Session II. Effects of Mild Maternal Hyperglycemia on Fetal Development

Perinatal Islet Function in Gestational Diabetes: Assessment by Cord Plasma C-Peptide and Amniotic Fluid Insulin

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We have attempted to use perinatal islet performance as an index of the impact of maternal fuels during fetal development. Accordingly, we analyzed cord plasma for C-peptide and amniotic fluid (secured several weeks before delivery) for immunoreactive insulin in infants of mothers with normal metabolism (NM), gestational diabetes (GDM), and tightly regulated White Class B or Class C insulin-dependent diabetes mellitus (IDDM). We tried to subdivide mothers with GDM on the basis of severity by distinguishing between those with fasting plasma glucose within the normal range for pregnancy (i.e., less than 105 mg/dl and those with fasting plasma glucose of 105 mg/dl or greater). Most of the latter were treated with insulin whereas the former received diet alone. We found that cord plasma levels of C-peptide in infants of mothers with GDM < 105, GDM ≥ 105, and IDDM did not differ significantly from one another; all were approximately twofold greater than in infants from mothers with NM. Values for insulin and ratios of insulin/glucose in amniotic fluid were also increased and to a similar degree in all three diabetic groups; all exceeded the findings in the NM group. Our results indicate that islet function is enhanced at birth in the offspring of mothers with even the mildest forms of untreated gestational diabetes (i.e., GDM < 105) to a degree that is not appreciably different than in more severe forms of diabetes receiving tightly regulated insulin therapy. The augmented B-cell secretion cannot be ascribed to intrapartum events, since similar changes were also found in amniotic fluid secured several weeks before delivery. We conclude that neonatal insulin secretion may constitute an exquisitely sensitive index of the effects of ambient fuels during intrauterine life. Neonatal B-cell function thereby may provide a useful yardstick for the retrospective evaluation of maternal glucoregulation during late gestation. DIABETES CARE 3: 425–429, MAY–JUNE 1980.

The preceding report from our laboratory indicates that even the mildest forms of gestational diabetes are associated with changes in all aspects of maternal fuel economy.[1] These changes can alter the quantitative and qualitative characteristics of the mixture of ambient nutrients to which developing fetal cells are exposed.[2–4] The implications are particularly relevant within the context of pregnancy as a "tissue culture experience,"[2,3] and it has been suggested that the impact of the altered fuels of maternal origin upon fetal development may well constitute the most meaningful consequence of gestational diabetes.[4]

To evaluate this concept in the clinical setting, more sensitive indices of structure and function of the offspring during the newborn period and later life will have to be developed and correlated with maternal metabolism during gestation. Birthweight has often been used as such an indicator, since women with gestational diabetes tend to have heavier babies.[5–8] The "hyperglycemia-hyperinsulinism" theory of Pedersen[5,8] and its recent modification to include other maternal nutrients besides glucose[2] have provided support for the use of birthweight as a retrospective yardstick of antepartum fuel economy in pregnancies associated with diabetes. However, the wide range of normal birthweight and the differences in estimating gestational age have compromised reliability. Greater specificity has been achieved by measuring skinfolds at birth, since these attempt to evaluate the impact of intrauterine events upon a single tissue, i.e., adipose

stores.[9] The present report summarizes our ongoing efforts to evaluate the consequences of maternal metabolism on the basis of function in an even more specific target site—the pancreatic B-cells in the offspring. We are attempting to assess whether neonatal islet performance may provide a useful, and perhaps semiquantitative, indicator of the ambient fuels to which all fetal cells had been exposed during intrauterine life. Numerous previous studies have suggested that this should constitute a promising approach. Thus, the infants of mothers with insulin-dependent diabetes mellitus (IDDM) have long been known to be hyperinsulinemic during the neonatal period and to secrete insulin exuberantly in response to glycemic challenge.[10–16] By contrast, infants of women with normal carbohydrate metabolism (NM) tend to secrete insulin in a more sluggish and/or attenuated manner.[12,13,15–19]

The heightened performance of pancreatic B-cell function in IDDM has been ascribed to increased stimulation of the developing fetal islets by glucose[2,5,8,20] and perhaps other nutrients[2,21] of maternal origin. The limits of sensitivity have not been probed, and characterizations of islet function in the infants of mothers with gestational diabetes mellitus (GDM) have been sparse.[12–17,19,22,23] In the present studies, concentrations of cord plasma C-peptide and glucose in infants of mothers with GDM have been secured and contrasted to similar observations in infants of mothers with NM or IDDM. In addition, amniotic fluids have been examined in these groups several weeks before delivery to assess whether the cord blood insulin/glucose relationships antedate intrapartum events. Our experiences have indicated that neonatal islet function appears to constitute an exquisitely sensitive indicator of the modifications that gestational diabetes introduces into the "tissue culture" aspects of pregnancy.

