Hyperinsulinemia and Macrosomia in the Fetus of the Diabetic Mother

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OBJECTIVE — To determine *1*) whether macrosomia in the fetus of the diabetic mother is related to fetal hyperinsulinemia and 2) whether hyperinsulinemia and macrosomia are related to maternal metabolic control.

RESEARCH DESIGN AND METHODS — Normal pregnant women (n = 95) were compared with insulin-treated pregnant women (n = 155), who were subdivided according to White's class, hypertension, and mode of delivery. All women were treated to achieve optimal metabolic control. HbA_{1c} was determined at each visit. At delivery, umbilical plasma was analyzed for glucose, insulin antibodies, total insulin, free insulin, C-peptide, proinsulin components, and total and individual amino acids.

RESULTS — Macrosomia, defined as >2 standard deviation units (97.75%), was found in 10–27% of the diabetic groups. It was not related to maternal mass or size, but was significantly correlated with umbilical total insulin, free insulin, and C-peptide. Proinsulin components were not different among groups. Amino acids also were not different. Glycosylated hemoglobin was a weak predictor of birth weight and fetal hyperinsulinism.

CONCLUSIONS — Macrosomia in the fetus of the diabetic mother remains inadequately explained. In a large population of pregnant women with strict metabolic control, macrosomia was mainly independent of glycosylated hemoglobin. Nevertheless, fetal hyperinsulinism remains the driving force for excessive fetal growth. The stimulus for fetal insulin excess in humans remains to be defined.

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PLC, proinsulin-like components; ANOVA, analysis of variance; AGA, appropriate for gestational age; LGA, large for gestational age; IDDM, insulin-dependent diabetes mellitus; BMI, body mass index; SDU, standard deviation unit; RIA, radioimmunoassay; PEG, polyethylene glycol; IGF-1, insulin-like growth factor 1.

The introduction of strict glycemic control of the diabetic pregnancy has resulted in a significant reduction of some morbidities, i.e., congenital anomalies and respiratory distress syndrome (1). However, macrosomia has continued to occur at an abnormally high rate (2,3). Macrosomia and selective organomegaly (brain and kidneys are spared) are best explained by the metabolic response to hyperinsulinemia (4). Macrosomia was a consistent response to in utero hyperinsulinemia in a normal fetal rhesus model (1,4).

Fetal hyperinsulinemia has been documented in diabetic pregnancies in humans by analysis of total insulin, free insulin, C-peptide, and proinsulin-like components (PLC) in umbilical plasma (5–7) or analysis of C-peptide by cordocentesis (8). Measurements of C-peptide in amniotic fluid, a correlate of fetal insulinemia, have been reviewed by Persson (9). The stimulus for fetal hyperinsulinemia is not well-established. Glucose appears to be a poor or weak secretagogue in utero (10,11), but several amino acids, including arginine and leucine, are potent both in utero and in the immediate postnatal period (10,12). The evidence in humans for in utero amino acid stimulation of insulin secretion is tenuous.

Although fetal macrosomia in humans has been correlated with fetal hyperinsulinemia, the stimulus for the latter remains unknown. Poor correlations with maternal glycohemoglobin have been observed (3). The best relationship, albeit weak, has been noted with postprandial maternal glucose concentration (3,13). Whether other nutrients and secretagogues are active is less clear. The multifactorial basis for macrosomia is undefined.

This prospective study was designed to ascertain whether a relationship between fetal hyperinsulinemia and macrosomia could be demonstrated in a population of diabetic women subjected to the current standards of strict glycemic control during pregnancy. Umbilical

plasma obtained at delivery was analyzed for insulin antibodies, total insulin, free insulin, PLC, and C-peptide. Substrates included glucose and all amino acids. Analysis of variance (ANOVA) and multivariate analysis were used to determine significant interrelationships.

