Evidence for impaired insulin production and higher sensitivity in stunted children living in slums

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The objective of the present study was to investigate the changes in glucose and insulin metabolism in nutritionally stunted children that can be involved in the appearance of chronic diseases in adulthood. For this purpose, sixty-one children were selected, thirty-five boys and twenty-six girls, residents of slums in São Paulo, Brazil. The children were classified according to the height-for-age as stunted (≤−1.5 Z-score; n 21) or non-stunted (≥−1.5 Z-score; n 40). The glucose and insulin plasma levels were determined and, from these values, the indexes that evaluate the pancreatic β-cell function (homeostasis model assessment (HOMA-B)) and insulin sensitivity (HOMA-S) were assessed. Stunted children showed lower values of fasting insulin than those of the non-stunted group (boys: 29·7 (SD 14·9) v. 50·4 (SD 29·2) pmol/l, P<0·01; girls: 34·4 (SD 12·6) v. 62·3 (SD 28·7) pmol/l, P=0·016) but the glucose levels were similar (boys: 4·6 (SD 0·3) v. 4·5 (SD 0·3) mmol/l; girls: 4·2 (SD 0·3) v. 4·4 (SD 0·3) mmol/l). Stunted children showed lower HOMA-B values (boys: 83 (SD 22) % v. 115 (SD 36) %, P<0·01; girls: 107 (SD 23) % v. 144 (SD 46) %, P=0·045) and higher HOMA-S values (boys: 196 (SD 92) % v. 120 (SD 62) %, P=0·014; girls: 159 (SD 67) % v. 98 (SD 57) %, P<0·016). The results show a decreased activity of β-cell function and increased insulin sensitivity in stunted children. The decreased β-cell function of this group may strongly predict type 2 diabetes.

Stunting: Undernutrition: Insulin sensitivity: β-Cell function: HOMA

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Previous studies on nutritionally stunted children living in the slums of São Paulo and, therefore, living in extreme poverty, showed a lower fasting RQ when compared with non-stunted children of the same age, indicating a tendency to preserve adipose tissue stores (Hoffman et al. 2000c). Their finding was confirmed when these children were re-evaluated 3 years later and a gain of fat mass as opposed to lean mass was observed in comparison with non-stunted children, showing a tendency towards overweight and obesity (Martins et al. 2004). Other metabolic changes were also observed in these and other stunted children in the same communities, such as a decrease in energy expenditure (Hoffman et al. 2000c; Grillo et al. 2005), impaired regulation of food intake (Hoffman et al. 2000a) and an increase in arterial hypertension (Fernandes et al. 2003).

Recently, Florêncio et al. (2001), studying the residents of slums on the outskirts of Maceió-Alagoas, demonstrated a high prevalence of overweight/obesity (30 %) in stunted women. This condition was verified even with an energy intake of only 65 % of the required intake adjusted to stature (Florêncio et al. 2003). An association between stunting and arterial hypertension was also observed and this association was even more evident in women who presented a higher content of abdominal fat (Florêncio et al. 2004).

Studies carried out on individuals after prenatal exposure to famine during the Second World War found glucose intolerance (Ravelli et al. 1998). Although the prevalence of type 2 diabetes in individuals that were undernourished in early life is not known, it is known that poor countries with an accelerated urbanization process are particularly vulnerable and have been experiencing a considerable increase in the prevalence of type 2 diabetes (Yajnik, 2004).

Classic studies involving hospitalized, severely undernourished children observed lower fasting insulin levels and hypoglycaemia, rising during recovery and peaking during the phase of rapid growth. A subnormal insulin response to a glucose load, which frequently persisted for many months after recovery, was also described in those children (Waterlow, 1992).

According to our knowledge no studies have been done that relate the increased susceptibility to fat gain and the metabolism of glucose and insulin in stunted children.

The objective of the present study was therefore to verify if the children living in slums and who are stunted because of chronic undernutrition during infancy show changes in production and sensitivity to insulin, which could lead to type 2 diabetes in adult life, and/or facilitate body fat gain.

Methods

Protocol

Sixty children were randomly selected among the 400 children screened in an anthropometric census performed in three slums in the south area of the city of São Paulo to participate in a cross-sectional study (Hoffman et al. 2000a,b,c) and after a period of 3 years, fifty-three of these children were located.
twenty-seven boys and twenty-six girls, to participate in a second phase analysis whose details were described elsewhere (Martins et al. 2004). The slums were randomly selected from among all the slums that are within 50 km of the Federal University of São Paulo, where the study was conducted. The present results represent the evaluation of the second phase of the study, therefore being cross-sectional.

The children were divided into two groups according to their height-for-age: the stunted group (height-for-age $\leq -1.5$ Z-score, $n = 21$) and the non-stunted group (height-for-age $>-1.5$ Z-score, $n = 32$). In addition, eight non-stunted boys of the same age, recruited from the same population to participate in another study, were included in the analysis to eliminate differences in pubertal stage between groups.

