Clinical characteristics of non-obese children with type 2 diabetes mellitus without involvement of β-cell autoimmunity

Tatsuhiko Urakami*, Remi Kuwabara, Masako Habu, Misako Okuno, Junichi Suzuki, Shori Takahashi, Hideo Mugishima

Department of Pediatrics, Nihon University School of Medicine, Tokyo, Japan

A R T I C L E   I N F O

Article history:
Received 22 August 2012
Received in revised form
11 November 2012
Accepted 23 November 2012
Published on line 20 December 2012

Keywords:
Non-obese type 2 diabetes mellitus
Children
Insulin resistance
Insulin secretory capacity
β-Cell autoimmunity

A B S T R A C T

Aims: We examined the clinical characteristics of non-obese Japanese children with type 2 diabetes mellitus (T2DM) not associated with β-cell autoimmunity.

Methods: Of 218 children who were diagnosed as having T2DM by a school urine glucose screening program in Tokyo, 24 were identified as being non-obese and were enrolled in this study. None of the children had any evidence of β-cell autoimmunity or genetic disorders.

Results: The mean ages at diagnosis and at the study were 12.5 ± 1.7 and 22.4 ± 5.7 years, respectively. Females were predominant (M/F ratio: 4/20). Family history of T2DM, mostly of the non-obese type, was present in 62.5% of the cases. In regard to the birth weight, 20.8% had a history of low birth weight, and 8.3% were large for gestational age. The mean fasting insulin level, HOMA-R, HOMA-β, and an insulinogenic index on the OGTT at the time of diagnosis were 11.8 ± 7.8 μU/ml, 5.4 ± 3.8, 96.1 ± 55.0 and 0.16 ± 0.14, respectively. Most patients were treated by either oral hypoglycemic drug (45.8%) or insulin (50.0%) therapy at the study, with the mean interval to the start of pharmacological treatment of 3.1 ± 2.3 years.

Conclusions: Non-obese children with T2DM seemed to show lower insulin secretory capacities with mild, but evident, insulin resistance even from the time of diagnosis, and also earlier requirement of pharmacological therapies during the clinical course. Some genetic factors not associated with autoimmunity may play a role in the etiology of T2DM in non-obese children.

© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

It is well known that the prevalence of type 2 diabetes mellitus (T2DM) is increasing worldwide, not only among adults, but also among young persons [1–3]. Asian populations, including Japanese people, have been found to show a higher incidence of T2DM than Caucasians [3]. School urine glucose screening programs to detect childhood diabetes mellitus (DM) have revealed an annual incidence of T2DM of approximately 3.0–3.5/100,000 in children aged 7–15 years, and of 6.0 or more/100,000, particularly in junior high school children aged 13–15

* Corresponding author at: Department of Pediatrics, Nihon University School of Medicine, 1-8-13 Kandasurugadai, Chiyoda-ku, Tokyo 101-8309, Japan. Tel.: +81 3 3293 1711; fax: +81 3 3293 1798.
E-mail addresses: urakami.tatsuhiko@nihon-u.ac.jp, turakami@med.nihon-u.ac.jp (T. Urakami).
years, during recent years in Japan [4,5]. We considered that the recent increase in the number of childhood cases of T2DM may be a consequence of the increased prevalence of obesity associated with westernization of the lifestyles and eating habits of children in Japan. It has been reported that 87% of Japanese children with T2DM are obese, with the frequency of obesity being higher in boys than in girls with T2DM. The remaining 13% of childhood cases of T2DM are reported to be non-obese at the time of diagnosis and to have no past history of overweight or obesity [5].

