Indices of Insulin Action, Disposal, and Secretion Derived From Fasting Samples and Clamps in Normal Glucose-Tolerant Black and White Children

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OBJECTIVE — To validate fasting indices of insulin sensitivity and secretion in a diverse pediatric population against gold standard estimates from euglycemic and hyperglycemic clamps.

RESEARCH DESIGN AND METHODS — A total of 31 children (mean BMI 25.1 ± 4.9 kg/m², mean age 8.7 ± 1.4 years, 15 girls and 16 boys, 12 black and 19 white) underwent euglycemic and hyperglycemic clamps 2–6 weeks apart to derive insulin sensitivity indices (SI_Eug clamp and SI_Hyper clamp). Fasting samples were used to derive the homeostasis model assessment of insulin resistance index (HOMA-IR), HOMA of percent β-cell function (HOMA-B%), quantitative insulin sensitivity check index (QUICKI), insulinogenic index, antilipolytic insulin sensitivity index, and C-peptide–to–insulin ratio.

RESULTS — The QUICKI correlated best with SI_Eug clamp (r = 0.69, P < 0.05) and had greater correlations to SI_Eug clamp than did either SI_Hyper clamp (r = 0.45, P < 0.05) or the HOMA-IR (r = –0.51, P < 0.05). Both fasting insulin and the insulinogenic index correlated well with first- and steady-phase insulin secretion (r’s from 0.79 to 0.86, P < 0.05). HOMA-B% was not as highly correlated (r = 0.69–0.72, P < 0.05). Fasting C-peptide–to–insulin ratio was not significantly correlated with clamp-derived metabolic clearance rate of insulin. ISI-FFA was not correlated with the degree of free fatty acid suppression obtained from the clamps.

CONCLUSIONS — The QUICKI, fasting insulin, and the insulinogenic index all closely correlate with corresponding clamp-derived indices of insulin sensitivity and secretion in this diverse pediatric cohort. These results, if replicated in similarly diverse populations, suggest that estimates based on fasting samples can be used to rank order insulin secretion and sensitivity in pediatric cohorts.

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Obesity and type 2 diabetes are diseases that have assumed considerable public health importance in the 21st century in both developed and developing countries (1–3). The increased prevalence of both these conditions in children adds an added dimension of seriousness to these modern-day epidemics (4). Since insulin resistance appears central to the development of the metabolic syndrome X (1,5), accurate quantification of insulin’s in vivo action, secretion, and disposal is necessary. While a combination of hyperglycemic and euglycemic-hyperinsulinemic clamp studies supplies the gold standard for quantifying these parameters (6), clamp studies are expensive and difficult tests to perform and require highly trained personnel. The difficulties with obtaining sequential clamp studies are even more pronounced for young children who may have more difficulty with clamp procedures.

For the purpose of epidemiologic studies, several indices based on fasting blood that estimate insulin sensitivity, secretion, and disposal have been developed for adults. The homeostasis model assessment of insulin resistance index (HOMA-IR), the HOMA of percent β-cell function (HOMA-B%), the insulinogenic index, and the QUICKI are among the best validated and most widely used (7–9). Validation for these indices in pediatric populations using gold standard clamp studies is largely lacking. Therefore, to examine the relationships between fasting indices of insulin sensitivity and secretion to clamp-derived estimates, we recruited a diverse population of children, obtained clamp-derived estimates, and compared the insulin profile indices from clamp studies with those derived from fasting blood.

RESEARCH DESIGN AND METHODS

Subjects
Overweight and nonoverweight children were recruited for metabolic studies through mailed notices to 6- to 12-year-old children in the Montgomery and Prince George’s Counties, Maryland school districts, as well as in the Washington D.C. area, and through local physi-
Fasting indices of insulin action in children

Clinical protocol

Subjects were studied at the Warren Grant Magnuson Clinical Center of the National Institutes of Health. Each subject had a full history and physical examination. BMI was calculated and BMI SD score (BMI SDS) was computed for each subject by using the formula BMI SDS = (actual BMI – mean BMI for age, race, and sex)/BMI SD for age, race, and sex based on established standards and norms (10). Breast development was recorded according to Tanner stages, and testicular volumes were measured according to methods of Prader (11). Weight was measured to the nearest 0.1 kg using a calibrated digital scale (ScaleTronix, Wheaton, IL). Height was measured in triplicate to the nearest 1 mm using a stadiometer calibrated before each set of measurements (Holtain Crymych, Wales, U.K.).

