OBJECTIVE — Because declines in acute insulin response (AIR) and insulin action (M) predict development of type 2 diabetes, we sought to determine childhood factors that predict insulin action and AIR using longitudinal data from young Pima Indian adults with normal glucose regulation.

RESEARCH DESIGN AND METHODS — Predictors of adult M, measured by the euglycemic-hyperinsulinemic clamp, and AIR, measured after a 25-g glucose bolus, were assessed in 76 individuals from a set of childhood data (BMI, systolic blood pressure [sBP] and diastolic blood pressure, cholesterol, fasting and 2-h insulin, and glucose levels during an oral glucose tolerance test).

RESULTS — After adjustment for sex, adult percent body fat, adult and childhood age, childhood BMI, and sBP were negative and independent predictors of adult M. A 5 kg/m² increase in childhood BMI was associated with a 7.4% decrease in adult insulin action (95% CI 12.7 to −1.8%, P = 0.01) and a 10-mmHg increase in childhood sBP with a 5.0% decrease in adult M (95% CI −8.4 to −1.4%, P = 0.007). After a similar adjustment with M as an additional covariate, childhood 2-h insulin was a positive predictor of adult AIR such that a 25% increase predicted a 7.3% increase in adult AIR (95% CI 1.5–13.5%, P = 0.014).

CONCLUSIONS — Childhood insulin response during an oral glucose challenge predicts adult AIR, indicating that β-cell capacity may be set early in life. Childhood measures related to adiposity predict adult insulin action, which may reflect common underlying mechanisms that may be amenable to modification through programs targeting prevention or treatment of childhood obesity.
with in utero exposure to diabetes (n = 5), a factor known to dramatically increase the risk of developing diabetes by the third decade (13) and to reduce acute insulin release in Pima Indians (14). In utero exposure to diabetes was defined by a confirmed date of diabetes diagnosis in the mother that preceded the child’s birth date or an OGTT during pregnancy with a 2-h value of $\geq 11$ mmol/l. In the final subset of 76 subjects (38% of eligible adult subjects), the childhood evaluations spanned the years 1979–1994, and the adult visits occurred over the years 1987–2004. Both study protocols were approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases.

**Childhood measurements**

All individuals were asked to arrive to the visit after an overnight fast. A 75-g OGTT was performed. Fasting and 2-h blood samples were drawn for measurement of glucose and insulin concentrations. Plasma glucose was measured by an automated analyzer using the potassium ferricyanide method (Technicon Instruments, Tarrytown, NY). Serum insulin concentrations were measured by radioimmunoassay using the Herbert modification of the Yalow and Berson method (15) or an automated analyzer (Concept 4; ICN Radiochemicals, Costa Mesa, CA). All insulin values were converted using regression equations to the earlier radioimmunoassay values for comparability. Height was measured using a stadiometer and weight using calibrated scales by a set of trained observers. No standard measures of puberty were available.

**Adult measurements**

OGTTs (75 g) were used to exclude people with diabetes before enrollment and to classify glucose tolerance. Normal glucose regulation was determined using 2003 ADA criteria, a fasting glucose $<5.6$ mmol/l, and a 2hG $<7.8$ mmol/l (11). All plasma glucose concentrations were determined by the glucose oxidase method (Beckman Instruments, Fullerton, CA). Upon admission to the clinical research unit, participants were given a weight-maintaining diet (50% carbohydrate, 30% fat, and 20% protein) and asked to abstain from strenuous exercise. Body composition was estimated by underwater weighing with determination of residual lung volume by helium dilution (20 participants) or by total-body dual-energy X-ray absorptiometry (DPX-L; Lunar Radiation, Madison, WI) (56 participants). Percent body fat measurements from the dual-energy X-ray absorptiometry scan were converted to underwater values using a conversion equation (16).

AIR was determined using an intravenous glucose tolerance test. A 25-g glucose bolus was given over 3 min, and blood was drawn every minute until the fifth minute for measurement of insulin levels. The AIR was calculated as the average increase in plasma insulin from baseline during this time period. Plasma insulin concentrations were determined by radioimmunoassay using an automated analyzer, either the Concept 4 analyzer described previously or an ACCESS analyzer (Beckman Instruments). All insulin measurements were normalized to the earliest radioimmunoassay using regression equations for comparability with childhood values.

