Body Composition, Adipocyte Size, Free Fatty Acid Concentration, and Glucose Tolerance in Children of Diabetic Pregnancies

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SUMMARY

Previous studies show that children of women who are diabetic during pregnancy are more obese and have a higher prevalence of non-insulin-dependent diabetes mellitus (NIDDM) than children of women who first developed NIDDM >1 yr after the pregnancy (prediabetic mothers) and children of women who have never developed diabetes (nondiabetic mothers). To determine whether lean and obese children of glucose-intolerant pregnancies can be distinguished from similar children of glucose-tolerant pregnancies, we measured body composition, abdominal and gluteal adipocyte size, fasting free fatty acid (FFA), and fasting and stimulated glucose and insulin concentrations during an oral glucose tolerance test in prepubertal children of glucose-intolerant and prediabetic mothers. Each group ranged in adipocity from 6 to 40% body fat. Age, weight, height, and percentage of body fat were similar in the two groups. There were no significant differences in adipocyte size or in glucose, FFA, C-peptide, and insulin concentrations between the groups. The correlation between abdominal adipocyte size and fasting insulin concentration (r = .91 and .18, t = 2.8, P = .01) was stronger in children from glucose-intolerant than from glucose-tolerant pregnancies, respectively. In terms of the parameters we measured, there are no major differences between children of glucose-intolerant and glucose-tolerant pregnancies. DIABETES 1986; 35:1077–80.

Offspring of women who are glucose intolerant during pregnancy have a high prevalence of both obesity and early onset of non-insulin-dependent diabetes mellitus (NIDDM).[1–3] Pettitt et al.[1] showed that 35% of 5- to 9-yr-old and >60% of 10- to 14-yr-old Pima Indian children are >140% ideal body wt. This is independent of both genetic predisposition to NIDDM and maternal body size. The prevalence rates of NIDDM are 6–10 times higher in children of diabetic mothers than in children of prediabetic and nondiabetic mothers,[2] and this has been shown to be independent of the obesity of the child (D. J. Pettitt, unpublished data). These studies demonstrate that environmental factors can greatly accelerate the development of NIDDM and obesity in genetically predisposed people.

The neonates of diabetic and glucose-intolerant pregnancies have a larger body size (macrosomia),[4,5] a higher percentage of body fat when matched for weight,[6,7] larger adipocytes,[8] and higher fasting and stimulated plasma insulin and C-peptide concentrations[9–12] than normal neonates. Although 7- to 11-yr-old children of glucose intolerant pregnancies maintain an increased body size, it is not known whether, when compared to equally obese children of glucose tolerant pregnancies, they continue to exhibit the same differences in body composition and glucose tolerance that they did as neonates.

Our study was designed to determine whether children from glucose-intolerant pregnancies can be distinguished in terms of body composition, adipocyte size, glucose tolerance, and free fatty acid (FFA) concentrations from children of glucose-tolerant pregnancies and to determine, if differences do occur, if they are the same as the differences that have been reported between neonates of glucose-intolerant and glucose-tolerant pregnancies.

We measured body composition, glucose tolerance, FFA concentrations, and fat cell size in two groups of children of similar age, height, and weight. The groups were selected from offspring of mothers who were glucose intolerant duringthe pregnancy and from offspring of mothers who had glucose-tolerant pregnancies but who developed glucose intolerance at least 1 yr after the pregnancy.

SUBJECTS AND METHODS

Twenty-six Pima Indian children from the Gila River Reservation were recruited. Each child fulfilled one of the following criteria: *1*) Group A: mother known to have NIDDM or impaired

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glucose tolerance (2-h glucose >140 mg/dl) before the pregnancy or found to have impaired gestational glucose tolerance (2-h glucose >120 mg/dl) or gestational diabetes mellitus (2-h glucose >165 mg/dl) during the pregnancy; five of the mothers of children of glucose-intolerant pregnancies were treated with insulin and eight were on diet treatment. Postprandial 2-h glucose concentration (mean ± SE) for these mothers during the third trimester was 169 ± 13 mg/dl, with a range of 99–273 mg/dl. 2) Group B: mother developed the first evidence of glucose intolerance or diabetes (2-h glucose >140 mg/dl) at least 1 yr after the pregnancy.

