Association of Acanthosis Nigricans With Hyperinsulinemia Compared With Other Selected Risk Factors for Type 2 Diabetes in Cherokee Indians

The Cherokee Diabetes Study

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OBJECTIVE — To report prevalence rates of acanthosis nigricans (AN) and hyperinsulinemia and the association of AN with hyperinsulinemia compared with other known or suspected risk factors for type 2 diabetes in young American Indians.

RESEARCH DESIGN AND METHODS — A random sample of Cherokee Nation members aged 5–40 years was invited to participate in the Cherokee Diabetes Study, a cross-sectional study of type 2 diabetes and its risk factors in a young American Indian population. Data were collected by personal interview, medical history, physical examination (including anthropometric and blood pressure measurements and examination of the neck for AN), and laboratory analyses of blood specimens. Levels of insulin, lipids, and glucose were measured on fasting blood specimens. Diabetes status was determined according to the American Diabetes Association criteria.

RESULTS — A total of 2,205 participants were examined. Overall prevalence rates for AN and hyperinsulinemia were 34.2 and 47.2%, respectively. In general, the rates for both increased with age and degree of Indian heritage and were higher in female subjects, overweight/obese individuals, those with type 2 diabetes, and those with a parental history of type 2 diabetes. In addition, both had significantly higher age- and sex-adjusted means for selected known or suspected risk factors for type 2 diabetes. AN remained significantly associated with hyperinsulinemia (P = 0.0001) in multivariate analysis.

CONCLUSIONS — AN is independently associated with hyperinsulinemia and therefore may be useful as an early indicator of high risk for diabetes.

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Type 2 diabetes is a chronic disease usually diagnosed in adults >40 years of age. Early in the 20th century, diabetes was rare in American Indians, but by 1940, prevalence approached that of the U.S. population (1). Since 1940, rates of type 2 diabetes continued to escalate in most tribes, reaching epidemic proportions among some American Indian groups (2–4). More recently, type 2 diabetes has been found in younger age groups, particularly in tribes with a high prevalence of the disease (5–7). Type 2 diabetes is more common in American Indians, and obesity frequently precedes its development (2,7). Risk factors for type 2 diabetes include age, hyperinsulinemia, impaired glucose tolerance, gestational diabetes, a family history of the disease, in utero exposure to gestational diabetes, both low and high birth weight, obesity, physical inactivity, and ethnicity (3).

Acanthosis nigricans (AN) is a skin condition that is clinically characterized by dark, coarse, and thickened skin. Although the darkness of the skin is frequently referred to as increased pigmentation (8–12), there are differing opinions as to the cause of the discoloration. AN has been reported by some to be a result of an increase in melanocytes and melanin (13), whereas others believe it to be more likely related to the thickness of the keratin-containing outer layers of the skin (14,15). AN was first reported in 1890 as a cutaneous sign of internal malignancy (8). Since then, it has been associated with obesity, insulin resistance, hyperinsulinemia, and type 2 diabetes (7,10–12,16). AN occurs in several areas where flexing, bending, and chafing of the skin by clothing occur but is commonly and consistently found on the back of the neck (16–18). AN is common among African-Americans, Hispanics, and American Indians but rare among whites (7,9–11,15,17) and has been reported to be strongly influenced by genetic factors (19). Previously thought to be rare in children and adolescents (14), AN is now common in young people, especially in populations with high rates of adult diabetes (7,10,11,16,18).

AN is reported to be a reliable predictor of hyperinsulinemia, a known precursor of type 2 diabetes (11,15); consequently, early screening of adolescent American Indians for AN would pro-
provide a relatively simple, inexpensive, and noninvasive tool to detect individuals prone to develop type 2 diabetes as well as other diseases associated with hyperinsulinemia. This study reports findings of the Cherokee Diabetes Study (CDS), in particular the prevalence of AN in young Cherokee Indians, and evaluates the relationship of AN with fasting plasma insulin and other risk factors for type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study population and design
The CDS is a cross-sectional population-based study designed to evaluate the prevalence of type 2 diabetes and its potential risk factors among a random sample of an estimated 24,000 age-eligible enrolled members of the Cherokee Nation who lived for at least the previous 6 months in a 5-county area of northeastern Oklahoma. A random sample of 5,600 was selected, which was stratified by age (seven strata, aged 5–40 years), sex, and degree of Indian heritage (four strata, <25% to 75%), with a goal of 75–80 participants (equal numbers of male and female subjects) in the 28 subgroups (~2,200 participants).

