Relation Between Acanthosis Nigricans and Insulin Sensitivity in Overweight Hispanic Children at Risk for Type 2 Diabetes

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OBJECTIVE — To investigate in a population of Hispanic children if 1) the presence of acanthosis nigricans (AN) is related to insulin sensitivity (SI) independent of adiposity and 2) scale scoring AN severity adds to the clinical estimation of insulin sensitivity, above and beyond the presence or absence AN alone.

RESEARCH DESIGN AND METHODS — The study population, 131 Hispanic overweight children (mean BMI percentile 97.0 ± 3.1, 72 boys, 59 girls, ages 8–13 years, mean Tanner stage 2.4 ± 1.5) with a family history of type 2 diabetes, underwent a physical examination of the neck to determine AN absence or presence (0–1), AN extent score (0–4 scale), AN texture score (0–3 scale), and an AN combined score (extent + texture; 0–7 scale). SI was measured by the frequently sampled intravenous glucose tolerance test and minimal modeling. Multivariate linear regression analysis was used to determine the role of BMI and AN in predicting SI.

RESULTS — BMI was the main predictor of SI, explaining ~41% of the variance. The presence of AN explained an additional 4% of the variability in SI; scale scoring AN extent or texture did not significantly improve the prediction.

CONCLUSIONS — Although AN is an independent risk factor for insulin resistance in overweight Hispanic children at risk for type 2 diabetes, body adiposity is the primary determinant of insulin sensitivity. In addition, scale scoring AN seems of minimal usefulness in clinically estimating the severity of insulin resistance over and above assessing the presence or absence of AN and calculating BMI.

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With the rise of overweight (1) and type 2 diabetes (2,3) in the pediatric population, especially in young Hispanics and other minority groups, early identification of those at elevated health risk is important so that appropriate interventions can be established. Insulin resistance is thought to be a major factor in the pathophysiology of type 2 diabetes in both adults and children (4–6). Acanthosis nigricans (AN), a skin disorder characterized by hyperpigmentation, hyperkeratosis, and papillomatosis, is a clinical marker that has been linked to surrogate markers of insulin resistance in adults (7,8) and adolescents (9), but only a few studies have explored the relation between directly measured insulin sensitivity (SI) and AN in children (10–12). Studies in adults have suggested that those with AN have elevated fasting insulin levels (13,14). However, these studies were limited in that they did not measure SI directly, neither did they evaluate the severity of AN. Burke et al. (8) proposed a classification system to grade the severity of AN and found that the severity of AN was associated with elevated fasting insulin and increased BMI. Previous studies that have examined the relation between SI and the severity of AN in adults and children have found mixed results (10–12). Thus, the interrelation between obesity, insulin resistance, and severity of AN still needs to be clarified, particularly in children at risk for diabetes.

Therefore, in this population of Hispanic children at risk of developing type 2 diabetes, our objectives were to 1) determine if the presence of AN was related to SI independent of adiposity and 2) ascertain if scale scoring AN severity added to the clinical estimation of SI above and beyond the presence or absence of AN alone.

RESEARCH DESIGN AND METHODS — The study population of 131 children (72 boys and 59 girls) was recruited through the Study of Latino Adolescents at Risk for Type 2 Diabetes (an ongoing longitudinal study of the pathophysiology of type 2 diabetes in Latino youth). The subjects were screened to meet the following inclusion criteria: 1) Hispanic origin (both sets of grandparents reported to be Hispanic), 2) a positive family history (sibling, parent, or grand-
parent) for type 2 diabetes, 3) age 8–13 years, 4) BMI ≥85th percentile for age and sex according to the Centers for Disease Control and Prevention growth charts (15), and 5) absence of diabetes, as established by an oral glucose tolerance test and according to Report of Expert Committee on the Diagnosis and Classification of Diabetes Mellitus diagnosis criteria (16,17). Children were excluded if they were taking medications known to affect insulin resistance or body composition or had been diagnosed with any major illness since birth. The subjects were recruited from greater Los Angeles County, California, through a combination of medical referrals (Los Angeles County + University of Southern California [USC] Pediatric Diabetes Prevention Clinic and other local physicians), local advertisements, local health fairs, and word of mouth. The subjects were of Mexican-American and Central-American heritage. The Institutional Review Board of the Health Sciences Campus, USC, approved the study. Informed consent was obtained from all parents and assent was obtained from all children after the nature of the procedures was explained and before testing commenced.