The pathophysiology of T2DM is still not clarified, however, insulin resistance is expected to be involved in obese patients with T2DM. Obese patients with T2DM are known to have a high prevalence of acanthosis nigricans, reflecting hypersecretion of insulin [1]. It is considered that insulin resistance in these patients is present even at the prediabetic stage before the development of overt clinical DM [6]. The evolution from normal to impaired glucose tolerance may be associated with deteriorating insulin resistance. It has been demonstrated that even obese people with normal glucose tolerance exhibit decreased insulin sensitivity, caused by the actions of some adipocytokines, such as TNF-α and IFN-γ derived from fat tissues [7]. Moreover, reduced serum levels of adiponectin have been reported to play an important role in decreasing insulin sensitivity in animal and human studies [8]. On the other hand, pancreatic β-cell failure may underlie the progression from insulin resistance to clinical DM among susceptible individuals [6,9,10]. Consequently, coexistence of insulin resistance and the compensatory insulin secretory failure is considered as the cause of T2DM among obese people. On the other hand, while a greater number of cases of T2DM in non-obese individuals are encountered among Asians than among Caucasians, the clinical characteristics of non-obese subjects with T2DM have not yet been well studied. Therefore, we studied the clinical characteristics of non-obese children with T2DM detected by a school urine glucose screening program in Tokyo, in an attempt to know the factors involved in the pathophysiology of T2DM in non-obese children.

2. Materials and methods

From 1974 to 2008, a total of 218 children were diagnosed as having T2DM by urine glucose screening of school children residing in Tokyo. Among the 218 patients, 194 were obese, with the % overweight in excess of 20% (23.4–88.8%), while the remaining 24 were non-obese, with a % overweight of less than 20% (–4.5 to 15.6%). The patient characteristics in the non-obese and obese children with T2DM are shown in Table 1. The mean age at the diagnosis and at the time of start of the study, and the mean duration of diabetes were not significantly different between the two patient groups. In regard to the gender, the percentage of females was significantly higher among the non-obese patients than among the obese patients (83.3% vs. 48.5%, P = 0.0012).

Since 1974, we have been conducting annual screening of primary school children aged 6–12 years and junior high school children aged 13–15 years residing in Tokyo for glucosuria, concomitantly with that for proteinuria and hematuria. If a urine test is positive for glucose, the urine test is repeated on another morning. When both the first and second test results are positive, an OGTT is performed to confirm the diagnosis of DM. However, some patients who show extremely high values of fasting plasma glucose (FPG) of more than 200 mg/dl and/or positive test for urinary ketone bodies, are immediately diagnosed as having DM without further confirmation by an OGTT. From 1974 to 2008, 266 children were detected as having T2DM by the school screening program. None of the patients diagnosed as having T2DM required insulin treatment to achieve optimal glycemic control for at least two years after the diagnosis [4,5]. None of the patients showed any positive test results for any diabetes-related autoantibodies, measured in stored serum samples, at the time of diagnosis and during the course of DM. HLA haplotypes also help to rule out a genetic predisposition to T1DM, however, HLA were not analyzed in most patients with T2DM of the present study.

No patients had genetic disorders for diabetes such as maturity-onset diabetes in the young (MODY) and mitochondrial diabetes. Three non-obese children diagnosed as DM by the urine glucose screening were identified to have genetic disorders; i.e. one with MODY3, one with MODY2 and one with mitochondrial DM. They were excluded from the study. No patients had insulin resistance syndrome, such as polycystic ovary syndrome.

As for treatment, most of the children diagnosed as having T2DM were initially treated by diet and exercise therapy. If successful glycemic control failed on the diet and exercise therapy, with HbA1c value remaining higher than 7.4% (NGSP), pharmacological means, including oral hypoglycemic drug (OHD) or insulin therapy, was introduced to the patients [10–12]. The first choice of OHD should be metformin, administered at the dose of 500–750 mg daily. In some cases, treatment was started with α-glucosidase inhibitors or sulfonylureas (SUs). Insulin treatment was finally initiated in children in whom the OHD therapy proved ineffective to obtain optimal glycemic control with HbA1c value less than 7.0%.