There were 13 children in each group; 6 children in group A and 5 children in group B were lean by the criterion of having <20% body fat by underwater weighing (see below). By the criteria of Marshall and Tanner,[13,14] 2 obese 11-yr-old girls in group A (B2-B3,Ph1 and B2-B3,PH2) had started puberty.

Our subjects were drawn from the same population used by Pettitt et al.,[1] although there are differences in the criteria by which the groups were chosen. In our study the criterion for inclusion in the group from glucose-intolerant pregnancies was a maternal 2-h glucose concentration >140 mg/dl, rather than 200 mg/dl as used by Pettitt. In the majority of cases this was based on a single estimate of maternal glucose tolerance during the third trimester. Because it is unlikely that the glucose tolerance test coincided with the time of most severe glucose intolerance during the pregnancy, it would tend to underestimate the maximum severity of glucose intolerance to which the fetus was exposed. Furthermore, the 500-g difference (see RESULTS) in birth weights between the children of glucose-intolerant and glucose-tolerant pregnancies, which is similar to that found in Pedersen's[4] study, suggests that our group A subjects came from pregnancies in which there was significant glucose intolerance.

The subjects were selected so that the groups would be as similar as possible in age, sex, height, and weight. Birth weights and plasma glucose levels of the mother during pregnancy were obtained by medical record review.

Written, informed consent to take part in the study was given by the parents or guardians, and the children gave written assent. The protocol was approved by the Clinical Research Committee of the National Institutes of Health, the Indian Health Service Research Committee, and the Gila River Tribal Council.

Each child, accompanied by one parent, was admitted to the research unit for 3 days. Diet was unrestricted and activity limited to the ward. A full medical history and physical examination were performed.

On the 2nd day, body composition was estimated by underwater weighing, with simultaneous measurement of lung volume by helium dilution.[15–17] In 22 of these children, body composition was estimated by both underwater weighing and deuterium oxide dilution techniques. The Pearson product-moment correlation coefficient between the two methods (r = .79, 99% confidence limit 0.52–0.93, $P < .0001$) was significant. The regression slope (.78 ± .14) was not significantly different from 1. We used the measurements of percentage of body fat obtained by underwater weighing in our analyses. Next, 20 mg of adipose tissue was obtained by percutaneous needle biopsy of periumbilical and gluteal fat depots with a 15-gauge needle through skin previously anesthetized with ethyl chloride and 2% Xylocaine.

On the 3rd day, after an overnight fast, a needle was inserted for venous blood sampling. An oral glucose tolerance test was performed with a glucose dose of 1 g/kg body wt up to a maximum of 75 g (Koladex; Custom Laboratories, Baltimore, MD); venous blood was sampled at −5, 30, 60, 120, and 180 min for measurement of plasma glucose, insulin, and C-peptide concentrations. Fasting FFA concentrations were also estimated.

Analyses. Plasma glucose concentration was measured by the glucose oxidase method with a Beckman glucose analyser (Beckman; Fullerton, CA). Plasma insulin concentrations were determined by the Herbert modification[18] of the radioimmunoassay of Yallow and Berson.[19] Serum C peptide was estimated with the radioimmunoassay of Hedin.[20] Concentrations of FFAs were measured by the microfluorometric method of Miles et al.[21]

Adipocyte sizing. Immediately after the biopsy, the fat specimens were fixed in 2% osmic acid for 48 h, and then a suspension of adipocytes in normal saline was prepared according to the method of Hirsch and Gallian,[22] modified by Cushman and Salans.[23] Adipocyte size was measured electronically with a Coulter channelyzer (model 2B, Coulter Electronics; Hialeah, FL) with a 400-μm aperture, equipped with a logarithmic-range expander, as described previously.[24]

Statistics. All data are expressed as means ± SE. The significance of differences between means was assessed by either Student's t test or analysis of covariance (AOC). The significance of differences between Pearson correlation coefficients (r) was calculated with the transformation of Fisher[25] and the two-tailed t statistic. The natural logarithms of the insulin concentrations and birth weights and of the estimates of weight, fat-free mass, and fat mass were used to normalize the distributions. The Shapiro-Wilk statistic[26] was used as a test of the normality of the distributions. These statistics were calculated with the Statistical Analyses System (SAS Institute; Cary, NC).