The children were brought to the university in the morning for the anthropometric and clinical evaluations and collection of a fasting blood sample (20 ml) for the biochemical analyses. Before the experimental protocol began, preliminary blood, faecal and urine tests were performed to ensure that none of the children had anaemia, parasites or infections at the time of the study.

All families signed the Free and Informed Consent Form and the study was approved by the Research Ethics Committee of the Federal University of São Paulo.

Anthropometry
The height and weight were obtained for each child in light clothing and stocking feet. Body weight was determined with an electronic scale (model SD-150; Country Technologies, Gay Mills, WI, USA) with a capacity of 150 kg and a precision of 100 g. A standard stadiometer was used to determine the height of each child and the value closest to 0.1 cm was taken; each child stood against the vertical board of the stadiometer with arms hanging by the sides of the body and the head in the Frankfort plane. Weight-for-age (Z-score), height-for-age (Z-score), BMI (kg/m²) and BMI-for-age (Z-score) were calculated by the program EPI-INFO for Windows, using the National Center for Health Statistics reference (Centers for Disease Control, 2000).

Pubertal stages
The pubertal stage was evaluated by a trained physician using the Tanner classification (Tanner, 1962). The cut-off points recommended by the World Health Organization (1995) were used to classify the children into prepubertal or pubertal, that is, breast-stage 2 for girls and genitalia-stage 3 for boys.

Biochemical analyses
Plasma levels of glucose and specific insulin (without C-peptide) from the blood samples collected were determined by the colorimetric enzyme method (Bayer Inc., New York, USA) and the enzyme immunoassay method (TOSOH Inc., Tokyo, Japan) respectively.

The homeostasis model assessment (HOMA) model was used to evaluate pancreatic $\beta$-cell function and insulin sensitivity based on plasma and fasting levels of glucose and insulin. This technique uses a nonlinear mathematical model that reflects the balance between the glucose produced by the liver and basal insulin, maintained by a glucose–insulin feedback system between the liver and the pancreatic $\beta$-cells (Levy et al. 1998). The computer model is used to determine insulin sensitivity (HOMA-S) to the pancreatic $\beta$-cell function (HOMA-B) (Wallace et al. 2004).

Although the euglycaemic hyperinsulaemia and the hyperglycaemic clamps are frequently referred to as the ‘gold standard’ methods for determining insulin resistance and pancreatic $\beta$-cell insulin production, the HOMA model, since its formulation (Wallace et al. 2004), has been validated by a number of studies that showed a good correlation between the estimates of insulin resistance and the euglycaemic clamp (Bonora et al. 2000), and between the estimates of $\beta$-cell function and the hyperglycaemic clamp (Matthews et al. 1985). Uwaifo et al. (2002) used the euglycaemic and hyperglycaemic clamps to validate the use of HOMA in well-nourished children. Gungor et al. (2004) validated the model in non-diabetic children and adolescents.

Statistical analysis
Separate analyses were made for boys and girls in order to test the differences between the group means. Analyses were done with the entire sample (n = 61) and stratified by pubertal stage only for boys as only one girl of each group did not reach puberty.

The studied variables were age (years), group (stunted and non-stunted), gender (boys and girls), weight (kg), height (cm), weight-for-age (Z-score), height-for-age (Z-score), BMI (kg/m²), BMI-for-age (Z-score), pubertal stage (pubertal and prepubertal), fasting glucose (mmol/l) and insulin (pmol/l), and the calculated HOMA-B (%) and HOMA-S (%) indexes. The differences between the groups were tested with the Mann–Whitney test. Fisher’s exact test was used to verify the differences in pubertal stage distribution between the groups.

The association of the HOMA-B and HOMA-S indexes with group was tested by multiple regression analysis for the entire sample (boys and girls), independent of pubertal stage and gender. The dependent HOMA-B and HOMA-S (in distinct models) variables were logarithmically transformed because they did not have a normal distribution. Pearson correlation coefficients between HOMA-B or HOMA-S and nutritional status variables were calculated in the children overall and including only pubertal children.

The analyses were performed with the software SPSS for Windows, version 11.0 (SPSS Inc., Chicago, IL, USA), with $\alpha = 0.05$ for all the analyses.

Results
The characteristics of the children are presented in Table 1. Boys and girls in the stunted group showed values significantly lower for weight, height, weight-for-age and height-for-age, but only the group of stunted boys showed significantly lower BMI and BMI-for-age values. The mean height-for-age was $< -2.0$ Z-score for stunted boys and girls.

