Prevalence of Type 1 Diabetes auto-antibodies (GADA, IA2, IAA) in overweight and obese children

Running Title: Type 1 Diabetes auto-antibodies in obese children

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Background: Little is known on the prevalence of beta-cells auto-antibodies in children with excess body weight.

Research Design and Methods: The prevalence of type 1 diabetes auto-antibodies and its relation with hyperglycemia was analyzed in 686 overweight/obese children and adolescents. All children underwent OGTT and anti-GAD, anti-IA2 and anti-IAA auto-antibodies were measured. Auto-antibodies prevalence was evaluated in 107 normal weight children for comparison.

Results: A single auto-antibody was present in 2.18% overweight/obese and in 1.86% normal weight subjects (p=NS). Post-load glycemia was significantly higher in antibody-positive children (133±69.9 vs. 105.4±17.7 mg/dl; p<0.0001) compared to auto-antibody-negative subjects. No difference in auto-antibodies distribution was seen when our cohort was stratified by age, sex, SDS-BMI, pubertal stage and HOMA-IR.

Conclusions: The 2.18% prevalence of type 1 diabetes auto-antibodies is very similar to that reported in non obese children. This study provided evidence that excess body weight and insulin-resistance do not influence auto-antibodies frequency.
Over the last 60 years a striking increase in the incidence of childhood type 1 diabetes has been observed consistently in almost all populations. EURODIAB (1) reported an overall increase of 3.2% per annum in Europe between 1989–1998. There have also been considerable changes in childhood nutrition, which have resulted in changes to growth. Increased weight, height and BMI in children have all been associated with a higher risk of type 1 diabetes (2). The so-called “accelerator hypothesis” argues that obesity causing overwork of beta-cells underlies both type 1 and type 2 diabetes, and that these ‘types’ are only distinguished by how the body responds to this growth-induced beta-cell stress. This hypothesis therefore attributes the rise in type 1 diabetes to an increase in child obesity (3). A variation of the hypothesis suggests that, once initiated, islet autoimmunity progresses more rapidly in the context of ‘overload’ of the beta-cells due to increased insulin-resistance (4).

Sardinia has one of the highest incidence of type 1 diabetes worldwide, second only to Finland (5). Moreover, Sardinian children and adolescents are experiencing the same increase in obesity as other European populations (6). To date little is known on the prevalence of auto-antibodies against beta-cells in children with excess body weight.

The aim of our study was to analyze the prevalence of type 1 diabetes auto-antibodies in a cohort of Sardinian overweight/obese children and adolescents, and to evaluate their distribution in relation to the presence of glucose abnormalities.

**RESEARCH DESIGN AND METHODS**

686 overweight/obese Italian children and adolescents were studied, all attending the Pediatric Endocrine Unit for the presence, in all cases, of excess body weight. Exclusion criteria were the presence of endocrine disorders or genetic syndromes, including syndromic obesity. A second group of normal weight children (n=107) was collected for antibody prevalence comparison. Clinical characteristics of all 793 subjects are shown in table 1.

**Clinical and metabolic parameters:** All overweight/obese subjects underwent oral glucose tolerance test (OGTT). OGTT was performed according to clinical recommendations for children (1.75g per kilogram-body weight, up to 75g). Plasma glucose and insulin were measured at 0 and 120 minutes. Subjects were classified according to ADA criteria in normal glucose tolerant (NGT), with impaired fasting glycemia (IFG), impaired glucose tolerance (IGT) or diabetes. Impaired glucose regulation (IGR) defined the presence of any category of glucose abnormality (IFG, IGT, and diabetes). Diagnosis of type 1 diabetes was made in the presence of diabetic hyperglycemia and at least one beta-cell auto-antibody. In all 793 children, anti-GAD, anti-IA2 and anti-IAA auto-antibodies (GAD-Ab$^{125}$I-Radioassay, IA2-Ab$^{125}$I-Radioassay and IAA-Ab$^{125}$I-Radioassay were assessed, all from DLD Diagnostika-GMBH, Germany). The upper normal limit for anti-GAD and anti-IA2 is ≤1 U/ml, for anti-IAA is ≤0.4 U/ml. Anti-GAD assay has an intra-assay coefficient of variation (CV) of 3.6% and an inter-assay CV between 4.9-7.0%. Anti-IA2 assay has intra- and inter-assay CVs between 2.5-2.8% and between 3.3-5.3%, respectively. Anti-IAA
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assay has intra- and inter-CVs between 3.0-5.8% and between 4.2-6.7%. Our laboratory achieved 76% sensitivity and 95.7% specificity for anti-GAD and 64% sensitivity and 98.9% specificity for anti-IA2 at the latest Diabetes Antibody Standardization Program (DASP2009). Anti-IAA results were confirmed by a second method based on a competitive fluid-phase radioimmunoassay (7).