Study methods
Informed consent was obtained from each participant or his/her parent or legal guardian before the examination, which consisted of a personal interview and a physical examination performed by trained staff. The personal interview included demographic and lifestyle information, personal and family medical history, and Indian heritage. The physical examination included anthropometric and blood pressure measurements and examination, both visually and by palpation, of the back of the neck only to identify the presence or absence of AN (regardless of severity), using American Indian–specific visual aids that were provided for identification and comparison purposes (C.A. Stuart, personal communication). Fasting blood samples were drawn for laboratory analysis.

Blood specimens were analyzed for fasting glucose, insulin, lipids, and lipoprotein cholesterol using a glucose-UV kit (Abbott, North Chicago, IL), an Abbott analyzer for total cholesterol and triglycerides by enzymatic methodology (20), a precipitation procedure (21) for HDL cholesterol, the Friedewald formula (22) for LDL cholesterol, and radioimmunoassay (23) for fasting insulin.

New diabetes status was determined using the American Diabetes Association (ADA) criteria (24). Self-reported cases of previously known type 2 diabetes were verified by a review of medical records to determine physician diagnosis and current therapy. In adults >19 years of age, overweight was defined as BMI ≥25 and <30 kg/m² and obese as BMI ≥30 kg/m². These cut points correspond to the 85th and 95th percentiles, respectively, for adults who participated in the 1999 NHANES (National Health and Nutrition Examination Survey) (25). In children and adolescents <20 years of age, at risk for overweight was defined as BMI ≥85th and <95th percentiles for age and sex and overweight as BMI ≥95th percentile for age and sex (26). Because plasma levels of insulin indicative of hyperinsulinemia are not well established, hyperinsulinemia was defined as fasting plasma insulin greater than or equal to the third quartile in participants free from diabetes and hypertension and who were not overweight (aged 5–19 years) or obese (aged 20–40 years) (aged 5–19 years, fasting insulin ≥17.8 μU/ml; aged 20–40 years, ≥16.5 μU/ml). These cut points were selected as being more representative than the recommended range for the assay, which was given by the sample processing lab as 8 ± 3 μU/ml.

Statistical analyses
Statistical analyses were performed using SAS version 6.12 (SAS Institute, Cary, NC). Point prevalence rates of AN and hyperinsulinemia by risk factor and sex and age group were evaluated using ANOVA with the Bonferroni option and χ² analysis, and variable means by AN and hyperinsulinemia status, adjusted for age and sex, were tested for significance by ANCOVA in a general linear model. Frequency variables by AN and hyperinsulinemia status were tested for significance by χ² analysis. To assess the association between AN, hyperinsulinemia, and other risk factors for diabetes in a multivariate analysis, a stepwise logistic regression procedure was performed.

RESULTS — During the study examination period (29 November 1995 through 31 July 1999), 2,205 participants were examined. After the study began, it was determined that, of the original list of 5,600, almost 1,400 prospective participants could not be recruited due to incorrect address, death, or ineligibility (too old or too young, moved out of the study area, etc.), leaving just over 4,200 to be recruited, for a participation rate of ~52%.

Of the 2,205 participants, there were 1,243 women and 962 men, the overall mean age was 22.1 years (989 <20 years of age), and the self-reported degree of Indian heritage ranged from 0.10 to 100%, with 15% full heritage, 61.4% ≥50% heritage, and a mean of 55.7%. Overall, the unadjusted prevalence of type 2 diabetes (ADA) was 4.3%; 4.2% in female and 4.4% in male subjects. The rate was 1.1% among participants aged <20 years and 7.0% in those >19 years of age. For participants aged 5–19 years, 51.6% were overweight or at risk for overweight, and 31.6% were overweight only. In the group aged 20–40 years, 78.1% were overweight or obese and 47.7% were obese only. Among participants with type 2 diabetes, only 9% were of normal weight.