RESEARCH DESIGN AND METHODS

— Pregnant women were recruited from the high-risk population of Departments I and II of Obstetrics and Gynecology, University Central Hospital in Helsinki, Finland from October 1983 to April 1990. This population is ethnically and socially homogeneous. Only insulin-treated diabetic patients were recruited and grouped according to White's class (Table 1). Gestational diabetes (White's class A/B) was diagnosed using World Health Organization criteria, and only those who needed insulin in addition to a diet regimen were included in the study. Insulin therapy was added to the diet therapy when the 24-h blood glucose profile had at least two preprandial blood glucose values >5.5 mM or one preprandial >5.5 mM and one postprandial >7.8 mM.

Nondiabetic control groups with appropriate for gestational age (AGA) newborn infants were selected from healthy women without risk factors for diabetes (14). Another nondiabetic control group was selected from women who had a normal oral glucose tolerance test but whose infants were large for gestational age (LGA). This group, however, was excluded from the regression analyses.

A control group studied previously, in which 3rd trimester HbA_{1c} and infant weight were obtained, has been used for selective analyses (15). This group represented a similar population. HbA_{1c} was determined by the same method in the same laboratory.

Diabetic management was provided by two perinatologists (V.H. and K.A.T.) and one internist consultant at the outpatient clinic every 2–4 weeks. The goal of therapy was to achieve normalization of metabolism, especially glucose, as early as possible. The type of insulin therapy was variable so that early recruits received beef-pork, pork, or human insulin. Toward the end of the study, only human insulin was used. Approximately one-third of the insulin-dependent diabetes mellitus (IDDM) women were seen at the prepregnancy clinic before the pregnancy by the same team for counseling and improvement of diabetic control when necessary. Diabetic patients measured blood glucose at home five times daily at least twice weekly. Most patients did daily measurements that were not available for statistical analysis. In addition, HbA_{1c} was determined at each prenatal visit. Patients who were hospitalized had 24-h blood sugar measurements every 4 h that were available for analysis. These included one overnight fasting sample, one postprandial (1-h) sample, and five preprandial samples.

Patients were subgrouped for statistical analysis as follows: group I, nondiabetic control subjects for AGA infants; group II, nondiabetic control subjects for LGA infants; group III, gestational diabetic subjects, diet and insulin treated White's class A/B IDDM patients and; group IV, White's classes B, C, and D combined; group V, IDDM patients, White's class F/R.

Among the maternal characteristics recorded were prepregnancy weight and height, which permitted calculation of maternal body mass index (BMI) (kg/m^2). Because hypertension, especially that found in group V subjects, may be associated with growth retardation, another subset analysis separated nonhypertensive from hypertensive subjects.

Infant size was determined by reference to a Finnish newborn population of 74,766 singletons born 1978–1982 (16). Using infant birth weight, gestational age and sex, each infant's relative birth weight was expressed in standard deviation units (SDUs). The AGA infants were between −2 SDU and 2 SDU (within 97.75% of the population). Macrosomic infants were >2 SDU (97.75%). Finnish infants in the reference population are larger than those in North America. Thus, term infants at 90–95% weigh 4,500 g rather than 4,000 g. The present definition is stringent compared with the 90th or 95th percentile. BMI of the newborn infants was calculated from birth weight and birth length (kg/m^2) (17).

Biochemical analysis

Maternal HbA_{1c} was determined by a well-standardized cation-resin technique in the clinical chemistry laboratory of the Women's Hospital (18). Plasma and serum samples were stored at −70°C until they were shipped on dry ice to Rhode Island without any difficulty.

Plasma glucose was measured using a YSI Model 23A glucose analyzer (Yellow Springs, OH). Serum insulin was measured in a double antibody radioimmunoassay (RIA) using ^{125}I-monoiodinated A14 insulin and unlabeled human insulin standards (provided by Dr. Bruce Frank, Lilly, Indianapolis, IN). Guinea pig anti-porcine insulin antisera and goat anti-guinea pig antisera were obtained from Linco Research (Eureka, MO). Percent PLCs were determined on serum samples subjected to gel filtration on a Sephadex G-50 column (Pharmacia LKB, Uppsala, Sweden) and eluted with 1 M acetic acid, 0.05% bovine serum albumin as described by Gorden and Roth (19). Selected samples, demonstrated to have no insulin antibodies, were fractionated on a Fractogel TSK-HW50S column (EM Science, Gibbstown, NJ) to achieve higher resolution for size separation. Proinsulin is expressed as the percentage of total immunoreactivity of the fractions that elute in the same position as purified proinsulin and/or proinsulin conversion intermediates (also supplied by Dr. Bruce Frank) (Fig. 1).