Subjects arrived at the USC General Clinical Research Center at ~0800 after an overnight fast for the outpatient visit. The subjects underwent a comprehensive physical examination, which included Tanner staging (18,19), anthropometric measurements (height and weight were recorded to the nearest 0.1 cm and 0.1 kg, respectively), and neck examination for the absence or presence of AN.

Subjects were admitted to the research center in the afternoon 1–2 weeks after the outpatient visit. A whole-body dual-energy X-ray absorptiometry (DEXA) scan was performed to determine whole-body composition using a Hologic QDR 4500W. The subjects were given dinner and an evening snack, with all food being consumed before 2000. Only water could be consumed between 2000 and testing the following morning.

**Determination of AN neck severity**

AN of the neck was assessed in all subjects by a single trained clinician (M.J.W.) as part of the comprehensive physical examination. The neck alone was used because of ease of access and higher reproducibility and because the neck is always involved when other areas are affected (20).

If AN was present, it was scored using the Burke neck AN scoring methods (8). AN severity was thus expressed by four separate scoring scales: AN dichotomous score (absent/present), AN extent score (0–4 scale), AN texture score (0–3 scale), and AN combined score (extent + texture; 0–7 scale). The AN combined scale represents the simple sum of the Burke scores for AN extent and AN texture.

**Insulin-modified frequently sampled intravenous glucose tolerance test**

At ~0630, a topical anesthetic (EMLA cream; AstraZeneca, Wilmington, DE) was applied under occlusion to the antecubital fossa of both arms; flexible intravenous catheters were then placed in both arms at ~0730. Two fasting blood samples were drawn at ~15 and ~5 min for determination of basal glucose and insulin. At t = 0, glucose (25% dextrose, 0.3 g/kg body wt) was administered intravenously over 1 min in one arm, and blood samples from the contralateral arm were collected at the following time points: 2, 4, 8, 19, 22, 30, 40, 50, 70, 100, and 180 min. Insulin (0.02 units/kg body wt; Humulin R regular insulin for human injection; Eli Lilly, Indianapolis, IN) was injected intravenously at 20 min. Plasma was analyzed for glucose and insulin, and values were entered into the MINMOD MILLENIUM 2003 computer program (version 5.16; Richard N. Bergman) for determination of $S_i$ and the acute insulin response (21,22). Insulin was assayed in duplicate with a specific human insulin enzyme-linked immunosorbent assay kit from Linco (St. Charles, MO). The insulin interassay coefficients of variation from our laboratory are 7–10%; the insulin intra-assay coefficient of variation is <2%. Glucose was measured in duplicate using a YSI 2700 Analyzer (YSI, Yellow Springs, OH).

**Statistical analysis**

Sex differences in metabolic and physical characteristics were explored using independent samples t tests. Spearman correlations were used to identify associations among $S_i$, anthropometric and body composition variables, and the four AN scoring scales. Because $S_i$ data were not normally distributed, this variable underwent log transformation before being entered as the dependent variable into the regression models. Multivariate regression analysis was used to determine which measure of AN explained the most variance in $S_i$, independent of body composition, age, and sex. In models 1–5, the log-transformed $S_i$ was entered as the dependent variable and sex, Tanner stage and BMI were included as the independent variables. In addition, models 2–5 included the following measures of acanthosis: AN dichotomous, AN extent, AN texture, and AN combined, respectively. $P < 0.05$ was considered to be significant. Statistical analysis of the data was performed using Statistical Packages for Social Sciences (SPSS) version 11 and EpiInfo software (version 6.0; Centers for Disease Control and Prevention, Atlanta, GA).