MATERIALS AND METHODS

In our clinic, we have been employing the oral glucose tolerance criteria of O'Sullivan and Mahan[24] to diagnose GDM. We have defined GDM as carbohydrate intolerance with onset of recognition in the latter half of pregnancy. We have attempted to distinguish between gradations of severity in GDM on the basis of fasting plasma glucose.[1,2,25] Thus, we have been differentiating between GDM with normal fasting plasma glucose concentrations (i.e., values less than the 105 mg/dl, which constitutes two standard deviations above mean fasting plasma values in mothers with normal carbohydrate tolerance[24]) and GDM with fasting plasma glucose concentrations of 105 mg/dl or greater. The former (GDM $<$ 105) have been treated with diet alone whereas insulin therapy has been instituted for most of the latter (GDM $\geq$ 105). The IDDM for the present studies were Class B or Class C patients according to the criteria of White.[26] Since late 1970, the insulin therapy for most gravida with IDDM in our clinic has consisted of soluble insulin with every major meal in addition to daily administration of intermediate-acting insulin.[25] All subjects were being followed throughout their pregnancies at the Diabetes in Pregnancy Center of Northwestern University Medical School and gave informed consent for all test procedures.

Cord blood was collected from infants at the time of delivery and plasma was separated for immediate determination of glucose concentration. Additional cord plasma samples were frozen for later C-peptide analysis. Amniotic fluid from women with GDM and IDDM was collected at the time of routine amniocentesis for determination of fetal lung maturity at 36–40 wk of gestation; amniotic fluid from women with NM was secured during amniocentesis before repeat elective cesarean section at term. All other NM data were derived from designated control women and infants.

Concentrations of cord plasma C-peptide were determined following precipitation with polyethylene glycol to remove insulin and proinsulin-binding antibodies[27] as described previously.[15] Immunoreactive insulin in amniotic fluid was estimated directly by double antibody precipitation.[28]

We used the Student's unpaired t test for analysis of data.[29] For determining the significance of differences between groups in the ratios for insulin/glucose and C-peptide/glucose, we employed log transformations.[30]

RESULTS

Table 1 depicts findings at birth. Birthweights of the infants from mothers with GDM $<$ 105, GDM $\geq$ 105, and IDDM did not differ from one another but significantly exceeded those of the infants from mothers with NM.

Cord plasma C-peptide concentrations were not significantly different in the infants from mothers with GDM $<$ 105, GDM $\geq$ 105, and IDDM. Values in all three groups were approximately twofold greater than the values in infants from mothers with NM (Table 1). Although exogenous glucose had been administered to some diabetic mothers during labor and/or delivery, attempts had been made to preserve normoglycemia. The minimal differences in mean values for cord plasma glucose indicate that this goal had been attained. Thus, the ratios for C-peptide/glucose in the infants of mothers with GDM $<$ 105, GDM $\geq$ 105, or IDDM were almost twice as great as in the infants of mothers with NM (Table 1), thereby suggesting that feedback relationships between ambient glucose and pancreatic insulin release were altered at the time of delivery in all three diabetic populations.

To document that insulin secretion at birth reflects alterations that antedated labor or delivery, we examined insulin/glucose relationships in amniotic fluid that had been secured earlier in pregnancy. Immunoreactive insulin was measured directly because we have been unable to find meaningful amounts of insulin-binding antibody in the amniotic fluid of IDDM. Results are summarized in Table 2. Amniotic fluid glucose concentrations in GDM $<$ 105 and IDDM (but not in GDM $\geq$ 105) significantly exceeded values for NM ($P < 0.01$). Amniotic fluid concentrations of immunoreactive insulin in all three diabetic groups signifi-

TABLE 1
Characteristics of infants at birth

Groups	N	Birthweight (g)	Cord plasma		Cord plasma
			C-peptide (ng/ml)	Glucose (mg/dl)	C-peptide/glucose (ng/mg)
GDM < 105	17	3638 ± 160*	2.72 ± 0.43*	126.2 ± 7.6*	2.12 ± 0.27*
GDM ≥ 105	18	3707 ± 180*	2.15 ± 0.32*	93.6 ± 7.2	2.31 ± 0.22*
IDDM	13	3780 ± 186*	2.56 ± 0.47*	121.0 ± 15.3	2.43 ± 0.47†
NM	22	3170 ± 74	1.22 ± 0.12	95.8 ± 3.8	1.31 ± 0.12