A hyperglycemic clamp study was subsequently performed, and 2–6 weeks later, subjects underwent a euglycemic clamp study. Hyperglycemic and euglycemic clamp studies were carried out using a modification of the methods described by DeFronzo et al. (6) with serial measures of insulin, glucose, C-peptide and free fatty acids (FFAs) (12). Fasting glucose, insulin, C-peptide, and FFAs were obtained at the beginning of each of these studies in triplicates and the means were used for the derived indices.

The euglycemic clamp studies involved a continuous infusion of regular insulin (Humulin S; Eli Lily, Indianapolis, IN) at a rate greater than 40 mU · m² body surface area · min⁻¹ during the 180-min duration of the test. This rate was chosen to achieve sustained plasma insulin levels above 1,500 pmol/l in order to completely suppress endogenous hepatic glucose output. Plasma glucose during the studies were maintained within the “normal” range of 5.3–5.8 mmol/l with a continuous infusion of variable amounts of 20% dextrose as previously described (6). Infusion adjustments were made every 5 min and steady-state hyperinsulinemia (plasma insulin >1,500 pmol/l) with coincident euglycemia (plasma glucose between 5.3 and 5.8 mmol/l) was achieved for all subjects in the study between 120- to 180-min periods of the test, which was the designated steady-state period. The procedure for the hyperglycemic clamp studies has been published previously (12).

Plasma FFAs were measured during the clamps using an enzymatic colorimetric assay (Wako Laboratories, Richmond, VA). Plasma glucose was concurrently measured using a glucose analyzer (Yellow Springs Instrument, Yellow Springs, OH), calibrated to within 5% of multiple glucose standards (50, 100, 125, 150, 250, and 500 mg/dl) before each study and using a Hitachi 736-30 analyzer (Boehringer Mannheim, Indianapolis, IN). Plasma insulin was measured by the TOSOH two-site immunoenzymometric assay (Covance, Vienna, VA). The cross-reactivity of the assay with proinsulin and C-peptide are both <1% while the mean inter- and intra-assay coefficients of variation are 5.8 and 3.6%, respectively. Serum C-peptide was assayed during the same time points of the studies using the analyte-specific reagents immunochromatometric assay (ICMA) method (Mayo Medical Laboratories, Rochester, MN).

All subjects also had an oral glucose tolerance test (OGTT) performed. This involved administration of 1.75 g/kg body wt of glucose (as a cola syrup called glucola) at the initiation of the test. The maximum dose administered was 75 g, and the samples for plasma glucose and insulin were obtained at baseline and 2 h later. The details of the methodology are as previously described (12). The definitions for normal, impaired fasting glucose, impaired glucose tolerance, and diabetes based on the OGTT were based on the established American Diabetes Association criteria (13).

Derived indices from clamp studies

Whole-body glucose uptake from the hyperglycemic clamp studies was estimated as the metabolic rate (M), defined as the infusion rate of exogenous glucose administered, corrected for urinary glucose losses and the glucose space correction (6,14). As a measure of insulin sensitivity (SI hyper clamp), the ratio of metabolic rate to steady-state insulin (M/I) was calculated (6,14). Whole-body glucose uptake from the euglycemic clamp studies was estimated as the metabolic rate (M), defined as the infusion rate of exogenous glucose administered, corrected for urinary glucose losses and the glucose space correction (6,14). The metabolic clearance rate (MCR) for insulin was computed as the insulin infusion rate divided by the increase in plasma insulin concentration above baseline (6). As a measure of insulin sensitivity (SI Eugl clamp), the ratio of metabolic rate to steady-state insulin (M/I) was calculated (6,14). The first-phase and steady-state insulin and C-peptide levels were derived from the hyperglycemic clamp study as indices of pancreatic β-cell secretory capacity (6,15). The C-peptide–to–insulin molar ratios for the first-phase and steady-state phase of the hyperglycemic clamp study were derived as indices of dynamic hepatic insulin clearance, respectively (15,16). The degree of FFA suppression from baseline during the clamp studies was utilized as an index of insulin sensitivity as an antilipolytic (17,18).