We examined the following clinical characteristics of non-obese children with T2DM and compared them with those of obese children with T2DM: (1) family history of T2DM in first- and second-degree relatives, (2) birth weight, (3) laboratory data at the time of urine glucose screening at school, including FPG, HbA1c, fasting plasma insulin (IRI), homeostasis model assessment of insulin resistance

<p>| Table 1 – Patient characteristics of non-obese and obese children with T2DM diagnosed by urine glucose screening conducted at schools in Tokyo. |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Non-obese</th>
<th>Obese</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>24</td>
<td>194</td>
<td></td>
</tr>
<tr>
<td>Age at the diagnosis</td>
<td>12.5 ± 1.7</td>
<td>12.8 ± 2.9</td>
<td>NS</td>
</tr>
<tr>
<td>Age at the study</td>
<td>22.4 ± 5.7</td>
<td>23.8 ± 5.8</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>10.4 ± 5.4</td>
<td>11.0 ± 5.8</td>
<td>NS</td>
</tr>
<tr>
<td>% of female</td>
<td>83.3</td>
<td>45.5</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

NS: not significant.
* Non-obese vs. obese patients.
(HOMA-R) and pancreatic β-cell function (HOMA-β), and an insulinogenic index on an OGTT, (4) current treatment at the time of the study, and (5) prognosis, i.e., the frequencies of microvascular complications at the time of the study. In regard to the laboratory data, measurement of IRI was not performed at 30 min on an OGTT in most obese children, therefore, data on the insulinogenic index calculated using the IRI at 30 min were not available. Consequently, the insulinogenic index could not be compared between the obese and non-obese children with T2DM.

Percent overweight is more commonly used than the body mass index (BMI) in Japan to evaluate obesity in children and adolescents. Therefore, we used the percent overweight as an index of obesity in this study. The percent overweight was calculated as (current weight – sex-, age- and height-matched ideal weight)/sex-, age- and height-matched ideal weight × 100 (%). Subjects with a % overweight in excess of 20% were judged to be obese [13].

As for diabetes-related autoantibodies, we measured islet cell antibodies (ICA) and anti-glutamic acid decarboxylase antibodies (GAD-A). ICA were detected by an indirect immunofluorescence method on unfixed cryostats of blood O type human pancreas; positive: more than 5 Juvenile Diabetes units (JDF U). GAD-A were measured using radioimmunoassay kits employing recombinant human GAD (Cosmic Corporation, Tokyo, Japan); positive: more than 1.5 units/ml. As mutation analysis for MODY, HNF1A, HNF 1B, GCK and HNF4A genes were analyzed, with kind collaboration of Dr. Tohru Yorifuji, Department of Pediatric Endocrinology and Metabolism, Children’s Medical Center, Osaka City General Hospital, Osaka, Japan. The mitochondrial 3243A→G mutation was tested by polymerase chain reaction (PCR)-restriction fragment length polymorphism, with collaboration of Dr. Tohru Yorifuji also.

HOMA-R for estimating insulin resistance was calculated as FPG (mg/dl) × fasting IRI (μU/ml)/405 [14]. HOMA-β for estimating endogenous insulin secretion was calculated as 360 × fasting IRI (μU/ml)/FPG (mg/dl) – 63 [14]. We used the insulinogenic index on the OGTT to evaluate the early insulin response to a rise of blood glucose, and the insulinogenic index was calculated as ΔIRI (30 min on OGTT)/ΔPG (30 min on OGTT) [15].

PG was measured by the glucose oxidase method and IRI by radioimmunoassay. HbA1c was measured by a HPLC method, and the value of HbA1c (%) was estimated as an National Standardization Program for Glycylated Hemoglobin (NSPG)-equivalent value (%), calculated using the formula, HbA1c (%) = HbA1c (Japan Diabetes Society: JDS) + 0.4%, considering the relational expression of HbA1c (JDS) measurement by the previous Japanese standard substance and measurement method and HbA1c (NSPG) [16].

2.1. Statistical analysis

The results were expressed as the mean values ± SD. SPSS, ver. 12, was used for the statistical analyses. Mann–Whitney’s U test and χ² test were used to detect the statistical significance of differences between the two groups, and P < 0.05 was considered as statistical significant.

3. Results

3.1. Family history of T2DM in the first- and second-degree relatives

A positive history of T2DM in first- and second-degree relatives was obtained in 62.5% of the non-obese children with T2DM and 58.5% of the obese children with T2DM. The difference in the frequency between the two groups was not statistically significant. It was of interest that 75.0% of the diabetic relatives of the non-obese children with T2DM were also non-obese with the BMI of less than 25 (18.9–24.2).