Insulin action was assessed using a euglycemic-hyperinsulinemic clamp. After an overnight fast, a primed continuous insulin infusion was administered through an antecubital vein at 40 mU/m² per minute for 100 min. The plasma glucose concentrations were measured every 5 min after the start of the insulin infusion, and a variable infusion of 20% dextrose was used to maintain plasma glucose at 5.6 mmol/l. The rate of glucose required to maintain euglycemia, as a measure of insulin sensitivity (M), was calculated for the last 40 min of the insulin infusion and corrected for both steady-state plasma insulin levels and endogenous glucose production. Endogenous glucose production was determined at baseline (120 min) and during the insulin infusion using a primed continuous infusion (0.3 μCi/min) of [3-3H]glucose. M values were normalized to estimated metabolic body size, or fat-free mass $+17.7$ kg (17). The average coefficient of variation for M was 2.2% in this subset of individuals.

**Statistical analysis**

Subject characteristics are presented as means ± SD if normally distributed and as median with 25th and 75th percentiles if skewed. M, AIR, and insulin levels were log$_{10}$ transformed to meet the assumptions of linear regression. Linear regression models were used to determine subsets of childhood predictors of adult M and AIR using backward selection, i.e., all factors were initially placed in the model, and the weakest factors were sequentially rejected until only those with a $P < 0.1$ remained. Significance level was set at $P < 0.05$. Adult and childhood age, sex, and percent body fat were not made available to the stepwise procedure and thus, by default, were included in the final model. Additionally, M was forced into the model for AIR. Because of concerns about the small sample size relative to the number of predictor variables, the models were also run using forward selection, i.e., the variables most strongly related to the outcome are entered into the model one at a time until none of the remaining variables improve the model, with identical results. The validity of the selected models was assessed using bootstrapping (18) with 1,000 replicates, each with a sample size of 76, created by sampling with replacement from the original dataset. Bootstrapping provides an estimate of what might be observed with multiple repetitions of a study. The backward selection procedure was applied to each of the 1,000 replicates to verify the stability of the chosen predictors in the final models. The final model for AIR was also applied to adult 30-min insulin levels during the OGTT, a frequently used surrogate for AIR. All analyses were performed using SAS Enterprise Guide 4.

**RESULTS**

**Subject characteristics**

Subject characteristics for the 76 individuals (63% male) included in this study during both childhood and adulthood are presented in Table 1. The median duration of follow-up time between the childhood and adult evaluations was 13.4 years (range 6.5–21.0). A total of 55% had at least one parent who developed type 2 diabetes before the age of 45 years. However, parental diabetes before age 45 years, if included in either model, was not a significant predictor of M or AIR in this subset. At the childhood visit, the individuals included in this analysis compared with those 1,332 children not included were of a similar age but had significantly lower average BMI, cholesterol, sBP, fasting insulin, and 2hIns levels. The differences in sBP and 2hIns levels were attributable to the BMI differences. On average, the individuals in this analysis, as adults, were relatively young and obese with an average BMI of 34.2 kg/m² and body fat of 32.3%. This is not substantially different from the complete group of full heritage adult Pima Indians who have participated in our metabolic studies. By
Early predictors of insulin action and release

Table 1—Subject characteristics during childhood and adulthood

|                  | Childhood | Adult |
|------------------|-----------|-------|
| Age (years)      | 11.7 ± 2.6| 25.3 ± 3.2|
| BMI (kg/m²)      | 21.3 ± 5.0| 34.2 ± 7.7|
| % Body fat       |           | 32.3 ± 8.2|
| sBP (mmHg)       | 99 ± 15   | 112 ± 11|
| dBP (mmHg)       | 56 ± 10   | 66 ± 9|
| Cholesterol (mmol/l) | 3.4 ± 0.5 |       |
| Fasting glucose (mmol/l) | 5.0 ± 0.4 | 4.7 ± 0.4|
| 2hG (mmol/l)     | 5.5 ± 1.2 | 6.1 ± 0.9|
| Fasting insulin (pmol/l) | 128 (90–208) | 270 (207–360)|
| 2hIns (pmol/l)   | 576 (319–056) | 1,039 (680–1,379)|
| AIR (pmol/l)     | 1,787 (1,241–2,387) |       |

Insulin action (mg glucose/kg EMBS per minute) 2.31 (2.10–2.91)

Data are means ± SD or medians (25th to 75th percentile). The median duration of follow-up between evaluations was 13.4 years (range 6.5–21.0). EMBS, estimated metabolic body size.

Design, all in our study population had normal glucose regulation and normal blood pressure. As expected, there was a high degree of correlation between the childhood factors that were evaluated as predictors of M and AIR (Table 2). The highest correlations were between BMI and insulin levels as well as glucose and insulin levels, with no difference between sexes.