For the individual variables considered in this study, data availability rates ranged from 94 to 100%, with AN data available for >97% of the participants. For the multivariate analysis, data availability was almost 83%.

Prevalence of AN
Overall, the prevalence rate of AN was 34.2% (37.1% in female and 30.5% in male subjects). Table 1 shows rates of AN for female and male subjects by age group, percent of Indian heritage, BMI category, and type 2 diabetes and hyperinsulinemia status. Analyses were performed by risk factor category for male and female subjects separately and by sex within each category.

Among all participants, the prevalence of AN increased with age. In female subjects, the rate of AN more than doubled between the groups aged 5–9 and 20–29 years (P < 0.05), with an even greater difference between the group aged 5–9 and those aged 30–40 years (P < 0.05). In male subjects, the age effect was not significant. Female subjects had a higher prevalence of AN than male subjects in each age group after 9 years of age; however, the only significant difference
As with age, the overall prevalence rate of AN increased with increasing percent of Indian heritage. Although the prevalence of AN was higher in female than in male subjects in each Indian heritage quantum, the only significant differences by sex were in the 25–49.9 and 50–74.9% categories (P = 0.02 for both).

Female subjects had a significantly higher rate of AN than male subjects among those who were overweight/obese only. The rates for both sexes increased significantly (P < 0.05) with increasing BMI; the rates for both overweight/obese female and male subjects were over four times higher than the rate among individuals whose BMI was normal.

Among all individuals with type 2 diabetes, the prevalence of AN was 73.3%. The only nonsignificant difference between male and female subjects was in the impaired fasting glucose category. The prevalence of AN was 32.5% in those with normal fasting glucose (NFG) and no type 2 diabetes and only ~13% in participants who had NFG levels, no diabetes, and normal weight.

The prevalence of AN was almost three times higher in female subjects with hyperinsulinemia compared with those with normal insulin values and was almost 2.5 times higher in male subjects for the same comparison. Among nondiabetic participants with NFG levels and normal weight, almost 15% of those with AN had hyperinsulinemia.

Analyses were performed on other known or suspected risk factors. Individuals with AN had significantly higher age and sex-adjusted means for waist circumference, waist-to-hip ratio, fasting plasma glucose, triglycerides, LDL cholesterol, and systolic and diastolic blood pressure and significantly lower HDL cholesterol (P = 0.0001). Furthermore, the percent of parental history of diabetes among participants with AN was almost twice that of individuals without AN (46.5 and 25.6%, respectively, P = 0.001).

**Prevalence of hyperinsulinemia**

The overall prevalence of hyperinsulinemia was 47.2%; the rate for female subjects was significantly greater than that for male subjects (49.5 vs. 44.3%, P < 0.05). Rates by age group were 20.4, 49.6, 52.5, and 51.1% for participants aged 5–9, 10–19, 20–29, and 30–40 years, respectively. In general, the prevalence of hyperinsulinemia increased with increasing percent Indian heritage, from a low of 42.1% in the <25% quantum to a high of 53% in the ≥75% quantum. The prevalence of hyperinsulinemia was 77.0% among all overweight/obese individuals (aged 5–40 years). 38.4% in at-risk for overweight (aged 5–19 years) or overweight (aged 20–40 years) participants, and 18.1% among all participants of normal weight. Hyperinsulinemia was present in 76.7% of individuals with previously as well as newly diagnosed type 2 diabetes. Among nondiabetic participants with NFG, no AN, and normal weight, the prevalences of hyperinsulinemia were 7.5, 22.2, 30.1, and 13.9% for groups aged 5–9, 10–14, 15–19, and 20–40 years, respectively.