3.2. Birth weight

The mean birth weight of the non-obese children with T2DM was 2865 ± 480 g, and that of the obese children with T2DM was 3002 ± 250 g, with no significant difference of the mean birth weight between the two groups. On the other hand, small for gestational age and large for gestational age were encountered in 20.8% and 8.3% of the non-obese children with T2DM, and 8.5% and 10.3% of the obese children with T2DM. The frequency of small for gestational age was significantly higher in the non-obese children with T2DM than in the general Japanese population [17] (n = 1,221,289, 6.3% for small for gestational age, P < 0.0001).

3.3. Laboratory data at the time of the school urine glucose screening (Table 1)

The mean levels of FPG and HbA1c at the time of the school urine glucose screening in the non-obese children were 133.5 ± 86.5 mg/dl and 8.4 ± 4.2%, respectively. There were no significant differences of these values between the two patient groups.

The mean values of fasting IRI, HOMA-R, HOMA-β and an insulinogenic index at the time of the school urine glucose screening in the non-obese children were 11.8 ± 7.8 μU/ml, 5.4 ± 3.8, 96.1 ± 55.0 and 0.16 ± 0.14, respectively. In regard to the indices of insulin resistance, fasting IRI and HOMA-R in the non-obese children were significantly lower than those in the obese children (P = 0.0003, P = 0.0026, respectively). On the other hand, the mean value of HOMA-β in the non-obese

| Table 2 – Laboratory data of non-obese and obese children with T2DM at the time of the urine glucose screening. |
| -------- |--------|--------|--------|
|          | Non-obese patients | Obese patients | P-value* |
| FPG (mg/dl) | 133.5 ± 86.5 | 146.5 ± 28.0 | NS |
| HbA1c (%) | 8.4 ± 4.2 | 8.6 ± 1.1 | NS |
| Fasting IRI (μU/ml) | 11.8 ± 7.8 | 30.0 ± 18.7 | 0.0003 |
| HOMA-R | 5.4 ± 3.8 | 13.3 ± 8.0 | 0.0026 |
| HOMA-β | 96.1 ± 55.0 | 162.3 ± 107.4 | 0.0005 |
| Insulinogenic index | 0.16 ± 0.14 | ND | |

ND: no data; NS: not significant.

* Non-obese vs. obese patients.
children was significantly lower than that in the obese children \( (P = 0.005) \) (Table 2).

### 3.4. Current treatment at the time of study

Among the 24 non-obese children with T2DM enrolled in this study, 22 (91.7%) were treated by only therapeutic modification of the diet and exercise habit, while the remaining 2 (8.3%) children were treated with OHDs, one with metformin and the other with \( \alpha \)-glucosidase inhibitors, immediately after the diagnosis. Subsequently, optimal glycemic control with HbA1c value less than 7.0% could be maintained with diet and exercise therapy in only one of the children (4.2%), while the remaining children (95.8%) needed to be switched to pharmacological treatment during the study: OHD therapy in 11 children (45.8%) and insulin therapy in 12 children (50.0%). The mean interval to the start of pharmacological treatment was \( 3.1 \pm 2.3 \) years (\( 2.2 \pm 2.1 \) years for OHD therapy, and \( 4.8 \pm 2.9 \) years for insulin therapy). The details of the current treatment of T2DM in the non-obese children are shown in Table 3.

On the other hand, among the 192 obese children with T2DM, we could obtain information on the current status of treatment in only 78 children, because the remaining 114 were being treated at the other hospitals or dropped out during the management with diet and exercise therapy. Analysis of the data of the 78 children revealed that 40% of the obese children received pharmacological treatment with either an OHD, mainly metformin, or insulin during the study. The frequency of progression to the need for pharmacological treatment during the study was significantly higher in the non-obese than in the obese T2DM children \( (P = 0.0052) \).