**RESULTS**

**Physical and metabolic characteristics of subjects**

The physical and metabolic characteristics of the children in this study are presented in Table 1. There were no significant physical or metabolic differences across sex, except that girls tended to be of a higher Tanner stage compared with boys ($P < 0.01$). Within this sample, 96 of 131 (73%) subjects had AN. Those with AN subsequently underwent severity scoring as described in RESEARCH DESIGN AND METHODS. All AN measures correlated negatively with $S_i$ (correlation coefficients $r_{i} = 0.45$ to 0.61; $P < 0.01$) and positively with all measures of body composition (correlation coefficients 0.478–0.750; $P < 0.01$) (Table 2).

**Multiple linear regression analysis to establish the contribution of AN measures to $S_i$, independent of BMI**

Multivariate linear regression analysis showed that BMI was significantly ($P < 0.001$) and negatively related to $S_i$ after adjustment for sex and Tanner stage and, all together, contributed ~41% of the variance in $S_i$ (model 1, $r_{i}^{2} = 0.41$) (Table 3). When this same regression was calculated to include AN as a dichotomous variable, the model explained 44.6% of the variance in $S_i$ (Model 2). However, when AN was entered into the regression model without BMI, it could explain only 24.1% of the variance in $S_i$. When the remaining AN scales were substituted for the AN dichotomous scale, the models explained 46.3–47.3% of the variance in $S_i$ (models 3–5). All AN measures were negatively and significantly related to $S_i$ and
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Table 1—Physical and metabolic characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th>Girls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>72</td>
<td>59</td>
<td>131</td>
</tr>
<tr>
<td>Age (years)</td>
<td>11.3 ± 1.6</td>
<td>11.0 ± 1.7</td>
<td>11.1 ± 1.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>151.0 ± 10.8</td>
<td>149.2 ± 12.3</td>
<td>150.2 ± 11.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.7 ± 17.2</td>
<td>66.1 ± 23.0</td>
<td>65.3 ± 19.9</td>
</tr>
<tr>
<td>Tanner stage*</td>
<td>1.9 ± 1.3</td>
<td>3.0 ± 1.4</td>
<td>2.4 ± 1.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.0 ± 4.9</td>
<td>28.8 ± 6.1</td>
<td>28.4 ± 5.5</td>
</tr>
<tr>
<td>BMI percentile</td>
<td>97.0 ± 3.1</td>
<td>96.7 ± 3.1</td>
<td>96.9 ± 3.1</td>
</tr>
<tr>
<td>Total fat mass (kg)</td>
<td>23.4 ± 8.9</td>
<td>26.6 ± 8.8</td>
<td>24.8 ± 10.3</td>
</tr>
<tr>
<td>Total lean tissue mass (kg)</td>
<td>39.0 ± 10.1</td>
<td>37.0 ± 11.2</td>
<td>38.0 ± 10.6</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>93.9 ± 6.3</td>
<td>93.0 ± 5.7</td>
<td>93.4 ± 6.0</td>
</tr>
<tr>
<td>2-h glucose (mg/dl)</td>
<td>124.2 ± 18.9</td>
<td>123.2 ± 15.9</td>
<td>123.8 ± 17.6</td>
</tr>
<tr>
<td>Fasting insulin (μU/ml)</td>
<td>18.2 ± 11.3</td>
<td>21.9 ± 14.9</td>
<td>19.9 ± 13.1</td>
</tr>
<tr>
<td>Insulin sensitivity (×10⁻⁴ min⁻¹·μU⁻¹·ml⁻¹)</td>
<td>1.9 ± 1.1</td>
<td>2.0 ± 1.4</td>
<td>1.9 ± 1.3</td>
</tr>
</tbody>
</table>

AN dichotomous (absent/present) [%] 21/51 (70.8) 14/45 (76.3) 35/96 (73.3)

Data are means ± SD unless otherwise noted. *Tanner stage of boys vs. girls, P < 0.01.

Independent of BMI (models 2–5) (Table 3). Results were not appreciably different if total fat and total lean tissue mass measured via DEXA were included as body composition variables in place of BMI (data not shown).

