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β -Cell autoimmunity in overweight non-diabetic youth: any implications?

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Abstract

Background and objective—The presence of β -cell antibodies is associated with a high risk of type 1 diabetes. With increasing rates of obesity, the distinction between obese T1DM and T2DM has become difficult. Moreover, increasing body mass index (BMI) in at-risk children has been proposed not only as a possible contributor to T1DM by increasing insulin resistance, but also as exerting an effect via the immunomodulatory properties of certain adipokines. This study aimed to determine prevalence of β -cell autoantibodies (AA) in overweight non-diabetic children and assess insulin sensitivity and secretion derived from an oral glucose tolerance test (OGTT) in those with vs. without β -cell AA.

Research design and methods—A total of 357 overweight (BMI > 85%) youths underwent OGTTs, dual energy X-ray absorptiometry (DEXA) and measurement of GAD65 and IA-2 AA according to the NIDDK harmonization assay. Using the same methodology, AA were measured in 90 normal weight, non-diabetic individuals.

Results—About 1.9% of overweight and 4.4% of control normal weight children had evidence of β -cell autoimmunity, with GAD65 AA detected in all subjects but none with IA-2. Youth with positive vs. those with negative AA had higher leptin/adiponectin ratio, glucose at 60 min and C-peptide at 90 min.

Conclusions—These findings suggest that the prevalence of β -cell AA in overweight youth may be similar to that in non-overweight children. Further studies using standardized methods are required.

Keywords

autoimmunity; β -cell antibodies; children; obesity

The presence of β -cell autoantibodies (AA) is associated with a high risk of developing T1DM and they are present in the majority of children with this disease. **Glutamic acid decarboxylase 65** (GAD65) and IA-2 AA are reported to be present in 0.9 and 0.5%, respectively, of school children in the USA (1) with data from Europe showing a range between 0.5–2.4% for GAD65 and 0.15–2.7% for IA-2 AA (2–4). On the other hand, it has been suggested that changes in adipokines may confer an elevated risk for T2DM and cardiovascular disease (CVD) (5, 6). With increasing rates of youth obesity, the distinction between T1DM and T2DM has become difficult. Moreover, increasing body mass index (BMI) in at-risk children has been proposed not only as a possible contributor to T1DM by increasing insulin resistance (7), but also as exerting an effect via the immunomodulatory properties of certain adipokines (6–8). Leptin administration at pharmacological doses to prediabetic NOD female mice has been described to promote early inflammatory infiltrates of pancreatic islets, increases interferon gamma production by T cells, and early development of autoimmune diabetes (9). The possible effect of glucotoxicity secondary to increased blood glucose levels in those with insulin resistance can expose more antigens and accelerate the destruction of the β -cell. One study in adult individuals showed that increased BMI was related to increased concentrations of GAD65 AA, which would be consistent with a possible contribution of obesity in the presence of autoimmunity (10). This was most prominent not only in older women but also in middle-aged men. To our knowledge, however, there are no data regarding the prevalence of β -cell antibodies in obese, otherwise healthy, children. The purpose of this study was to (i) determine the prevalence of β -cell AA in overweight non-diabetic children and (ii) assess insulin sensitivity and secretion derived from an oral glucose tolerance test (OGTT) in those with vs. without β -cell AA.

Research design and methods

Overweight (BMI $\geq$ 85th percentile for age and gender) but otherwise healthy youth underwent assessment of glucose tolerance at the Pediatric Clinical and Translational Research Center at the Children's Hospital of Pittsburgh. Exclusion criteria included prior diagnoses of impaired glucose regulation, diabetes or any chronic illness (such as cystic fibrosis or Cushing's), syndromes associated with obesity and the use of medications that influence glucose, lipid metabolism and blood pressure. Study participants were recruited through several sources: Weight Management and Wellness Center, Pediatric Endocrinology Clinic, Primary Care Center (all part of Children's Hospital of Pittsburgh), National Youth Sports Program (federally funded project) and newspaper advertisements in the greater Pittsburgh area. The investigation was approved by the Institutional Review Board of the University of Pittsburgh. Parental informed consent and child assent were obtained. All participants underwent a physical examination with height and weight measurements, BMI calculation and assessment of pubertal development by Tanner criteria. Body composition was assessed by dual-energy X-ray absorptiometry (Lunar, Madison, WI).