### 3.5. Frequency of microvascular complications of diabetes at the time of the study

Table 4 shows the frequency of microvascular complications of diabetes during the study among the 24 non-obese and 78 obese children with T2DM for whom this information could be obtained. Two (8.3%) non-obese children and 4 (5.1%) obese children were found to have background retinopathy, and 2 (8.3%) non-obese and 3 (3.8%) obese children to have microalbuminuria, with a urinary albumin/creatinine excretion ratio exceeding 15 mg/g creatinine. Thus, there were no statistically significant differences in the frequency of the development of microvascular complications between the two groups. None of the children in either group showed progression to severe complications, such as proliferative retinopathy or overt proteinuria, during the study.

### 4. Discussion

While the clinical features of T2DM are heterogeneous, some of the more commonly recognized features include obesity, presence of a family history of T2DM, presence of evidence of insulin resistance, and absence of evidence of diabetes-related autoimmunity \([1,18]\). Pediatric obesity is believed to be the most prominent candidate involved in the onset of T2DM \([19]\). Obese children have lipid deposition in the peripheral and visceral compartments, and exhibit insulin resistance as a result of elevation of the serum levels of inflammatory adipocytokines, such as TNF-\( \alpha \) and IFN-\( \gamma \), and decrease of the serum levels of adiponectin \([7,8,19]\). Most Caucasian children with T2DM are reported to be obese \([1,18]\), while a substantial number of Asian children with T2DM are found to be non-obese. Namely, it is reported that half of Asian Indian urban children with T2DM have normal weight \([20]\), and that a half of Taiwanese children with T2DM are non-obese \([21]\). Moreover, approximately 10–30% of Japanese children with T2DM detected by school urine glucose screening conducted at various cities of Japan are also found to be non-obese \([4,5,11]\). Yoon et al. \([22]\) reported that even though the average BMI of Asians is lower than that of people of European descent, the prevalence of T2DM is higher in Asians. The waist circumference or waist-to-hip ratio may be a more appropriate index to evaluate obesity in Asian people. On the other hand, some studies have indicated that for the same BMI and/or waist circumference, Asians might have a greater amount of visceral adiposity than Caucasians \([23,24]\). We unfortunately did not measure the waist circumference or the visceral fat area on CT either at the time of diagnosis or during the course of treatment in most children. It might be necessary to examine these indices to evaluate the amount of visceral adiposity which could more precisely reflect insulin resistance in the children with T2DM (Table 5).

In the present study, we found some novel characteristics of the non-obese children with T2DM, as different from those of the obese children with T2DM, which are summarized in Table 4.

The present study demonstrated that the non-obese children with T2DM were predominantly females, unlike

### Table 3 – Current treatment at the time of the study in non-obese children with T2DM.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet and exercise</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>Insulin</td>
<td>12 (50.0)</td>
</tr>
<tr>
<td>Insulin alone</td>
<td>11</td>
</tr>
<tr>
<td>Insulin + Met</td>
<td>1</td>
</tr>
<tr>
<td>OHD</td>
<td>11 (45.8)</td>
</tr>
<tr>
<td>SUs alone</td>
<td>4</td>
</tr>
<tr>
<td>SUs + ( \alpha )-Gls</td>
<td>3</td>
</tr>
<tr>
<td>SUs + Met</td>
<td>2</td>
</tr>
<tr>
<td>SUs + DPP4-I</td>
<td>2</td>
</tr>
</tbody>
</table>

OHD: oral hypoglycemic drug, Met: metformin, SUs: sulfonylureas, \( \alpha \)-Gls: \( \alpha \)-glucosidase inhibitors, DPP4-I: DPP4 inhibitors.

### Table 4 – Frequency of microvascular complications at the time of the study in non-obese and obese children with T2DM.

<table>
<thead>
<tr>
<th>Complication</th>
<th>Non-obese patients</th>
<th>Obese patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background retinopathy</td>
<td>2 (8.3%)</td>
<td>4 (5.1%)</td>
<td>NS</td>
</tr>
<tr>
<td>Microalbuminuria*</td>
<td>2 (8.3%)</td>
<td>3 (3.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>Progressed complication</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

NS: not significant.