Derived indices from fasting blood samples

The HOMA-IR, QUICKI index, and fasting glucose–to–insulin ratios were derived as estimates of insulin sensitivity (7,8). HOMA-IR was computed using the following formula: (fasting insulin in mU/ml × fasting glucose in mmol/l)/22.5, while QUICKI was computed as 1/log fasting insulin in mU/ml + log glucose in mg/dl). In addition to the fasting C-peptide and insulin levels, the insulinogenic index and the HOMA-B% were derived as indices of pancreatic β-cell function (7,9). The insulinogenic index was computed as the ratio of fasting insulin in mU/ml and fasting glucose in mg/dl, while the HOMA-B% was computed as 20 × fasting insulin in mU/ml/fasting glucose in mmol/l – 3.5. The fasting C-peptide–to–insulin molar ratio was considered an index of hepatic insulin...
clearance. Estimates of insulin’s antilipolytic effect based on fasting insulin and FFA levels were obtained using the nomogram reported by Belfiore et al. (19,20).

Statistical analysis

The derived data were analyzed using JMP IN version 3.2.1 software for Windows (1989–1997, SAS Institute, Cary, NC) and StatView version 5.0.1 for Windows (1992–1998, SAS Institute). Standard tests of data symmetry using skewness and kurtosis were performed on all data, and normality was tested using the Shapiro-Wilk test. Nonnormal data could not be normalized by transformation procedures to achieve data symmetry and normality before use of parametric tests. Data that were transformed by common log or other transformation procedures were analyzed using equivalent nonparametric tests. Unless otherwise indicated, data are reported as mean ± SD. Correlations between parameters were evaluated using Spearman correlation coefficients. Comparisons between groups of data were done using unpaired Student’s t tests, ANOVA, or ANCOVA. P < 0.05 was considered significant for all the data analyses.

RESULTS — A total of 31 children (12 black and 19 white) were studied (Table 1). Of the study subjects, 81% were obese, having a BMI percentile ≥95th percentile for age, sex, and race (4).

All the children had normal OGTTs. There were no children with diabetes, impaired fasting glucose, or impaired glucose tolerance. The mean fasting glucose and insulin levels are as shown in Table 2 while the corresponding mean 2-h glucose and insulin values are 5.98 ± 0.87 mmol/l and 536.2 ± 578.5 pmol/l.

Figure 1—Correlation plot of fasting insulin sensitivity indices and BMI SDS. A: Correlation between QUICKI and BMI SDS. B: Correlation between HOMA-IR and BMI SDS.

was a predominantly obese cohort of children (20). Both QUICKI and HOMA-IR were significantly related to the BMI SDS (Fig. 1).

Table 3 shows the mean insulin sensitivity, secretion, and clearance parameters derived from the clamp studies and demonstrates the wide range of values observed. Spearman correlation coefficients between the fasting and clamp estimates (Table 4 and Fig. 2) demonstrate that the best fasting indices of pancreatic β-cell function correlate well with the clamp-derived estimates.

Table 2—Fasting indices of insulin secretory capacity, sensitivity, and hepatic insulin clearance

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<thead>
<tr>
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<th>Mean ± SD</th>
<th>Range</th>
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<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.86 ± 0.60</td>
<td>2.90–5.70</td>
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<tr>
<td>Fasting insulin (pmol/l)</td>
<td>82.50 ± 63.10</td>
<td>13.6–253.30</td>
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<tr>
<td>Fasting C-peptide (nmol/l)</td>
<td>0.70 ± 0.36</td>
<td>0.29–1.70</td>
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<tr>
<td>Insulinogenic index</td>
<td>0.13 ± 0.09</td>
<td>0.02–0.40</td>
</tr>
<tr>
<td>HOMA-B%</td>
<td>185 ± 120</td>
<td>24–523</td>
</tr>
<tr>
<td>Glucose/insulin ratio  (in conventional units)</td>
<td>12.30 ± 9.30</td>
<td>2.50–47.90</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.50 ± 2.00</td>
<td>0.4–7.6</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.354 ± 0.042</td>
<td>0.288–0.463</td>
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<tr>
<td>ISI FFA</td>
<td>0.64 ± 0.31</td>
<td>0.20–1.36</td>
</tr>
<tr>
<td>C-peptide/insulin molar ratio</td>
<td>10.80 ± 5.40</td>
<td>4.0–31.2</td>
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Table 1—Subject demographics