RESULTS
Type 1 diabetes auto-antibodies: In the 686 overweight/obese children a single auto-antibody (either anti-GAD, anti-IA2 or anti-IAA) was present in 15 subjects (2.18%). Anti-GAD were detectable in 13/686 (1.89%) children, anti-IA2 were present in 6/686 (0.87%) and anti-IAA were found in 0.43% (3/686) of the children. Two antibodies were found together in 5 (0.7%) subjects. All three auto-antibodies were found in only one subject. In the 107 normal weight children anti-GAD and anti-IA2 were found together in 2 subjects (1.86%, p=NS vs overweight/obese children).

No difference in auto-antibodies distribution was observed when our cohort was stratified by age, sex, SDS-BMI, pubertal stage and the insulin-resistance index HOMA-IR (data not shown).

IGR and auto-antibodies in overweight/obese children: Overall prevalence of IGR in our cohort of overweight/obese children was 11.37% (78/686). The frequency of IFG was 8.16% (56/686), IGT 3.2% (22/686) and diabetes 0.6% (4/686).

When divided on the basis of glucose regulation, the presence of autoimmunity was three times more prevalent in children with IGR (5.12%) compared to those with NGT (1.80%). The prevalence of glucose abnormalities in antibody-positive subjects was 27%, compared to 11% in antibody-negative children.

In the whole group anti-IAA titres correlated with post-load glycemia (p<0.03), which was significantly higher in antibody-positive children (133±69.9 vs 105.4±17.7 mg/dl; p<0.0001) when compared to antibody-negative subjects. Antibodies titres were not correlated to fasting glucose (93.5±16.2 vs. 89.6±7.4; p=NS).

DISCUSSION
In the present study, we found that the prevalence of auto-antibodies in overweight/obese children was similar (2.18%) to that found in our cohort of normal weight-matched subjects (1.86%, p=NS), as well as to that reported in the general population of schoolchildren (8, 9). When our cohort was stratified in subjects with normal and impaired glucose regulation, prevalence of auto-antibodies was higher in those with IGR (5.12%). This prevalence is very similar to that reported in non-obese hyperglycemic children (10).

We also found that antibody-positive subjects had a significantly higher 2h glycemia. Our results are in line with those recently demonstrated in the DPT-1 study (11) where the majority of subjects diagnosed with type 1 diabetes had impaired post-OGTT glucose levels, thus suggesting that OGTT in antibody-positive subjects may help to prevent acute-onset disease. With regards to this point, the prevalence of glucose abnormalities in our antibody-positive subjects was nearly 30%, and in all cases they were diagnosed as IGR by the 2h value.

CONCLUSION
In conclusion, this study provides evidence that excess body weight and
insulin-resistance do not influence the frequencies of auto-antibodies as postulated by the accelerator hypothesis, which is therefore not supported by our data. It also shows that an obese child can be at risk for type 1 diabetes as much as a normal weight child. However, the hypothesis of the ‘overload’ of beta-cells as a result of increased insulin demands linked to obesity warrants further studies.

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Disclosure: The authors have no relevant conflict of interest to disclose.
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3. Wilkin TJ. The accelerator hypothesis: a review of the evidence for insulin resistance as the basis for type I as well as type II diabetes. Int J Obes (Lond) 2009; 33(7): 716-26


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charts for height, weight and BMI (2 to 20 yr). J Endocrinol Invest 2006; 29: 581-593

Table. 1. Clinical and biochemical characteristics of overweight/obese and normal weight children and adolescents