* Urinary albumin/creatinine ratio exceeding 15 mg/g creatinine.
the obese children with T2DM, with a similar age at the onset of DM. Besides, several Caucasian studies which examined obese T2DM showed a higher frequency of females in childhood T2DM [1,2], while no gender difference was reported in Japanese studies which also examined the obese cases [5]. Of interest, Japanese children with a slowly progressive form of T1DM (SPT1DM), who are non-obese, also show female predominance in gender. Thus, femaleness may play a role in the development of some particular subtypes of DM in Japanese children.

Several studies have demonstrated that a positive family history of T2DM is strongly associated with a high frequency of development of T2DM while the frequency is relatively lower, at 50–70%, in Asians, including Japanese, as compared with that in Caucasians and African-Americans, at 74–100% [1,3,5,11]. In the present study, we also found a relatively lower frequency of T2DM of approximately 60% in first- and second-degree relatives. Besides, 75.0% of the relatives of the non-obese patients have a similar diabetic type; i.e. non-obese T2DM. This finding suggests that there might be a strong hereditary component, likely a multigenetic determinant, in the development of non-obese T2DM.

In regard to the role of the intra-uterine environment, we found a significantly higher frequency of small-for-gestational age history in the non-obese children with T2DM than in the general Japanese population. Sugihara et al. [17] reported that the frequencies of both low and high birth weights were higher among patients with T2DM than in a control group, producing a U-shaped distribution; in a multicenter study in Japan, the U-shaped curve was revealed to be more evident for children with non-obese T2DM. These results raise the possibility of a stronger link between intrauterine growth impairment and the risk of non-obese T2DM than that of obese T2DM.

Racial differences in insulin sensitivity have been reported in children with T2DM. The Bogalusa Heart Study demonstrated that African-American children without DM showed higher insulin responses on an OGTT than white Caucasian children, suggesting higher compensatory insulin resistance [25]. In a euglycemic clamp study, the insulin sensitivity was 30% lower in African-American adolescents as compared with that in white Caucasian adolescents [26]. This study suggests that children in minority ethnic groups with high prevalence of T2DM may have a genetic predisposition to insulin resistance. In the present study, we found that the fasting IRI and HOMA-R in the non-obese children with T2DM were significantly lower than those in the obese children with T2DM, who showed exaggerated compensatory secretion of insulin. Indeed, hormonal and physical changes during puberty increase insulin resistance. We previously demonstrated that Japanese adolescents had higher insulin levels than prepubertal children because of decreased insulin sensitivity during puberty [27]. Besides, we found that non-obese children with T2DM had significantly higher fasting IRI and HOMA-R than age- and pubertal stage-matched normal non-obese children; i.e. normal non-obese children: IRI 9.0 ± 3.6 μU/ml, HOMA-IR 2.0 ± 0.9, versus children with non-obese T2DM, P < 0.0001, respectively [28]. These findings suggest that non-obese children and adolescents with T2DM have mild, but significant, insulin resistance, which could raise a blood glucose concentration.

Regardless of the evidence that insulin resistance exists in the pathophysiology of T2DM, β-cell dysfunction and the consequent inability to maintain appropriate insulin secretion might be precipitating factors for the progression to clinical DM among susceptible individuals [6,9]. Kobayashi et al. [10] reported that obese adolescents with T2DM showed impaired β-cell insulin secretory responses to glucose stimulation, as demonstrated by a relatively low first-phase insulin response to decreased insulin sensitivity in an insulin-modified, frequently sampled intravenous glucose tolerance test, in contrast to adolescents with simple obesity. In the present study, we found that the mean value of HOMA-β in non-obese children with T2DM patients was substantially lower than that in obese children with T2DM, but was sustained to some degrees. Furthermore, non-obese children with T2DM showed low levels of the insulinogenic index with the mean value of 0.16 ± 0.14, and 93.3% of the non-obese patients showed this index of below 0.4; a cut-off point for early insulin secretion for adults [15]. From these data, it is considered that non-obese children with T2DM maintained insulin secretory capacity to some degree, but not exaggerately, and impaired early insulin secretory response to a rise of the blood glucose.