**Childhood predictors of adult insulin action**

The final regression model included childhood sBP (P < 0.007) and childhood BMI (P = 0.01) as negative predictors of insulin action after controlling for sex, adult percentage body fat, and child and adult age (F = 9.62, r² = 0.46, P < 0.0001) (Fig. 1). These two childhood measures explained an additional 14% of the variance in adult M over the reduced model. Because insulin action was log transformed, the clinical meaning of the relationship between M and the childhood factors is reported in terms of percent change in M. For every 5 kg/m² increase in childhood BMI, adult M decreased by 7.4% (95% CI −12.7 to −1.8%). For every 10 mmHg increase in childhood sBP, adult M decreased by 5.0% (95% CI −8.4 to −1.4%). Bootstrapping confirmed that childhood BMI and sBP were the two strongest childhood predictors of M (chosen in 812 and 768 of the 1,000 replicates). The other six predictors were chosen <40% of the time.

We did not have enough individuals or pubertal information to be able to separate children into pre- and postpubertal developmental stages; however, in the small group of children with data before the age of 12 years (n = 37), the relationships between childhood factors and adult measurements maintain the same directionality as those in the larger group, but only the association between childhood BMI and adult M was significant (data not shown).

**Childhood predictors of adult acute insulin secretion**

Both childhood 2hG (P = 0.086) and 2hIns (P = 0.014) were chosen for the final model of AIR, but only 2hIns was statistically significant (F = 2.68, r² = 0.23, P = 0.017) (Fig. 1). In a reduced model without 2hIns, neither the model (P = 0.09) nor 2hG (P = 0.85) were statistically significant. Additionally, without 2hG in the model, 2hIns was no longer significant (P = 0.08), indicating that 2hG is a suppresser confounder for 2hIns, i.e., a variable associated with both the risk factor and the outcome, which when adjusted for, allows the relationship between the factor and the outcome to become apparent (22). The insulin sensitivity index combining glucose and insulin measurements into a single factor was not significant in the model and did not improve the fit of the model. Childhood 2hIns explained an additional 8.6% of variance in adult AIR over the reduced model. Adjusting for childhood BMI in the final model also did not alter the results. For every 25% increase in childhood 2hIns, adult AIR increased 7.3% (95% CI 1.5–13.5%). Bootstrapping also confirmed that childhood 2hIns was the strongest childhood predictor of adult AIR (810 of 1,000 replicates). The other six factors were chosen in less than half the replicates. Notably, childhood fasting insulin was only chosen in 177 out of the 1,000 replicates. To further corroborate these findings, the final model was applied to adult 30-min insulin levels from the OGTT, a commonly used surrogate for AIR. Childhood 2hIns was significant in the model (β = 0.7, 95% CI 0.2–1.2) with (P = 0.007) and without (P = 0.04) 2hG, but the association was stronger when 2hG was included. Again, 2hG was not quite significant (P = 0.06).

**CONCLUSIONS** — In this longitudinal observational study, childhood factors predicted adult insulin action and secretion in adults with normal glucose regulation, i.e., before clinical evidence of glucose dysregulation. The main findings are first that childhood BMI and sBP are negatively associated with adult M and, second, that 2hIns during a childhood OGTT is positively associated with adult AIR after adjustment for childhood 2hG.

Both M and AIR predict type 2 diabetes in the Pima population (12), even in individuals with normal glucose regulation (4). In the development of type 2 diabetes, both insulin secretion and insulin action have already deteriorated by the time impaired glucose regulation manifests (1). These prior studies indicate that preventing diabetes should begin at an early stage and target both insulin resistance and insulin secretion. Our work expands on this idea by identifying early life measures that are associated with these pre-diabetic elements. We have previously shown that childhood metabolic factors predict development of type 2 diabetes at a young age in Pima Indians (5). This study found that 2hG, waist circumference, and BMI were the strongest predictors, whereas sBP and dBP, although predictors, were relatively weak. This study did not include 2hIns (5). An earlier study which did include 2hIns measurements found that in Pima Indians, parental diabetes, weight relative to height, and 2hG predicted adult risk of type 2 diabetes (10). In this study, weight relative to height and fasting and 2hG and insulin levels were associated with parental diabetes. We did not find parental diabetes to be an important contributor to either M or AIR in our study, but this may be due to use of M and AIR as outcomes in the present analysis and diabetes in the previous articles (5,10). Our analysis separates the physiologic components of diabetes to determine individual predictors in this population based dataset of longitudinal data. We show that these
Table 2—Pearson correlations between childhood metabolic factors evaluated as predictors of adult insulin action and AIR