GDM refers to mothers in whom gestational diabetes had been diagnosed according to the oral glucose tolerance criteria of O'Sullivan and Mahan.[24] We have further subdivided GDM on the basis of fasting plasma glucose values of less or greater than 105 mg/dl (i.e., GDM < 105 and GDM ≥ 105) as described in the text. N indicates the number of subjects in each group. Birthweight and values for cord plasma (mean ± SEM) denote observations secured in infants of those mothers at birth. The asterisk and dagger indicate significant differences from infants of NM (* $P < 0.01$; † $P < 0.05$).

cantly exceeded NM values ($P < 0.01$). Thus, ratios for insulin/glucose in amniotic fluid were significantly greater in GDM < 105, GDM ≥ 105, and IDDM than in NM (Table 2). These augmented insulin/glucose relationships in amniotic fluid appeared to parallel the C-peptide/glucose relationships in cord plasma and corroborated that islet secretory responsiveness in the offspring of GDM, as in those from mothers with tightly regulated IDDM, may also be increased in utero.

DISCUSSION

Heightened B-cell function in the infants of mothers with known diabetes has been well documented since the pioneer demonstration by Baird and Farquhar of increased insulin-like activity in cord plasma.[10] The observations have been confirmed repeatedly by more specific immunoassay procedures.[11–16] Despite some conflicting reports,[22] elevated plasma insulin (or C-peptide) concentrations at birth, and heightened islet responsiveness, have also been reported in a limited number of offspring of mothers with GDM.[12–17,19,23] However, attempts to ascertain how much of a disturbance in maternal metabolism is required to "trigger" increased neonatal islet responsiveness or to establish any quantitative correlations with the severity of maternal diabetes have been complicated by several factors. First, the antepartum regulation of maternal diabetes has not been described with sufficient detail in many instances. Particular difficulties have been encountered with GDM because of the variations in definitions and diagnostic criteria. Second, the estimates of insulin-like activity or immunoreactive insulin in offspring of insulin-treated mothers have often been compromised by analytical limitations. Although insulin itself does not cross the placenta,[31,32] the ready transfer of insulin-binding antibodies from mother to fetus,[33,34] and the prolonged persistence of such antibodies in the circulation, have precluded absolute precision.[35] Use of C-peptide to estimate endogenous insulin secretion has obviated some of these limitations, but artifactually high plasma or serum values may be obtained even with this approach unless additional steps are taken to remove the cross-reacting proinsulin, which is bound to the insulin-binding antibodies.[15,27]

TABLE 2
Amniotic fluid obtained at 36–40 wk gestation

Groups	N	Insulin (μU/ml)	Glucose (mg/dl)	Insulin/glucose (μU/mg)
GDM < 105	18	33.3 ± 10.0*	36.2 ± 5.4*	71.6 ± 17.5*
GDM ≥ 105	15	19.2 ± 4.2*	30.7 ± 4.7	67.5 ± 12.0*
IDDM	11	21.2 ± 4.8*	40.2 ± 8.3*	56.4 ± 13.2†
NM	37	7.4 ± 1.0	23.8 ± 1.8	31.9 ± 3.2

The groups have been designated as in Table 1. N denotes the number in each group. Insulin, glucose, and insulin/glucose relationship (mean ± 1 SEM) indicates values in amniotic fluid obtained at 36–40 wk gestation before onset of labor or cesarean section. Values for NM are for women with normal carbohydrate metabolism who underwent repeat cesarean section. These women differ from those who comprised the NM group in Table 1. The asterisk and dagger indicate significant differences from values for NM (*$P < 0.01$; † $P < 0.05$).

In the present studies, we tried to diminish both problems. We used rigid criteria to identify our GDM and sought to grade the severity of their disorder on the basis of fasting plasma glucose[25]—a distinction that has been strengthened by our recent demonstrations that metabolic abnormalities are more profound in GDM ≥ 105 than in GDM < 105.[1,2] For evaluating IDDM, we confined our observations to White Class B and C patients, since limitations in pelvic vasculature are not likely to impair the delivery of maternal fuels to the conceptus in these classes. Finally, all analyses for C-peptide in the present studies, as in our earlier efforts,[15] were preceded by polyethylene glycol precipitation,[27] so that we could compare islet secretory performance in the newborn of mothers with NM, DM, and IDDM directly without concern for antecedent insulin therapy.