In regard to the indices for evaluating insulin resistance and insulin secretory capacity, all of HOMA-R, HOMA-β and an insulinogenic index are not validated for pediatric subjects [29–33]. Schwartz et al. reported that HOMA-R was most widely used of the surrogate measures in children, and was highly correlated with fasting IRI in children [29]. Cutfield et al. [30], however, demonstrated that HOMA-R was a poor surrogate measure and was no better than a fasting IRI for evaluating insulin resistance in prepubertal children. Levy-Marchal et al. [33] have indicated in the review of insulin resistance in children that standards for insulin resistance in children have not established. This is due, in part, to lack of sufficient cohort sizes to establish normative distributions for insulin sensitivity, and lack of adequate longitudinal studies to relate definitions for insulin resistance to long-term outcomes. Fasting IRI and HOMA-R are not optimal tools for individual assessment of insulin sensitivity, and HOMA-β and an insulinogenic index are also not suitable measurements for evaluating insulin secretory capacity in pediatric subjects. Nevertheless, these indices may provide information regarding the degrees of hyperinsulinemia and insulin secretory failure. In the present study, we compared these indices between children with non-obese T2DM and those with obese T2DM, and found differences in insulin resistance and insulin secretory capacity. We also found differences in these indices as compared with age- and pubertal stage-matched Japanese non-obese school children. The cut-off points for these indices

<table>
<thead>
<tr>
<th>Table 5 - Summary of the clinical characteristics of children with non-obese T2DM.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Predominance in females</td>
</tr>
<tr>
<td>(2) Positive family history of non-obese T2DM</td>
</tr>
<tr>
<td>(3) High frequency of low birth weight</td>
</tr>
<tr>
<td>(4) Milder insulin resistance</td>
</tr>
<tr>
<td>(5) Lower insulin secretory capacity</td>
</tr>
<tr>
<td>(6) Earlier progression to pharmacological treatment</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 6 - Values of HOMA-IR and HOMA-β in non-obese children with T2DM.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Predominance in females</td>
</tr>
<tr>
<td>(2) Positive family history of non-obese T2DM</td>
</tr>
<tr>
<td>(3) High frequency of low birth weight</td>
</tr>
<tr>
<td>(4) Milder insulin resistance</td>
</tr>
<tr>
<td>(5) Lower insulin secretory capacity</td>
</tr>
<tr>
<td>(6) Earlier progression to pharmacological treatment</td>
</tr>
</tbody>
</table>

than prepubertal children because of decreased insulin sensitivity during puberty [27]. Besides, we found that non-obese children with T2DM had significantly higher fasting IRI and HOMA-R than age- and pubertal stage-matched normal non-obese children; i.e. normal non-obese children: IRI 9.0 ± 3.6 μU/ml, HOMA-IR 2.0 ± 0.9, versus children with non-obese T2DM, P < 0.0001, respectively [28]. These findings suggest that non-obese children and adolescents with T2DM have mild, but significant, insulin resistance, which could raise a blood glucose concentration. Regardless of the evidence that insulin resistance exists in the pathophysiology of T2DM, β-cell dysfunction and the consequent inability to maintain appropriate insulin secretion might be precipitating factors for the progression to clinical DM among susceptible individuals [6,9]. Kobayashi et al. [10] reported that obese adolescents with T2DM showed impaired β-cell insulin secretory responses to glucose stimulation, as demonstrated by a relatively low first-phase insulin response to decreased insulin sensitivity in an insulin-modified, frequently sampled intravenous glucose tolerance test, in contrast to adolescents with simple obesity. In the present study, we found that the mean value of HOMA-β in non-obese children with T2DM patients was substantially lower than that in obese children with T2DM, but was sustained to some degrees. Furthermore, non-obese children with T2DM showed low levels of the insulinogenic index with the mean value of 0.16 ± 0.14, and 93.3% of the non-obese patients showed this index of below 0.4; a cut-off point for early insulin secretion for adults [15]. From these data, it is considered that non-obese children with T2DM maintained insulin secretory capacity to some degree, but not exaggerately, and impaired early insulin secretory response to a rise of the blood glucose. In regard to the indices for evaluating insulin resistance and insulin secretory capacity, all of HOMA-R, HOMA-β and an insulinogenic index are not validated for pediatric subjects [29–33]. Schwartz et al. reported that HOMA-R was most widely used of the surrogate measures in children, and was highly correlated with fasting IRI in children [29]. Cutfield et al. [30], however, demonstrated that HOMA-R was a poor surrogate measure and was no better than a fasting IRI for evaluating insulin resistance in prepubertal children. Levy-Marchal et al. [33] have indicated in the review of insulin resistance in children that standards for insulin resistance in children have not established. This is due, in part, to lack of sufficient cohort sizes to establish normative distributions for insulin sensitivity, and lack of adequate longitudinal studies to relate definitions for insulin resistance to long-term outcomes. Fasting IRI and HOMA-R are not optimal tools for individual assessment of insulin sensitivity, and HOMA-β and an insulinogenic index are also not suitable measurements for evaluating insulin secretory capacity in pediatric subjects. Nevertheless, these indices may provide information regarding the degrees of hyperinsulinemia and insulin secretory failure. In the present study, we compared these indices between children with non-obese T2DM and those with obese T2DM, and found differences in insulin resistance and insulin secretory capacity. We also found differences in these indices as compared with age- and pubertal stage-matched Japanese non-obese school children. The cut-off points for these indices
have not been established, however, we could compare the degrees of insulin resistance and insulin secretory ability in the non-obese patients with those in the obese patients and normal non-obese school children.