<table>
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<tr>
<td>Sex (F/M)</td>
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<tr>
<td>Race (W/B)</td>
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<tr>
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<td>9/14 Tanner 1, 5 Tanner 2, 1 Tanner 3 (range 1–3)</td>
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<td>Girls’ breast Tanner stage</td>
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<tr>
<td>Boys’ Tannar pubic hair stage</td>
<td>9 Tanner 1, 4 Tanner 2, 2 Tanner 3 (range 1–3)</td>
</tr>
<tr>
<td>Age (years)</td>
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</tr>
<tr>
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<tr>
<td>BMI (kg/m2)</td>
<td>25.1</td>
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<tr>
<td>Waist circumference (cm)</td>
<td>73.8</td>
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<tr>
<td>Hip circumference (cm)</td>
<td>84.8</td>
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<tr>
<td>Data are n or means ± SD (range).</td>
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</table>
secretory capacity were the fasting insulin and insulinogenic index, rather than the HOMA-B%. The correlation coefficient between SI Eug clamp and the QUICKI (r = 0.69) was greater than that between SI Eug clamp and SI Hyper clamp (r = 0.45, P < 0.05) or between SI Eug clamp and HOMA-IR (r = −0.51, P < 0.05). The fasting measures of insulin’s lipid-modulating effect, however, were not significantly correlated with the degree of FFA suppression during the clamp studies. The fasting C-peptide-to-insulin ratio (an index of hepatic insulin clearance) was not significantly correlated with either the MCR of insulin (from the euglycemic clamp) or the steady-phase C-peptide-to-insulin ratio (from the hyperglycemic clamp), although it was correlated with the first-phase C-peptide-to-insulin ratio (from the hyperglycemic clamp).

CONCLUSIONS — We found in a diverse cohort of children (n = 31) aged 6.2–11.3 years that fasting indices of insulin sensitivity correlated well with estimates obtained from the gold standard method, the euglycemic-hyperinsulinemic clamp. In addition, the fasting insulin and insulinogenic indices were found to correlate well with the gold standard method for estimating pancreatic β-cell secretion, the hyperglycemic clamp. Although the hyperglycemic clamp is being increasingly used to estimate insulin sensitivity, it is crucial to note that this has significant limitations and potential caveats (14,21) and that the euglycemic clamp remains the most robust method for quantifying glycemic insulin sensitivity (14,21). Fasting estimates of insulin’s ability to modulate FFAs did not, however, correlate with the degree of FFA suppression from either clamp study. The fasting C-peptide-to-insulin molar ratio did not correlate with the MCR from the euglycemic clamp or with the steady-phase C-peptide-to-insulin ratio, but it did correlate with the first-phase C-peptide-to-insulin ratio, suggesting that this index may possibly be a useful surrogate of hepatic insulin clearance but not of total insulin clearance.

Although they clearly yield the most robust measures of insulin sensitivity, pancreatic β-cell secretion, and total insulin clearance (6), the combined clamp studies are difficult to perform, require sophisticated equipment and highly trained staff, and carry the potential risks of hypoglycemia if intravenous access is lost during the hyperinsulinemic clamp.