<table>
<thead>
<tr>
<th></th>
<th>ow n = 217</th>
<th>ob n = 469</th>
<th>p-value ow vs ob</th>
<th>ow/ob n = 686</th>
<th>nw n = 107</th>
<th>p-value ow/ob vs nw</th>
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<tbody>
<tr>
<td>age (years)</td>
<td>10.6 ± 3</td>
<td>10.2 ± 3.2</td>
<td>NS</td>
<td>10.3 ± 3.2</td>
<td>11.4 ± 3.2</td>
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<td>sex (n F/M)</td>
<td>124/93</td>
<td>236/233</td>
<td>NS</td>
<td>360/326</td>
<td>49/58</td>
<td>NS</td>
</tr>
<tr>
<td>pre-pubertal/pubertal (n)</td>
<td>150/67</td>
<td>329/240</td>
<td>NS</td>
<td>479/207</td>
<td>68/39</td>
<td>NS</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>25.1 ± 2.3</td>
<td>28.9 ± 4</td>
<td>&lt;0.0001</td>
<td>27.7 ± 4.5</td>
<td>17.9 ± 2.7</td>
<td>&lt;0.0001</td>
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<td>SDS-BMI</td>
<td>1.9 ± 0.7</td>
<td>3.2 ± 1.2</td>
<td>&lt;0.0001</td>
<td>2.8 ± 1.2</td>
<td>-0.46 ± 0.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>104.19 ± 13.4</td>
<td>107.53 ± 15.5</td>
<td>&lt;0.017</td>
<td>106.4 ± 14.9</td>
<td>105 ± 10.6</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>61.35 ± 7.9</td>
<td>62.38 ± 9.4</td>
<td>NS</td>
<td>62 ± 9</td>
<td>61.1 ± 5.4</td>
<td>NS</td>
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<tr>
<td>glycemia 0’ (mg/dl)</td>
<td>90.1 ± 7.8</td>
<td>89.4 ± 7.6</td>
<td>NS</td>
<td>89.6 ± 7.7</td>
<td>88.4 ± 8.4</td>
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<tr>
<td>insulin µU/ml</td>
<td>15.7 ± 7.2</td>
<td>16.9 ± 9.7</td>
<td>NS</td>
<td>16.6 ± 9</td>
<td>11.7 ± 6</td>
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<tr>
<td>HOMA-IR</td>
<td>3.5 ± 1.6</td>
<td>3.7 ± 2.3</td>
<td>NS</td>
<td>3.7 ± 2</td>
<td>2.6 ± 1.4</td>
<td>&lt;0.0001</td>
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<tr>
<td>total cholesterol (mg/dl)</td>
<td>168 ± 34.1</td>
<td>165.9 ± 32.2</td>
<td>NS</td>
<td>166.6 ± 32.8</td>
<td>165 ± 26.3</td>
<td>NS</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>51.4 ± 11.8</td>
<td>49.8 ± 11</td>
<td>NS</td>
<td>50.3 ± 11.3</td>
<td>59.4 ± 12.2</td>
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<tr>
<td>LDL (mg/dl)</td>
<td>105.1 ± 28.9</td>
<td>103.3 ± 28.2</td>
<td>NS</td>
<td>103.9 ± 28.4</td>
<td>96.4 ± 24</td>
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<tr>
<td>tryglicerides (mg/dl)</td>
<td>57.2 ± 39.2</td>
<td>60.8 ± 32.8</td>
<td>NS</td>
<td>59.7 ± 35</td>
<td>45.7 ± 29.5</td>
<td>&lt;0.0001</td>
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<tr>
<td>auto-Abs positive n (%)</td>
<td>7 (3.22)</td>
<td>8 (1.70)</td>
<td>NS</td>
<td>15 (2.18)</td>
<td>2 (1.86)</td>
<td>NS</td>
</tr>
<tr>
<td>anti-GAD positive n (%)</td>
<td>6 (2.76)</td>
<td>7 (1.49)</td>
<td>NS</td>
<td>13 (1.89)</td>
<td>2 (1.86)</td>
<td>NS</td>
</tr>
<tr>
<td>anti-IA2 positive n (%)</td>
<td>1 (0.46)</td>
<td>5 (1.06)</td>
<td>NS</td>
<td>6 (0.87)</td>
<td>2 (1.86)</td>
<td>NS</td>
</tr>
<tr>
<td>anti-IAA positive n (%)</td>
<td>2 (0.92)</td>
<td>1 (0.21)</td>
<td>NS</td>
<td>3 (0.43)</td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>

ow = overweight; ob = obese; nw = normal weight; M = male; F = female; SBP = systolic blood pressure; DBP = diastolic blood pressure; auto-Abs = anti-GADA and/or anti-IA2 and/or anti-IAA positive.

Overweight, obesity and SDS-BMI were defined according to Italian growth charts in people aged 2–20 yrs [12]. 1 standard deviation (SD) of BMI defines overweight, 2 SD of BMI defines obesity. Pubertal developmental stages were determined according to Tanner. Differences between variables were evaluated by two-tailed Student’s t-test or Mann-Whitney test. Categorical variables were compared by χ² or Fischer’s-exact tests.