Several Caucasian studies have reported that diabetes-related antibodies can be detected in some children exhibiting phenotypes of T2DM [24,34–37]. The SEARCH for Diabetes in Youth study [35] demonstrated that 21.2% of children with physician-identified T2DM were positive for antibodies to GAD 65. The TODAY study [36] also showed that 9.8% of children who were clinically diagnosed as having T2DM were positive for antibodies to GAD 65 and/or IA-2. Moreover, the antibody-positive patients were more likely to be Caucasian (40.7%) and male (51.7%) than the antibody-negative patients (19.0% white, 35.7% male). On the other hand, in the present study, both obese and non-obese children with T2DM were found to be negative for any diabetes-related autoantibodies at the time of diagnosis and during the course of DM. We also detected some children with SPT1DM by the urine glucose screening in school children. Approximately 80% of patients with SPT1DM showed positivity for diabetes-related autoantibodies at the time of diagnosis [38,39]. At the time of the urine glucose screening, it was difficult in some cases to distinguish between children with non-obese T2DM and SPT1DM. However, children with SPT1DM, characterized by impaired β-cell function, require continuous insulin treatment from within 18 months of the diagnosis. In contrast, the β-cell secretory capacity in non-obese children with T2DM is reasonably well maintained for at least 4–5 years after the diagnosis, the children, however, eventually becoming dependent on pharmacological therapies including insulin supplementation. Butler et al. [40] demonstrated impaired insulin secretion due to decrease in the β-cell mass in patients with T2DM, and that the underlying mechanism is increased β-cell apoptosis. They also indicated that the frequency of β-cell apoptosis was increased by 10-fold in non-obese T2DM patients and by 3-fold in obese T2DM patients as compared with that in a non-diabetic control group; on the other hand, the frequency of β-cell replication was very low in all cases and not significantly different among the groups. Therefore, we consider the cause of the diabetes in patients with SPT1DM is autoimmune destruction of β-cells, while that in patients with non-obese T2DM is β-cell apoptosis, without the involvement of autoimmunity.

In conclusion, the clinical characteristics of non-obese children with T2DM seemed to be different from those of obese children with T2DM. Non-obese children with T2DM tended to show lower insulin secretory capacities and milder, but evident, insulin resistance even from the time of diagnosis; in addition, they also showed earlier requirement of pharmacological therapies during the clinical course. Some genetic factors not associated with autoimmunity may play a role in the etiology of T2DM in non-obese children, however, further studies are needed to confirm these results and to clarify the pathophysiology of T2DM in non-obese children.

**Conflict of interest**

The authors declare that they have no conflict of interest.

**References**