| Adult AIR | Adult insulin | Adult BMI | Child BMI | Child sBP | Child dBP | Child insulin | Child 2hIns | Child 2hG | Child age | Child fasting cholesterol | Child fasting glucose | Child 2hGlu | Child BMI | Child waist circumference | Child aerobic capacity |
|-----------|---------------|-----------|-----------|-----------|-----------|---------------|-------------|------------|-----------|-------------------------|----------------------|-------------|-----------|-------------------------|-----------------------|
| 1.00      | -0.10         | 0.00      | 0.01      | 0.01      | 0.01      | 0.01          | 0.34        | 0.24       | 0.25      | 0.04                    | 0.05                 | 0.00         | 0.26      | 0.11                    | -0.03                 |
| 0.00      | 1.00          | 0.00      | 0.00      | 0.00      | 0.00      | 0.00          | 0.25        | 0.01       | 0.01      | 0.01                    | 0.00                 | -0.01        | 0.00      | 0.00                    | 0.00                  |
| 0.24      | 0.25          | 1.00      | 0.70      | 0.16      | 0.35      | 0.51          | 0.08        | 0.09       | 0.09      | 0.00                    | -0.01                | 0.01         | 0.00      | 0.00                    | 0.00                  |
| 0.34      | 0.35          | 0.70      | 1.00      | 0.41      | 0.16      | 0.13          | 0.11        | 0.03       | 0.03      | 0.00                    | 0.00                 | 0.00         | 0.00      | 0.00                    | 0.00                  |
| 0.25      | 0.05          | 0.01      | 0.01      | 1.00      | 0.01      | 0.01          | -0.01       | -0.01      | -0.01     | -0.01                   | 0.00                 | -0.01        | 0.00      | 0.00                    | 0.00                  |
| 0.01      | 0.18          | 0.00      | 0.00      | 0.00      | 1.00      | -0.01         | 0.08        | 0.08       | 0.08      | -0.01                   | -0.01                | 0.00         | 0.00      | 0.00                    | 0.00                  |
| 0.01      | 0.00          | 0.00      | 0.00      | 0.00      | 0.00      | 1.00          | 0.00        | 0.00       | 0.00      | 0.00                    | 0.00                 | 0.00         | 0.00      | 0.00                    | 0.00                  |
| 0.01      | 0.00          | 0.00      | 0.00      | 0.00      | 0.00      | 0.00          | 1.00        | 0.00       | 0.00      | 0.00                    | 0.00                 | 0.00         | 0.00      | 0.00                    | 0.00                  |

Data are Pearson correlation coefficient (r) values. Insulin action, AIR, and insulin levels were log10-transformed to approximate a normal distribution.
discovered reasons is unclear. It has been shown that insulin release in Pima Indians is similar to African Americans and exaggerated when compared with Caucasians (23). Although the prevalence of diabetes is much higher in the Pima Indian population, in general, diabetes in this population has been prototypic of type 2 diabetes in other populations.

Overall, our population had lower childhood BMIs and lower average sBP and 2hIns levels than the general Pima Indian population with childhood measures; the lower sBP and insulin levels were attributable to the lower BMI levels. This is likely due to a survivor effect given our selection of adults with normal glucose regulation, who may have had relatively lower childhood BMIs than those excluded for impaired glucose regulation or diabetes. Nevertheless, as M and AIR are predictors of type 2 diabetes, even in adults with normal glucose regulation, our results are still relevant. Another limitation of this study is the small number of participants who met our inclusion criteria for this analysis. This may have resulted in overfitting of our model given the number of childhood factors evaluated relative to our sample size. However, we did rerun the models using forward selection, which is better suited for dealing with small sample sizes (22) with identical results. The results from our analysis are also consistent with our bootstrapped analysis and with the literature previously mentioned. Nevertheless, given the small sample size, the findings should be interpreted cautiously pending replication in other studies.

In conclusion, abnormalities contributing to adult insulin resistance and release and, thus, risk of diabetes appear to begin in childhood or earlier. While it is important to implement preventive lifestyle measures once impaired glucose regulation develops, identifying childhood factors that contribute to or associate with pathological declines in M and AIR may uncover earlier preventive targets in advance of clinical evidence of dysfunction. In particular, our study implies that prevention of future declines in insulin action may be possible through modification of childhood body weight, but insulin secretion may not be modifiable through lifestyle interventions.

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No potential conflicts of interest relevant to this article were reported.

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