RESULTS

The sex distribution and mean age, height, weight at the time of the study, birth weight, and percentage of body fat for each group are shown in Table 1. Each group consisted of

TABLE 1
Sex distribution and age, height, weight, birth weight, and percentage of body fat of the two groups of children

Group	Age (yr)	Sex (male/female)	Height (cm)	Weight (kg)	Birth Weight (g)	Body fat (%)
Glucose-intolerant pregnancy	9.8 ± 0.5	8/5	139 ± 4	45.5 ± 5.9	3820 ± 220	22 ± 3
Glucose-tolerant pregnancy	9.1 ± 0.4	8/5	138 ± 3	44.4 ± 5.1	3305 ± 135	24 ± 2

Values are expressed as means ± SE.

TABLE 2
Fasting and stimulated glucose, insulin, and C-peptide levels in the two groups of children

Group	Glucose		Insulin		C peptide	
	Fasting (mg/dl)	Area above basal (mg · min · dl^{-1})	Fasting (μU/ml)	Area above basal (μU · min · ml^{-1})	Fasting (μmol/L)	Area above basal (μmol · min · L^{-1})
Glucose-intolerant pregnancy	100.8 ± 2.0	2,450 ± 800	33 ± 6	10,600 ± 3,250	0.78 ± 0.10	300 ± 60
Glucose-tolerant pregnancy	100.9 ± 2.0	2,550 ± 350	27 ± 4	11,900 ± 2,000	0.64 ± 0.1	345 ± 40

Values are expressed as means ± SE.

a wide range of lean and obese children, from 6 to 41% body fat. There was no significant difference between the groups in means for age, weight, height, and percentage of fat. The mean birth weight of the children from glucose-intolerant pregnancies was significantly greater than that of the children from glucose-tolerant pregnancies ($t = 2.1$, $P < .05$).

The groups did not differ significantly in fasting or stimulated glucose, insulin, or C-peptide concentrations (Table 2) or in abdominal or gluteal adipocyte sizes (Table 3). Analysis of covariance using body weight, fat mass, fat-free mass, or percentage of fat, respectively, as independent variables also showed no difference between the groups in adipocyte size or in glucose, insulin, and C-peptide concentrations. Fasting FFA concentrations were not significantly different between the groups of children from glucose-intolerant and glucose-tolerant pregnancies (320 ± 30 vs. 410 ± 30 μeq/L, respectively, $t = 1.9$, NS).

The correlation between fasting insulin concentration and abdominal adipocyte size (Fig. 1) is stronger in children of glucose-intolerant pregnancies than in children of glucose-tolerant pregnancies ($r = .90$ and .18, respectively; $t = 2.8$; $P = .01$).

There was no correlation between fasting insulin concentration and gluteal adipocyte size in either children of glucose-intolerant ($r = .26$, NS) or glucose-tolerant pregnancies ($r = .32$, NS).

DISCUSSION

Pettitt and co-workers[1,2] demonstrated in Pima Indians that among the offspring of glucose-intolerant pregnancies, there is an increased prevalence of obesity during childhood and a predisposition to early development of glucose intolerance. White et al.[3] made similar observations in a non-Indian population.

The data from our study show that glucose intolerance in the mother during pregnancy is not associated with changes in body composition in groups of prepubertal children of similar age, sex, height, weight, and genetic predisposition to diabetes. Although there were no significant differences between the groups in glucose, FFA, insulin, or C-peptide concentrations during the oral glucose tolerance test, it is possible that the elevation of fasting insulin and C-peptide concentrations in the children of glucose-intolerant pregnancies would be significant in a larger sample. There is a significant difference between the groups of children from glucose-intolerant and glucose-tolerant pregnancies in the extent of the correlation between abdominal adipocyte size and fasting insulin concentration.