Age- and sex-adjusted means of continuous variables and frequencies of classified variables by hyperinsulinemia status are given in Table 2. As also occurred with the prevalence of AN (data not shown), birth weight was the only clinical characteristic known or suspected to be associated with hyperinsulinemia and/or diabetes considered in these analyses that was not significantly different by participant hyperinsulinemia status.

To determine whether AN was independently associated with hyperinsulinemia after adjustment for other factors known or suspected to be associated with hyperinsulinemia, logistic regression was performed. Results with the dependent variable hyperinsulinemia (yes/no) are shown in Table 3. Because there was missing data in some variables, the population for the stepwise logistic regression was reduced to 1,793.

Independent variables included AN status, age, sex, three measures of obesity (BMI, waist circumference, and waist-to-hip ratio), lab values for triglycerides, HDL and LDL cholesterol and fasting plasma glucose, systolic and diastolic blood pressure, degree of Indian heritage, birth weight, and parental history of diabetes. All of the significantly associated independent variables that remained in the final model (AN status, sex, BMI, waist circumference, log triglycerides, fasting plasma glucose, and systolic blood pressure) were positively related to hyperinsulinemia. Odds ratios indicate that...
CONCLUSIONS — The prevalence of AN has been found to range from 3 to 74% in groups specifically examined for its presence (11,12,18,27). However, most of the early reports of AN are from studies that were limited in size, including case studies and groups with underlying health conditions, such as obesity or other metabolic conditions, or in underprivileged populations of mixed ethnicity.

The first large-scale study of AN in an American Indian population found AN in 38% of 260 members (10–70 years of age) of the Alabama-Coushatta tribe in Texas and 1,141 children (3–19 years of age) of the Winnebago/Omaha tribe in Nebraska (16). In addition, they found a twofold higher fasting insulin concentration in subjects who exhibited AN than in weight-matched subjects who did not have the condition, concluding that AN was highly prevalent among American Indians and that its presence suggests insulin resistance and an increased risk for type 2 diabetes.

The CDS is the first large study (n = 2,205) with wide ranges for age (5–40 years) and degree of Indian heritage (0.10–100%) to report on AN in a population of American Indians selected only for age, sex, geographic location, and enrollment in the Cherokee Nation with documented Indian heritage. The data presented in our report add to the evidence that AN is highly prevalent in American Indians and that AN is associated with hyperinsulinemia. The overall prevalence of AN in the CDS was 34.2%; however, AN was present in >73% of the CDS participants with type 2 diabetes. The prevalence of hyperinsulinemia was 47.2%, using our definition given earlier. If the reference range given by the lab had been applied, the prevalence of hyperinsulinemia would have been >70%. Among the nondiabetic participants who had NFG and were not overweight (aged 5–19 years) or obese (aged 20–40 years), the age- and sex-adjusted mean log insulin level was significantly greater in those who had AN compared with those who did not (2.61 vs. 2.48 μU/ml, P = 0.0022). Furthermore, among this subgroup, the only variables that were not significantly different by AN status were systolic and diastolic blood pressure. Overall, the results of multivariate analysis showed that AN, as an independent variable, was significantly associated with hyperinsulinemia.

The ADA convened a panel of experts in childhood diabetes to develop recommendations for diabetes testing in children. The panel recommended that any

### Table 2 — Known or suspected diabetes risk factors by hyperinsulinemia status

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Hyperinsulinemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Age- and sex-adjusted least squares means of continuous variables</td>
<td></td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3.46</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.6</td>
</tr>
<tr>
<td>Degree Indian blood (%)</td>
<td>59.7</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>97.6</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.90</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dl)</td>
<td>92.0</td>
</tr>
<tr>
<td>Log fasting plasma insulin (μU/ml)</td>
<td>3.42</td>
</tr>
<tr>
<td>Log triglycerides (mg/dl)</td>
<td>4.35</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>39.0</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>100.3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>118.7</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>74.6</td>
</tr>
<tr>
<td>Frequency of classified variables (%)</td>
<td></td>
</tr>
<tr>
<td>Parent with history of diabetes</td>
<td>42.3</td>
</tr>
<tr>
<td>Overweight (&lt;20 years of age)</td>
<td>57.8</td>
</tr>
<tr>
<td>Obese (≥20 years of age)</td>
<td>71.6</td>
</tr>
<tr>
<td>AN</td>
<td>50.6</td>
</tr>
</tbody>
</table>