We have thus been able to show that B-cell responsiveness at birth, as judged by the relationships between glucose and C-peptide in cord plasma, is unequivocally enhanced even in the offspring of mothers with the mildest forms of gestational diabetes (i.e., GDM < 105). We cannot affix a precise time

to the onset of this heightened islet function, but we examined amniotic fluid obtained several weeks before delivery to see whether the increased secretion of insulin preceded intrapartum events. We found that immunoreactive insulin, in absolute terms and in relation to the concurrent levels of glucose, is already elevated in such amniotic fluids. While the exact route for insulin entry into amniotic fluid is unclear, most investigators believe that the insulin is of fetal origin, since maternal insulin does not cross the placenta[31,32] and insulin is first detectable in amniotic fluid[36] shortly after its appearance in extracts of fetal pancreas[37] and in fetal serum.[38] Accordingly, it does not seem unreasonable to conclude that enhanced fetal B-cell function is already operative in utero, at least in the few weeks before delivery, even when GDM is minimal.

The data represent the largest series of C-peptide estimates in the offspring of mothers with GDM that has been reported to date. However, C-peptide data concerning all populations are limited. Block et al.[39] found values for total C-peptide in 5 offspring of insulin-treated gestational diabetic women and 10 infants of IDDM to be higher than the values in infants from mothers with NM; absolute comparisons are precluded because the C-peptide estimates were not preceded by attempts to remove the obfuscating insulin-binding antibodies. Sosenko et al.[16] reported values similar to our findings in two infants from White Class A mothers (? GDM) in whom cord serum C-peptide concentrations were determined after polyethylene glycol precipitation; however, lack of paired estimates of cord serum glucose complicates attempts to correlate these measurements with concurrent ambient glucose. They also reported that mean values for cord serum C-peptide were increased to an even greater extent in infants from 16 Class B, 32 Class C, and 24 Class D, F, and R mothers.[16] Again, individual correlations with circulating glucose were not cited, although the reported values of 208 mg/dl for mean cord glucose in 43 of these infants would suggest that stimulated rather than basal levels of C-peptide secretion were being evaluated. By contrast, in our series, we attempted to maintain physiologic glucose levels throughout delivery so that neonatal secretory performances could be compared under relatively basal conditions. Under such circumstances we found that values for cord plasma C-peptide did not differ significantly in infants from mothers with GDM < 105, GDM ≥ 105, and IDDM of the White Class B and C variety; in all three groups, the values were approximately twofold greater than in infants of mothers with NM.

It should be emphasized that our GDM < 105 had been treated with diet alone whereas most of our GDM ≥ 105 and all our IDDM had been given insulin in amounts designed to achieve optimal regulation of their diabetes. Insofar as neonatal insulin secretion correlates with the antepartum exposure of the fetus to maternal fuels, it would appear that these therapies had effected roughly equivalent levels of metabolic control in all our diabetic mothers. By the same token, one also would have to conclude that none of our diabetic group had been fully normalized, as judged by the significantly lower values for birthweight, cord plasma C-peptide, and C-peptide/glucose ratios in the infants of our control mothers with NM. Thus, the present studies still do not establish the minimal degree of metabolic abnormality in the mother necessary to affect the fetus. Our experiences with the offspring of mothers with GDM < 105 would indicate that it need not be very much. Prospective longitudinal studies correlating most major metabolic fuels in the mother during late gestation with neonatal islet secretory performance are underway to answer this key question more precisely. The present studies have affirmed that B-cell performance of the newborn may constitute a superbly sensitive tool for this type of inquiry and that neonatal estimates of C-peptide may also prove useful for the retrospective evaluation of antepartum therapeutic manipulations.

However, in a more theoretical sense, our findings also reinforce the thesis that maternal metabolism exquisitely impinges upon the functional properties of developing cells. Elsewhere it has been suggested that the "tissue culture" formulation of pregnancy may be of particular importance for cells with limited replicative potentialities.[2,4] Thus, the possibility that the heightened development of B-cells during fetal life may have long-range implications warrants consideration. It should be recalled that Aerts and Van Assche[40] have recently reported that islet cell function during adult life is diminished in the offspring of rats in whom streptozotocin-induced diabetes was present during pregnancy—a finding that suggests that B-cell performance may be permanently compromised as a consequence of exogenous blood sugar challenges during intrauterine development rather than intrinsic genetic endowment.

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