Thus, although there may be physiological differences between these groups of children, we could not demonstrate definitive evidence of glucose intolerance or insulin resistance, such as elevated glucose or insulin concentrations[27,28] or increased adipocyte size,[29] in children of glucose-intolerant pregnancies.

There are no previous reports of a correlation between fasting insulin concentrations and abdominal adipocyte size. The reason the correlation between these variables is so strong in children of glucose-intolerant pregnancies compared with children of glucose-tolerant pregnancies is not clear. Possibly insulin mediates growth of abdominal fat in the former through a dominant effect on adipocyte size and in the latter through an effect on both adipocyte size and number. Because growth of fat tissue through an increase in cell number occurs by recruiting small preadipocytes, the mean adipocyte size may fall and disguise the effect on increasing adipocyte size. A change in insulin's effect from

TABLE 3
Abdominal and gluteal adipose cell sizes in the two groups of children

Group	Abdominal cell size (μg lipid/cell)	Gluteal cell size (μg lipid/cell)
Glucose-intolerant pregnancy	0.60 ± 0.05	0.69 ± 0.03
Glucose-tolerant pregnancy	0.62 ± 0.05	0.68 ± 0.04

Values are expressed as means ± SE.

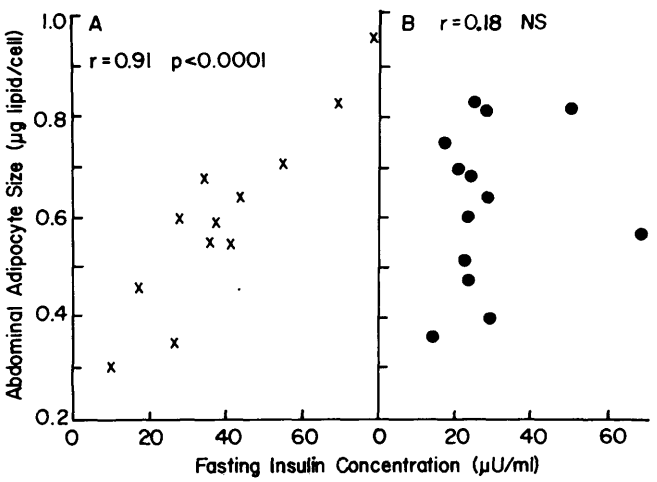

FIG. 1. Relationship between abdominal adipocyte size and fasting insulin concentrations in groups of children from glucose-intolerant pregnancies (*A*; $r = .91$, $P < .0001$) and glucose-tolerant pregnancies (*B*; $r = .18$, NS).

increasing cell number to increasing cell size is an interesting model that should be explored further.

Previous reports of a positive correlation between adipocyte size and insulin concentration in adults have measured gluteal adipocyte size.[30,31] Brook and Lloyd[32] showed a weak correlation between total postprandial insulin area and gluteal adipocyte size in 26 English children, and Hager et al.[33] showed a correlation between fasting insulin concentration and gluteal adipocyte size in obese girls, neither of which are present in either of our groups of children. This could be due to racial or dietary differences between the samples.

Other investigators have shown that neonates of glucose-intolerant pregnancies have a higher percentage of body fat than normal neonates of similar weight.[7] Fasting and stimulated insulin levels are also higher in neonates of glucose-intolerant pregnancies,[9–12] but these data are not from weight-matched groups. The finding of similar percentages of body fat in our weight-matched groups of children suggests that the increased adiposity of macrosomic neonates of glucose-intolerant pregnancies does not persist into childhood. Whether there is persistence of the higher insulin and C-peptide concentrations is not clear.

In summary, we found that in addition to the associations with an increased prevalence of obesity during childhood and an increased predisposition to NIDDM, offspring of glucose-intolerant pregnancies may have a change in the statistical relationship between abdominal adipocyte size and fasting insulin concentration. There was no evidence consistent with these offspring being more insulin resistant. It is possible that this minor difference is a reflection of the mechanisms that lead to the increased rate of growth and prevalence of NIDDM, although it is not clear what these mechanisms are. We suspect that metabolic and growth responses to insulin may be important. Furthermore, the data suggest that the high percentage of body fat and high insulin concentrations typical of neonates of glucose-intolerant pregnancies do not persist into childhood.

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