Participants underwent an OGTT (1.75 g/kg, maximum 75 g). Blood samples were obtained at 0, 15, 30, 60, 90 and 120 min for determination of glucose, insulin and C-peptide. Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) were defined according to American Diabetes Association (ADA) criteria (11). Insulin sensitivity was calculated using the whole-body insulin sensitivity index (WBISI), homeostasis model assessment of insulin resistance (HOMA_{IR}) and the fasting glucose (mg/dL)/insulin (μ U/mL) ratio. Insulin secretion was estimated using the insulinogenic index. The glucose disposition index (GDI) was used to adjust insulin secretion for the degree of insulin resistance. All these methods were described by us previously (12). Glucose and insulin area under the curve (AUC) during the OGTT were calculated by the trapezoidal rule. The same indices were evaluated using C-peptide.

Ninety non-overweight youths recruited from newspaper advertisements in the greater Pittsburgh area underwent a physical examination with height and weight measurements, BMI calculation and measurements of β -cell antibodies to serve as controls.

Biochemical measurements

Plasma glucose, insulin and C-peptide were measured as described previously (12). Plasma lipids were measured in the Nutrition Laboratory of the University of Pittsburgh and certified by the National Heart, Lung and Blood Institute standardization program. Adiponectin and leptin were measured using a commercially available radioimmunoassay kit (Linco Research, St. Louis, MO). They were all measured in fasting conditions. Proinsulin and high sensitivity C-reactive protein (hs-CRP) were measured at the Esoterix Endocrinology Laboratory (Calabasas Hills, CA). For hs-CRP, values >10 mg/L were excluded as these may be a sign of occult infection or other systemic inflammatory process (13).

Antibody testing

Detection of GAD65 and IA-2 AA was performed at the Northwest Lipid Metabolism and Diabetes Research Laboratories, University of Washington (Seattle, WA) using a standardized assay protocol and a common serum calibrator developed by the NIDDK-sponsored standardization group. Results are expressed as DKU/mL. Based on analysis of 550 samples, the cutoff values for positivity/negativity are 33 DKU/mL for GAD65 and 5 DKU/mL for IA-2. The calculated assay specificity is 97 for GAD65 and 99 for IA-2, whereas the sensitivity is 76 and 64, respectively.

Statistical analysis

Data are presented as mean $\pm$ standard deviation for normally distributed continuous variables, median (interquartile range) for non-normal continuous variables and n (percentage) for categorical variables. Independent *t*-tests were used to compare normally distributed continuous subject characteristics as well as Pearson's chi-square test to compare proportions. Non-parametric analyses were used when appropriate. Two-tailed *p* values of 0.05 were considered statistically significant.

Results

Of the 357 participants, 188 were Caucasians, 146 were African Americans and 23 were Biracial; 143 were males and 214 were females with a mean age of 12.7 ± 2.6 yr, mean BMI percentile of 98.2 ± 1.8 and mean percent body fat of $42.2 \pm 7.2\%$.

Seven (1.9%) children had evidence of autoimmunity, all of them positive for GAD65, none for IA-2 AA. There was no relationship between IFG or IGT status and presence of antibodies. None of the children with IFG ($n = 7$) or IGT ($n = 13$) had positive AA.

Using the same methodology, GAD65 and IA-2 AA were measured in 90 normal weight, non-diabetic individuals (57% Caucasians, 52% males, with a mean age of 11.0 ± 2.2 yr and mean BMI percentile of 56.3 ± 18.9). Four subjects were positive for GAD65 AA but none for IA-2 giving a prevalence of 4.4% which was not significantly different when comparing the prevalence of β -cell AA in those who were overweight ($p = 0.17$). There were no statistically significant differences in most characteristics between those with β -cell AA compared to those with none (Table 1). However, those with at least one antibody had a higher leptin/adiponectin ratio, glucose level at 60 min and C-peptide levels at 90 min.

Conclusions

Our findings in overweight non-diabetic children demonstrate that (i) the prevalence of β -cell antibodies was similar to that of non-overweight controls from the same population; (ii) the presence of β -cell antibodies was associated with higher leptin/adiponectin ratio, without significant differences in sex, race, age, BMI percentile or percent body fat; and (iii) those with β -cell antibodies had significantly higher glucose at 60 min and higher C-peptide level at 90 min with no differences in OGTT-derived indices of insulin sensitivity or insulin secretion.