Conclusions — The major finding of this study was that although AN was indeed a statistically significant contributor to S₁ independent of BMI, its contribution was relatively small and only added ~4–6% to the overall variance in S₁. Scale scoring of AN severity yielded little additional clinically relevant information beyond the simple determination of the presence or absence of AN. Thus, body adiposity rather than AN appeared to be the primary determinant of variance in insulin resistance in this population.

Our study differed from previous studies that have explored the relation between AN and insulin resistance in a number of ways. First, we used a direct measure of whole-body S₁ rather than fasting insulin. Second, we determined whether the severity of AN was more strongly related to insulin resistance. Third, we studied a population of Hispanic youth at high risk for developing type 2 diabetes. Previous studies have inferred the relation between insulin resistance and AN through the use of indirect indexes, such as fasting insulin (7–9, 23–27). The studies that have used direct measures of S₁ compared with AN include one in girls with premature adrenarche (10) and another in women with hyperandrogenism (12). These studies examined the effect of androgen excess and AN on S₁. Both found that subjects with AN had decreased S₁ levels that were independent of adiposity. These studies were limited by their small sample sizes (12 and 11 subjects, respectively) and did not address AN severity. Nguyen et al. (11) examined S₁ in overweight children with AN and found that AN correlated with decreased S₁, but after adjusting for BMI, this association was no longer significant. However, Nguyen et al. did not stratify the subjects by AN severity; if they had, it could have unmasked a subtle contribution of AN to S₁ that would otherwise have been obscured by the very large effect of adiposity. Our study attempted to address the limitations of previous studies while focusing on a much understudied segment of the population, overweight Hispanic adolescents.

Our analysis showed that AN was inversely related to directly measured S₁ independent of adiposity. Although body composition was found to be the strongest predictive variable of S₁, the presence of AN was indicative of modestly greater insulin resistance as compared with subjects who did not have the skin pathology. Because AN and S₁ were both strongly related to BMI, it is important to consider more specific measures of body fat. In the current study, we were able to examine whether a convenient clinical indicator of adiposity (BMI) provided similar results to a more sophisticated research measure of body fat (DEXA). Our data showed that the relation between AN and S₁ independent of adiposity was very similar, even when the more sophisticated measures of body composition were used in the analysis.

An assessment of whether grading of AN severity adds to the clinical estimation of S₁ is important to determine if the added time and effort required to scale score AN is justified. Although all regression models showed that AN was an independent correlate of S₁ beyond that of adiposity, the differences between the various severity scales were minimal. Of the AN severity scoring scales, the combined scale (0–7) had the strongest association with S₁. However, this scale explained only ~3% more variance in S₁ than the dichotomous (absent or present) scale. Given the small differences between the regression models, we believe evaluating AN using scale scoring provides little additional clinical benefit in estimating S₁ and therefore is unlikely to aid in the medical decision-making process. However, our findings support the American Diabetes Association criteria for screening type 2 diabetes in children with elevated...
Model 1: $r^2 = 0.410$
- Sex: 0.05 ± 0.10
- Tanner stage: −0.05 ± 0.04
- BMI: −0.08 ± 0.01

Model 2: $r^2 = 0.446$
- Sex: 0.05 ± 0.10
- Tanner stage: −0.04 ± 0.04
- BMI: −0.07 ± 0.01
- Dichotomous (0–1): −0.27 ± 0.11

Model 3: $r^2 = 0.463$
- Sex: 0.07 ± 0.10
- Tanner stage: −0.04 ± 0.04
- BMI: −0.06 ± 0.01
- Neck extent (0–4): −0.11 ± 0.35

Model 4: $r^2 = 0.467$
- Sex: 0.10 ± 0.10
- Tanner stage: −0.02 ± 0.04
- BMI: −0.06 ± 0.01
- Neck texture (0–3): −0.18 ± 0.06

Model 5: $r^2 = 0.473$
- Sex: 0.09 ± 0.10
- Tanner stage: −0.03 ± 0.04
- BMI: −0.05 ± 0.01
- Combined scale (0–7): −0.08 ± 0.02

**References**
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12. Stuart CA, Peters EJ, Prince MJ, Richards...
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