Table 2 shows the distribution of the children according to the pubertal stage classification. Half of the boys in the stunted group had not yet reached puberty while 35% of the boys in the non-stunted group were classified as pubertal, but the
The association between group and the HOMA-B and HOMA-S indexes remained significant in HOMA-S (Fig. 1) even after including gender and pubertal stage. In the two models tested by multiple linear regression analysis, independent of HOMA-B and HOMA-S indexes (after logarithmic transformation) was significant (boys: 196 %, P = 0.045) at the same time that their values for HOMA-S were significantly greater (boys: 196 %, P = 0.014; girls: 159 %, P = 0.016).

When the analysis was stratified by pubertal stage, the difference between groups in HOMA-B and HOMA-S values was no longer significant for pubertal boys (P = 0.302 and P = 0.424, respectively), while for prepubertal ones it remained significant in HOMA-S (P = 0.029) but not HOMA-B (P = 0.081). Among pubertal girls, the difference in the HOMA-B values between the groups was marginally significant (P = 0.061) and the significance remained for the HOMA-S values (P = 0.016).

The association between group and the HOMA-B and HOMA-S indexes (after logarithmic transformation) was tested by multiple linear regression analysis, independent of gender and pubertal stage. In the two models tested (HOMA-B and HOMA-S as dependent variables), the regression coefficient of the group variable remained significant (P = 0.001) even after including gender and pubertal stage variables, with the $R^2$ values being equal to 0.35 and 0.39, respectively (P < 0.001 in the two models).

The analyses of the relationship of the HOMA-B and HOMA-S indexes with the indicators of nutritional status for the children in the entire and pubertal children-only samples are described in Table 4. The HOMA-B index associates positively with weight-for-age, height-for-age, BMI and BMI-for-age in both samples, the one including all the children and the one including only the pubertal children. On the other hand, the HOMA-S index is negatively associated with all the evaluated indicators of nutritional status, both for the entire and for the pubertal children-only samples. The Pearson correlation coefficient was highest in the relationship between HOMA-B and HOMA-S indexes and BMI.

### Discussion

The results of the present study show changes in insulin production and sensitivity in nutritionally stunted children. Specifically, stunted boys and girls showed lower fasting plasma insulin when compared to non-stunted children and there were no differences in plasma glucose levels between the groups. The calculated HOMA-B and HOMA-S indexes showed that stunted children have lower pancreatic b-cell function and higher insulin sensitivity when compared to non-stunted children.

The effect of puberty was controlled by multiple linear regression analysis and the group effect remained significant in the HOMA-B and HOMA-S indexes, regardless of gender and pubertal stage.

The HOMA model is a useful tool to estimate b-cell function and insulin sensitivity through simple measurements, such as insulin and glucose plasma levels, and has been validated by comparison with other models (Wallace et al. 2004). Yet, this is a qualitative index, so it is not possible to identify

### Table 1. Characteristics of the study children

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th></th>
<th>Girls</th>
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<tbody>
<tr>
<td></td>
<td>Non-stunted</td>
<td>Stunted</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Age (years)</td>
<td>11·9</td>
<td>2·4</td>
<td>13·0</td>
<td>1·3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>40·9</td>
<td>9·2</td>
<td>33·3</td>
<td>6·4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>149·8</td>
<td>10·6</td>
<td>140·4</td>
<td>7·7</td>
</tr>
<tr>
<td>Weight-for-age (Z-score)</td>
<td>0·8</td>
<td>−1·7</td>
<td>0·7</td>
<td>&lt;0·001</td>
</tr>
<tr>
<td>Height-for-age (Z-score)</td>
<td>0·1</td>
<td>0·9</td>
<td>−2·0</td>
<td>0·7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>18·0</td>
<td>1·7</td>
<td>16·8</td>
<td>1·9</td>
</tr>
<tr>
<td>BMI-for-age (Z-score)</td>
<td>0·0</td>
<td>−0·9</td>
<td>0·9</td>
<td>0·003</td>
</tr>
</tbody>
</table>

### Table 2. Pubertal stage distribution

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<th>Boys</th>
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<th>Girls</th>
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<tbody>
<tr>
<td></td>
<td>Non-stunted</td>
<td>Stunted</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Pre-puberty</td>
<td>8</td>
<td>35</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>Puberty</td>
<td>15</td>
<td>65</td>
<td>6</td>
<td>50</td>
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the mechanisms involved in the changes found in insulin sensitivity. The increase in insulin sensitivity may be due to a higher number of insulin peripheral receptors, especially in the adipose and muscle tissues. This increase in sensitivity may establish a counter-regulation mechanism to compensate for the low levels of insulin, which could have contributed to the preferential increase of fat with age observed in these children and described in a previous study (Martins et al. 2004).