Serum samples for total insulin were extracted with HCl and polyethylene glycol (PEG) according to the method of Kuzuya et al. (20). Serum samples for free insulin were extracted with PEG im-

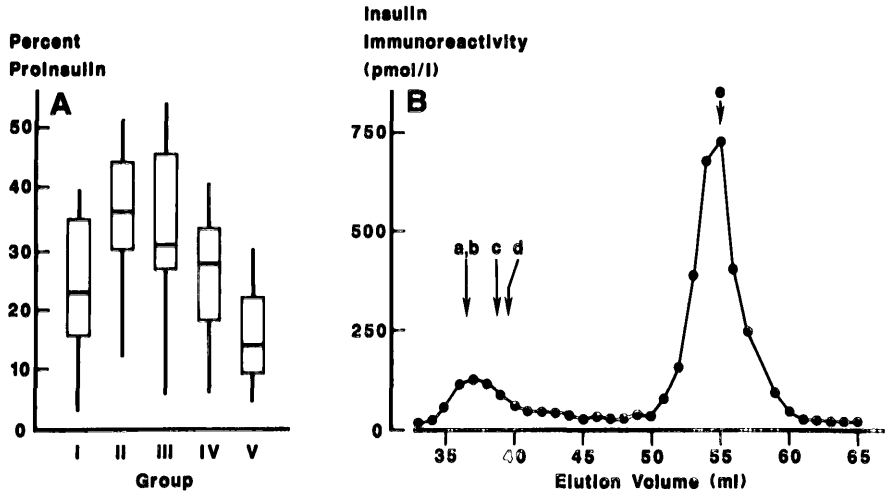

Figure 1—*Box and whisker plot (Tukey). The box includes the lower edge at the 25th percentile and upper edge at the 75th percentile. The intermediate line within the box represents the median (mean for normally distributed data). The outer lines are ± 2.7 SD from the mean of normally distributed data. A: distribution of percent PLCs in five groups of cesarean section–delivered subjects. No statistical differences were found among groups. B: a representative chromatograph of elution pattern of serum insulin immunoreactivity from a Fractogel column. Peak elution pattern of standards are as follows: a, split 32,33 proinsulin; b, des 31,32 proinsulin; c, des 57–65 proinsulin; d, intact proinsulin; and e, insulin.*

mediately after sample collection and stored for analysis at −70°C to minimize the loss of free insulin in the presence of insulin antibodies (21). PEG was added to insulin standards for total and free insulin assays. The presence of insulin antibodies was determined by incubation of insulin free sera (22) with ^{125}I-A14 insulin and expressed as the percentage bound of labeled insulin. Plasma C-peptide was determined by double antibody RIA using ^{125}I-tyrosylated C-peptide and standards obtained from Lilly and antisera from Linco Research. Quantitative plasma amino acids were analyzed using a Beckman ion-exchange system Model 7300.

Statistical analysis

We used several forms of the general linear model for the statistical analysis (23,24). Differences in the outcomes of interest among groups I-V were examined using two-way ANOVA to control for possible confounding with blood pressure status (hypertensive vs. normotensive). The ANOVAs were stratified on delivery mode. The alternative, which is to add a delivery mode to the ANOVA, could produce results that would be difficult to interpret owing to the absence of groups II and V among subjects delivered following labor. Tukey's approach, with Kramer's modification for unequal sample sizes, was used for pair-wise comparisons of means in ANOVAs that produced statistically significant results.