Since its initial description (7), the HOMA-IR has been validated in diverse adult populations (22–28). There are, however, few data on its utility in pediatric populations (29) and, to our knowledge, no pediatric information on its validation against clamps. Our cohort showed a significant correlation between the HOMA-IR and the SI estimates from
both the euglycemic and hyperglycemic clamps ($r = 0.51$ and $r = 0.56$, respectively). These correlation coefficients were comparable to that between the $\text{SI}_{\text{Hyper clamp}}$ and the $\text{SI}_{\text{Eug clamp}}$. However, the more recently developed QUICKI index, which has been suggested to have excellent correlation with clamp-derived insulin sensitivity estimates in adults (8, 30, 31), had a significantly greater correlation with $\text{SI}_{\text{Eug clamp}}$. As with the HOMA-IR, there are to our knowledge no validation data of the QUICKI with clamp indices in children (29). However, a recent report in abstract form documents similar trends in a large cohort of children on whom fasting data and euglycemic clamps were obtained (32). Silfen et al. (33) have, however, previously found that in a cohort of prepubertal girls with premature adrenarche and/or obesity, QUICKI correlated well with OGTT-derived estimates of insulin sensitivity.

Among the measures of pancreatic $\beta$-cell–secretory capacity, the first-phase and steady-state insulin secretion from the hyperglycemic clamp studies are believed to give the most robust estimates (6). The HOMA-B% first described by Matthews et al. (7) correlated reasonably well with these measures; however, the simpler insulinogenic index and fasting insulin levels had even higher correlations ($r = 0.79–0.86$). This finding has also been described in several adult studies (9, 34–36).

Based on known secretion and kinetics patterns of C-peptide and insulin, (15, 37, 38) the molar C-peptide–to–insulin ratio has been suggested as a good surrogate of hepatic insulin clearance. Our data did not show a significant correlation between the fasting C-peptide–to–glucose ratio and MCR from the euglycemic clamp. Thus, the C-peptide–to–insulin ratio cannot be recommended as a surrogate for the euglycemic clamp MCR. Similarly, the ISI-FFA, which has been validated in adults (20), did not correlate with the degree of FFA suppression during clamp studies and thus appears to be an inadequate substitute for the clamp-derived measures.

Overall, our study results suggest that the fasting-derived QUICKI index and insulinogenic index have significant predictive value for estimating both insulin sensitivity and pancreatic $\beta$-cell secretion in children and could thus be used in large epidemiologic studies of pediatric populations. However, there are some important caveats to mention in interpreting these findings. First, there is biological variability in fasting glucose and insulin levels, and some have expressed concerns regarding the degree of repeatability of the fasting data upon which these indices are dependent (39). In the current study, we found no statistically significant differences between measures obtained from the two clamp studies (performed 2–6 weeks apart). Second, reports in adult cohorts suggest limitations in the utility of the HOMA-IR in men with impaired glucose tolerance (40) and in the QUICKI’s ability to detect changes in insulin sensitivity brought about by exercise training (41). As no pediatric subjects with impaired glucose tolerance were studied, and given that no data were obtained in relation to exercise training, we do not know if these limitations will also apply to these indices in children. Third, the relatively small sample size of our cohort makes it insufficiently powered to perform subgroup analyses of the potential confounding effects of sex, pubertal status, obesity versus leanness, and ethnicity on these findings. Finally, it is known that by measuring only the fasting glucose concentration (which is largely dependent on baseline hepatic glucose output), one cannot identify patients who may have impaired glucose tolerance and/or diabetes despite having normal fasting glucose (42, 43). There is a similar discordance between fasting and postprandial insulin levels, which could thus result in fasting-based indices that underestimate insulin resistance (44). Based on these important caveats, it seems that indices based exclusively on fasting data rather than dynamic data are best restricted to use in large epidemiologic studies rather than smaller intervention and/or screening metabolic studies.

In summary, for a diverse group of lean and overweight children, the QUICKI correlated most closely with the $\text{SI}_{\text{Eug clamp}}$ and the insulinogenic index, and fasting insulin correlated closely with both first-phase and steady-phase hyperglycemic clamp insulin secretion. Fasting estimates of hepatic insulin clearance did not, however, correlate with MCR of insulin, nor did fasting estimates of insulin’s antilipolytic effect correlate with the degree of FFA suppression from clamp studies. If these findings are replicated in larger, similarly diverse pediatric cohorts.
it would suggest a place for the use of these fasting indices in large epidemiological surveys. However, while these fasting indices of insulin sensitivity and secretory capacity might be suitable for large epidemiological studies of pediatric populations, their use cannot fully substitute for more accurate measures of insulin sensitivity and secretory capacity.

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