Reports evaluating the prevalence of β -cell AA in non-diabetic school children around the world have fluctuated between 0.5–2.4% for GAD65 AA and between 0.15–2.7% for IA-2AA (1–4). Different methodologies and cut points were used in the different studies, making comparisons hard. In the USA, data from Washington State showed a prevalence of 0.9 and 0.5% of GAD65 and IA-2 AA, respectively, in school children (1). No reference to their BMI appears in any of these publications. There is no information, to our knowledge, on prevalence of β -cell AA in healthy overweight children. Based on our results, with the current NIDDK standardized method, the proportion of overweight non-diabetic children shown to have evidence of β -cell AA seems to be similar than the one reported in school-age children in the USA. When comparing the prevalence of AA in this group of overweight children to that of non-overweight controls from the general population in our area, using the same methodology developed by the NIDDK-sponsored standardization group, no difference in prevalence was seen. Clearly, more studies are needed to be able to draw a conclusion using standardized methods, larger number of controls and careful phenotyping of BMI and glucose tolerance.

Similar to what has been reported previously, GAD65 seems to be more frequently present than IA-2 AA. This is probably not surprising as IA-2 AA have been observed to be the last appearing AA in most cases prior to the development of diabetes (14).

Is there any significance to these findings? Even if few children had evidence of autoimmunity, the presence of antibodies appeared to be associated with changes in the leptin/adiponectin ratio. Whether or not this reflects an immunomodulatory property of adipokines remains to be elucidated.

Those with β -cell antibodies also had significantly higher glucose at 60 min and higher C-peptide level at 90 min despite normal OGTTs. Whether these differences reflect very early and subtle abnormalities in glucose homeostasis cannot be discerned at the moment without prospective longitudinal observations. Furthermore, even though the insulinogenic index was 60% lower in obese youths with antibodies compared with those without antibodies, the difference was not statistically significant. This is most likely due to inadequate power. With such low prevalence rates of AA positivity in obese youths, large numbers of obese children need to be tested for pancreatic autoantibodies in order to have sufficient numbers of positive subjects which would provide adequate power for the analysis of metabolic outcomes. A pilot study ($n = 756$) in Swedish non-diabetic adults above 30 yr of age participating in the WHO MONICA study showed that GAD65 AA correlated with insulin resistance (measured by HOMA) within a group defined as insulin sensitive, suggesting that insulin resistance could serve as an initiator or a progression factor of the autoimmune process in subjects predisposed to autoimmunity (15). Moreover, it was found that high levels of GAD65 AA were correlated with high BMI, independent of OGTT results, implying a correlation between the metabolic syndrome and T1DM risk markers. As the authors argue, obesity was associated with insulin resistance and perhaps resulting in increase in beta cell activity. Therefore, GAD65 AA in obese subjects may mark subtle

dysfunction and insulin resistance in some of their subjects (10). Our results did not show any differences in insulin sensitivity most likely due to very few children with positive antibodies. However, the observed glucose and C-peptide differences during the OGTT may be an early indication of metabolic differences consequent to the presence of autoantibodies.

In summary, healthy overweight children seem to have similar prevalence of β -cell AA than non-overweight children when using the same standardized assay. With the recent advent of the NIDDK harmonization act for pancreatic AA testing, prevalence rates of AA across different populations can be assessed more reliably, using the same methodology. Moreover, large-scale longitudinal studies, using the same methodology across populations and centers, will help decipher the natural history and the implications of β -cell antibodies in overweight youth in the development of diabetes.

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Table 1Subject characteristics by presence or absence of β -cell antibodies