Hyperinsulinemia was defined as fasting plasma insulin ≥3rd quartile in nondiabetic normotensive participants of normal weight: fasting plasma insulin, aged 5–19 years ≥17.8 μU/ml; aged 20–40 years ≥16.5 μU/ml. Overweight was defined by Centers for Disease Control growth charts (U.S. 2000 BMI-for-age percentiles): overweight, ≥95th percentile; obese by National Heart, Lung, and Blood Institute 1998 definition using BMI: obese, BMI ≥30 kg/m².

### Table 3 — Stepwise logistic regression analysis for the relationship of hyperinsulinemia and factors known or suspected to be associated with hyperinsulinemia in Cherokee Indians

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN (yes/no)</td>
<td>1.660</td>
<td>1.274–2.163</td>
<td>0.0002</td>
</tr>
<tr>
<td>Sex (F versus M)</td>
<td>1.826</td>
<td>1.402–2.378</td>
<td>0.0001</td>
</tr>
<tr>
<td>BMI (per 8 kg/m²)</td>
<td>2.121</td>
<td>1.510–2.979</td>
<td>0.0001</td>
</tr>
<tr>
<td>Waist circumference (per 2 cm increase)</td>
<td>2.102</td>
<td>1.496–2.955</td>
<td>0.0001</td>
</tr>
<tr>
<td>Log triglycerides (per 0.59 mg/dl increase)</td>
<td>1.567</td>
<td>1.366–1.797</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fasting plasma glucose (per 25 mg/dl increase)</td>
<td>1.537</td>
<td>1.267–1.863</td>
<td>0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure (per 14 mmHg increase)</td>
<td>1.290</td>
<td>1.095–1.520</td>
<td>0.0023</td>
</tr>
</tbody>
</table>

Independent variables that were deleted as not significant during the stepwise regression analysis included waist-to-hip ratio, HDL and LDL cholesterol, diastolic blood pressure, percent Indian heritage, birth weight, and parental history of diabetes. Hyperinsulinemia was defined as fasting plasma insulin ≥3rd quartile in nondiabetic normotensive participants of normal weight: fasting plasma insulin, aged 5–19 years ≥17.8 μU/ml, aged 20–40 years ≥16.5 μU/ml. The model was also adjusted for age within the two age groups used to define hyperinsulinemia.
child who is overweight and has any two of the following risk factors should be screened every 2 years, beginning at 10 years of age or the onset of puberty: 1) has a first- or second-degree relative with type 2 diabetes; 2) belongs to a certain ethnic group, including American Indians; and 3) exhibits signs of insulin resistance, including AN, hypertension, dyslipidemia, and polycystic ovarian syndrome (7).

Our results suggest the need for further refinements of this algorithm. In ethnic groups such as American Indians, whose insulin resistance may be genetically determined and whose prevalence of type 2 diabetes is high, perhaps the initial screening should be for the presence of overweight and/or AN. Looking only at children who are already overweight may over look some cases of hyperinsulinemia. The results of our study showed that in the group aged 10–14 years, 37.2% of the participants who had AN, NFG, and no diabetes were not overweight (data not shown). Furthermore, more than 1/ third of this subgroup had hyperinsulinemia, which may reflect either the transient increase in resistance to insulin that occurs developmentally during puberty or an increased risk for development of type 2 diabetes. Because it is now well documented that modification of lifestyle habits can delay or possibly prevent the onset of the disease (29), early risk detection using simple and noninvasive methodology may reduce the heavy burden of diabetes (and its many serious complications) at both the individual and community level in American Indian populations.

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