economic stress [2]. However, in 2000s, due to the global spread of fast food chains and low physical activity, the percentage of overweight/obese children in Russia stably increases, particularly in urban areas.

Pediatric obesity is considered as a serious risk factor for several metabolic complications including insulin resistance (IR), glucose intolerance, and type 2 diabetes (T2D). IR is a common feature of childhood obesity and represents an important link between obesity and other metabolic complications [3]. In obese children, the adipose tissue plays a central role in the pathophysiology of IR by releasing several adipokines and metabolites. In adiposity, adipocytes release increased levels of non-esterified (free) fatty acids that suppress glucose metabolism via impaired intracellular insulin signaling [4]. A spectrum of adipokines released by the

* Corresponding author at: Department of Medical Nanobiotechnology, Pirogov Russian State Medical University, Ulitsa Ostrovityanova 1, 117997 Moscow, Russia. Tel.: +7 495 434 13 01; fax: +7 495 434 14 22.
E-mail address: dimitry.chistiakov@lycos.com (D.A. Chistiakov).

http://dx.doi.org/10.1016/j.dsx.2014.07.002
1871-4021/

adipose tissue in obese children is biased toward increased production of leptin and resistin that leads to the activation of proinflammatory response, proatherogenic changes in lipid profile, impaired balance between energy intake and expenditure, and altered eating behavior [5]. In contrast, production of adiponectin, a cytokine, which possesses insulin-sensitizing effect associated with anti-atherogenetic properties, is down-regulated in adiposity [6]. In obesity, adipocytes start to release significantly more proinflammatory cytokines such as tumor necrosis factor-α and interleukin-6 that induce obesity-related inflammation and further contribute to IR progression [7].

T2D is a disease in which IR is necessarily accompanied with dysfunction of insulin-producing β-cells [8]. IR with compensatory hyperinsulinemia is thought to be the earliest impairment that precedes the next stage associated with alterations in secretion of insulin and finally leads to chronic hyperglycemia and clinically manifested diabetes [9,10]. Several studies have shown that insulin sensitivity in obese youth with T2D may be significantly (by 2–3 fold) decreased compared to non-diabetic obese adolescents matched by age, body mass index, and fat deposition [11,12]. In obese children, induction of impaired oral glucose tolerance (IGT) may be associated with IR and lower 1st phase insulin response while 2nd phase of insulin response remains preserved [13,14]. In obese diabetic children, β-cell function is fully impaired, with altered 1st and 2nd phase of insulin response [14].

Childhood obesity is a complex multifactorial disease resulting from interaction of susceptibility genetic variants with an obesogenic environment. To date, according to the results of multiple genome-wide association studies (GWAS) and meta-analyses over 30 loci conferring susceptibility to adult obesity and obesity-related traits have been found [15]. Several GWAS and meta-analyses performed in pediatric populations so far revealed a total of 20 loci associated with increased BMI, with the strongest impact of the fat mass and obesity associated (FTO) gene [16]. Interestingly, a total of 7 loci that increase obesity risk and BMI in adults have been shown to be significantly associated with various diabetes-related traits in a French population [17]. Obesity-associated genetic variants have been found to contribute to T2D risk through increased IR and enhanced β-cell function as a compensatory response against IR [17].

In this study, we studied three groups of obese children (obesity only, obesity + IGT, and obesity + T2D) in order to find clinical, metabolic, and genetic factors involved in progression from normal to impaired glucose tolerance and then to diabetes in a Russian population.

2. Experimental procedures

2.1. Patients

The subjects were recruited by the Moscow Endocrinology Research Center. All patients studied were the residents of Moscow and Moscow region. The study protocol was approved by the Review Board of the Endocrinology Research Center, and all participants (or their parents) provided written informed consent. The study was performed according to the WMA Declaration of Helsinki (2008) that considers ethical principles for medical research involving human subjects. Permission to conduct the study was provided by the local Ethical Committee of the Endocrinology Research Center. A total of 151 obese and 210 non-obese normal children were enrolled. Body Mass Index (BMI) was calculated for each subject according to the normal values typical for each age and gender and was presented as standard deviation score (SDS). SDS-BMI was calculated as $X - X'7SD$ where X is the logarithm of patients' BMI, X' is the logarithm of BMI that is average for a given age and gender, and SD is the logarithm of standard deviation of BMI for a given age and gender. Overweight was defined as SDS-BMI of higher 1.5 and less or equal 2.0; obesity was defined as SDS-BMI of higher 2.0 [18]. Among obese children, 35 had impaired glucose tolerance and 62 were affected with T2D. The remaining 54 obese children had normal glucose tolerance (NGT).

Type 2 diabetes was diagnosed using oral glucose tolerance test (OGTT) and measuring glycated hemoglobin ($HbA1_C$). An $HbA1_C$ level of 6.5% or higher on two separate tests indicated diabetes. A result of 5.7–6.4% was considered as IGT. According to OGTT, the presence of plasma glucose at level of 11.1 mmol/L and higher 2 h later after glucose intake corresponded to diabetes, whereas glucose levels between 7.8 and 11.1 mmol/L were considered as presence of IGT. Fasting glucose level of 6.9 mmol/L and higher indicated diabetes, while fasting glucose levels ranged between 5.6 and 6.9 mmol/L corresponded to IGT. Clinical characteristics of three groups of obese patients are presented in Table 1.

2.2. Biochemical measurements

HbA1c was measured using an ion-exchange high performance liquid chromatography (normal reference range: 4.1–6.4%). Blood serum glucose was measured using the Glucose Assay Kit (BioVision, Mountain View, CA, USA). Plasma insulin levels were determined by means of an enzymatic immunoassay (Insulin Assay Kit, CisBio Bioassays, Bedford, MA, USA). Fasting cholesterol, high density lipoproteins (HDL), cholesterol, and triglycerides were

Table 1
Clinical characteristics of obese patients with normal glucose tolerance, impaired glucose tolerance, and type 2 diabetes.

Characteristic	Obesity + NGT ($n = 54$)	Obesity + IGT ($n = 35$)	Obesity + T2D ($n = 62$)
M/F, n	29/25	22/14	31/31
Age, years	11.7 ± 3.2	12.5 ± 2.2	13.1 ± 2.2
SDS-BMI	3.4 ± 0.57	2.94 ± 0.68	3.11 ± 0.5
Cholesterol, mmol/L	4.5 ± 0.69	4.43 ± 0.88	4.45 ± 0.78
Triglycerids, mmol/L	1.27 ± 0.67	1.33 ± 0.63	1.47 ± 0.57
LDL, mmol/L	3.57 ± 0.82	2.74 ± 0.78	2.72 ± 0.65
HDL, mmol/L	0.97 ± 0.28	1.16 ± 0.32	1.07 ± 0.24
HOMA-IR	4.1 ± 2.4[a]	4.21 ± 2.25[b]	7.5 ± 3.1
WBISI (Matsuda)	2.8 ± 1.75[c]	2.33 ± 1.2	2.4 ± 1.6

Results are shown in percentages as mean ± S.D. The variables were compared using the unpaired Student's t-test. SDS-BMI, standard deviation score of body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; HOMA-IR, homeostasis assessment model of insulin resistance; WBISI, whole body insulin sensitivity index; NGT, normal glucose tolerance; IGT, impaired glucose tolerance; T2D, type 2 diabetes.

[a] HOMA-IR: obesity + NGT vs. obesity + T2D, $P < 0.001$.

[b] HOMA-IR: obesity + IGT vs. obesity + T2D, $P < 0.001$.

[c] WBISI (Matsuda): obesity + NGT vs. obesity + IGT, $P < 0.05$.