Variable	One β -cell antibody (n = 7)	No β -cell antibody (n = 350)	p-value
Sex (Male/female) (%)	3/4 (43/57)	140/210 (40/60)	0.88
Race (C/AA/B) (%)	5/2/0 (71/29/0)	183/144/23 (52/41/7)	0.55
Age (yr)	13.2 $\pm$ 2.1	12.8 $\pm$ 2.7	0.66
Tanner stage (I/II–IV/V) (%)	2/1/4 (28/15/57)	81/131/136 (23/38/39)	0.44
Family history of diabetes (first-degree relative) (%) [*]	0 (0)	60 (36)	0.14
Family history of diabetes (any relative) (%)	5 (71)	238 (68)	0.78
BMI percentile	99.1 (98.1–99.7)	99.0 (97.8–99.4)	0.54
Percent body fat (%)	40.3 $\pm$ 12.9	42.2 $\pm$ 7.1	0.49
Adiponectin (μ g/mL) [†]	4.6 $\pm$ 3.1	7.7 $\pm$ 4.3	0.11
Leptin (ng/mL) [‡]	54.7 $\pm$ 38.3	41.1 $\pm$ 25.6	0.29
Leptin/adiponectin ratio [‡]	17.5 $\pm$ 17.8	6.7 $\pm$ 5.7	0.001
Fasting glucose (mg/dL)	91 $\pm$ 7	87 $\pm$ 7	0.14
Glucose at 60 min (mg/dL)	151 $\pm$ 32	127 $\pm$ 8	0.02
Glucose at 90 min (mg/dL)	126 $\pm$ 32	121 $\pm$ 20	0.52
Two-hour glucose (mg/dL)	113 $\pm$ 19	115 $\pm$ 15	0.73
Fasting insulin (μ IU/mL) [§]	18.4 (12.6–49.8)	23.7 (16.9–34.5)	0.98
Insulin at 60 min (μ IU/mL) [§]	166.2 (79.9–435.4)	128.0 (80.1–205.9)	0.24
Insulin at 90 min (μ IU/mL) [§]	134.1 (49.5–629.8)	117.0 (77.7–173.7)	0.11
Two-hour insulin (μ IU/mL) [§]	170.0 (75.6–258.3)	104.7 (65.2–180.3)	0.54
Fasting C-peptide (ng/mL) [¶]	3.4 $\pm$ 1.3	2.8 $\pm$ 1.1	0.24
C-peptide at 60 min (ng/mL) [¶]	12.9 $\pm$ 7.5	9.2 $\pm$ 6.4	0.20
C-peptide at 90 min (ng/mL) [¶]	11.6 $\pm$ 9.6	8.5 $\pm$ 3.2	0.05
Two-hour C-peptide (ng/mL) [¶]	10.1 $\pm$ 2.2	8.5 $\pm$ 3.5	0.32
WBIS [§]	2.1 $\pm$ 1.3	2.0 $\pm$ 0.9	0.77
HOMA _{IR} [§]	6.6 $\pm$ 4.7	6.2 $\pm$ 4.3	0.87
Fasting glucose (mg/dL)/insulin (μ IU/mL) ratio [§]	4.5 $\pm$ 2.4	4.1 $\pm$ 2.3	0.72
Insulinogenic index (Δ I30/ Δ G30) [§]	2.8 $\pm$ 2.0	4.1 $\pm$ 4.4	0.55
GDI (with insulinogenic index at 30 min) [§]	0.51 $\pm$ 0.49	0.79 $\pm$ 1.0	0.59
Glucose AUC 0–120 min	18 379 $\pm$ 2026	16 330 $\pm$ 1991	0.02
Insulin AUC 0–120 min [§]	27 343 $\pm$ 18 605	19 428 $\pm$ 12 997	0.23
C-reactive protein (mg/L) ^{**}	1.0 (0.4–2.4)	1.5 (0.62–3.2)	0.41
Proinsulin (pml/L) ^{††}	30 $\pm$ 21	26 $\pm$ 29	0.77
Triglycerides (mg/dL) ^{‡‡}	101 $\pm$ 47	109 $\pm$ 60	0.74
HDL (mg/dL) ^{‡‡}	37 $\pm$ 12	41 $\pm$ 9	0.36
LDL (mg/dL) ^{‡‡}	90 $\pm$ 31	96 $\pm$ 29	0.60

Variable	One β -cell antibody (n = 7)	No β -cell antibody (n = 350)	p-value
VLDL (mg/dL) ^{††}	19 $\pm$ 9	20 $\pm$ 11	0.80
TG/HDL ^{††}	3.1 $\pm$ 2.4	2.9 $\pm$ 2.1	0.82

Data are presented as n (%), mean $\pm$ SD, or median (interquartile range). AA, African American; AUC, area under the curve; B, biracial; BMI, body mass index; C, Caucasian; GDI, glucose disposition index; HOMA_{IR}, homeostasis model assessment of insulin resistance; WBISI, whole-body insulin sensitivity index.

* Data available on 172 subjects.

[†] Data available on 233 subjects.

^{††} Data available on 218 subjects.

[§] Data available on 238 subjects.

[¶] Data available on 229 subjects.

** Data available on 203 subjects.

^{††} Data available on 167 subjects.

^{†††} Data available on 319 subjects.