According to our knowledge, this is the first time that a study shows a decrease in pancreatic β-cell function associated with an increase in insulin sensitivity in nutritionally stunted children by use of the HOMA index. In his review about protein-energy undernutrition and the resultant metabolic changes, Waterlow (1992) had already documented classic studies showing the occurrence of lower levels of plasma insulin, as well as hypoglycaemia, in severely undernourished children.

In the present study, the most likely explanation for the decrease in insulin production by the pancreatic β-cells is the presence of a lower number of cells due to undernutrition. As far as we know, the only data available are from intrauterine undernutrition. By making a morphological analysis of the pancreas of fetuses presenting intrauterine growth retardation, Van Assche et al. (1977) observed a reduction in endocrine pancreatic tissue and insulin-producing β-cells. Recently, Holemans et al. (2003) described many studies involving man and animals with intrauterine undernutrition, where a lower secretion of insulin was associated with a delay in the development of pancreatic β-cells. Holemans et al. (2003) speculate that these changes could lead to an impaired adaptation of the pancreas to diets with higher glucose content and to insulin resistance in adult life. Cianfarani (2003) also suggested that intrauterine undernutrition would affect the number of stem cell precursors of pancreatic β-cells, which could lead to an early exhaustion of organ function, especially when the demand for insulin increases.

In the present study, besides a decrease in the HOMA-B index, we found an increase in insulin sensitivity in stunted children. Similar to what was observed in the present study, studies in animals observed a decrease in insulin secretion and an increase in the insulin-mediated total glucose uptake in rats that were subject to food restriction in early postnatal life (Picarel-Blanchot et al. 1995; Moura et al. 1996, 1997). Picarel-Blanchot et al. (1995) attributed their results to an adaptation that would limit the deterioration to glucose tolerance. Nevertheless, other studies showed that the insulin sensitivity pattern changes with age (Ozanne & Hales, 2002). During the early phases of adult life, rats born to dams fed a low-protein diet showed better glucose tolerance when compared to a control group (Langley et al. 1994; Hales et al. 1996; Holness 1996). Yet, at

**Table 3. Plasma glucose and insulin levels of stunted and non-stunted boys and girls**

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<thead>
<tr>
<th></th>
<th>Boys</th>
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<th>Girls</th>
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<tbody>
<tr>
<td></td>
<td>Non-stunted</td>
<td>Stunted</td>
<td>Non-stunted</td>
<td>Stunted</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.6 0.3</td>
<td>4.5 0.2</td>
<td>6.3 0.3</td>
<td>4.2 0.3</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>50.4 29.1</td>
<td>29.7 14.9</td>
<td>62.3 28.7</td>
<td>34.4 12.6</td>
</tr>
</tbody>
</table>

*For details of procedures, see p. 997.
44 weeks of age, the glucose tolerance was similar between the two groups (Petry et al. 1997) and at 15 months of age, the relationship inverted, with the protein-restricted group showing a lower glucose tolerance than the control group (Hales et al. 1996). At 17 months, diabetes mellitus was detected in these rats (Petry et al. 2001). Studies in isolated rat tissues suggested that these changes in sensitivity seem to be associated with variations in the number of insulin receptors (Ozanne et al. 1996, 2003).

The findings of the present study may be especially harmful to poor individuals living in urban areas and facing the challenges of the high glycaemic index of modern food types. Studies in adults with low birth weight have been showing a greater insulin resistance than normal individuals (Phillips, 1996; Lithell et al. 1996; Jaquet et al. 2000). A study in 20-year-old young adults who had a history of undernutrition in the first year of life showed that a larger amount of abdominal fat (measured by computed tomography) was more detrimental to insulin sensitivity (evaluated by euglycaemic and hyperinsulinaemic clamp) than a higher degree than in individuals that had never been undernourished (Boule et al. 2003). Another study involving Indian children showed that the highest levels of insulin resistance were found in children who had a low birth weight and a higher fat mass at 8 years (Bavdekar et al. 1999). On the other hand, Moore et al. (2001) did not find changes in the glucose–insulin axis in individuals of the rural area of Gambia that were undernourished in childhood but remained lean in adult life.

The present study showed a decrease in pancreatic β-cell function and an increase in insulin sensitivity in children who have, as has previously been shown, increased body fat gain (Martins et al. 2004). In addition, the present findings on the metabolism of glucose and insulin may be involved in the metabolic changes that are responsible for the appearance of chronic diseases in adult life, such as obesity and type 2 diabetes. The large proportion of poor people living in urban areas underlines the importance of preventing stunting and therefore future non-communicable diseases later in life, in developing countries.

References
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Hoffman DJ, Sawaya AL, Coward WA, Wright A, Martins PA, de Nascimento C, Tucker KL & Roberts SB (2000b) Energy...
expenditure of stunted and nonstunted boys and girls living in the shantytowns of Sao Paulo, Brazil. Am J Clin Nutr 72, 1025–1031.