Associations among continuous variables were examined using correlation and linear regression. Analysis of covariance was used to determine if the relationships varied among groups. Least-square means were used for pair-wise comparisons of regression lines where statistically significant variation among groups was detected.

The residuals from all models were examined for departures from the assumption of normality. Log transforms of the outcome variables proved effective for correcting such departures. All model fitting took place in SAS (25). Significance was attributed at $P < 0.05$.

RESULTS— The mean ages of diabetic subjects in the various combined cesarean section and labor groups are shown in Table 1. Because maternal size has been related previously to infant size at birth (26), prepregnancy weight and maternal BMI (kg/m^2) were examined and summarized in Table 1. Prepregnancy weight in group III (gestational diabetic) mothers was significantly increased (75.8 kg). Prepregnancy BMI was similar among groups with only group III being increased (28.3 vs. 22.2 kg/m^2). Thirty-eight percent of the combined (cesarean section and labor) group III mothers had BMI that exceeded mean + 2 SD of the control population. A significant number were overweight by the criterion of a BMI >27 (27). In general, women in class A/B were larger than those in any other group except control mothers of macrosomic infants. BMI was also different for group III vs. II delivered by cesarean section (Table 1).

Mean maternal HbA_{1c} in the 3rd trimester exceeded the upper level of control subjects (HbA_{1c} 5.4 ± 0.6%, i.e., 6.6%; mean + 2 SD) in both groups B, C and D, and F/R. Sixty percent of diabetic subjects were within the control range. Glucose control, as indicated by normalization of HbA_{1c}, was best for group III (A/B). Glycohemoglobin was more variable for groups IV and V, which did not differ from each other statistically. Of the total diabetic population, the mothers of the 120 AGA infants had mean HbA_{1c} of 6.39 ± 1.06%, whereas the corresponding HbA_{1c} was 6.91 ± 0.86 in the 32 mothers with macrosomic infants. An unpaired Student *t* test indicated a significant difference ($t = 2.57$; $P = 0.011$).

Gestational age at delivery varied from 228 to 299 days for the entire population (Table 2). Because of the relatively high frequency of pregnancy complications, the class F/R group was the youngest with a mean (± SD) of 255 ± 10.6 days compared with the AGA control subjects and the LGA control subjects. No significant differences for the other groups were found. Infant relative birth

Table 1—*Maternal data*

Group	White class	Number total/section	Maternal age (years)	Prepregnancy maternal weight (kg)	Prepregnancy index BMI (kg/m^2)	Maternal HbA_{1c} (%)
I	Control subjects	89/66	32.1 ± 5.6	59.7 ± 10.2	22.2 ± 3.4	5.40 ± 0.6*
	AGA		16–45	43.0–92.0	17.4–34.6	
				(87)	(87)	
II	Control subjects	6/6	32.2 ± 2.3	63.8 ± 8.8	23.7 ± 2.8	—
	LGA		30–35	55.0–78.0	20.4–27.6	
III	A/B	40/19	31.9± 6.4	75.8 ± 18.9	28.3 ± 6.8	5.88 ± 0.69
			18–41	50–140	19.5–54.7	4.70–7.30
				(39)	(39)	(39)
IV	B,C,D	98/76	28.8 ± 5.3	60.7 ± 10.1	22.5 ± 3.4	6.67 ± 1.10
			18–42	46.0–117.0	17.2–43.0	4.30–10.1
				(96)	(96)	(96)
V	F/R	17/17	27.9 ± 5.7	59.9 ± 5.4	22.3 ± 1.8	6.90 ± 0.77
			20–45	52.0–68.0	19.0–26.2	5.60–8.2
				(16)	(16)	

Data are means ± SD and range; (*n*), different from total *n*. Number total/section, total number per group and number delivered by cesarean section. * Previous study.

weight expressed in SDU was not different among groups I, III, and V. Group II contained only macrosomic control infants by selection (>2 SDU). Among the diabetic groups, group IV (White's classes B, C, and D) had the largest infants relative to AGA control subjects (1.38 vs. −0.05 SDU) (Table 2) and the greatest incidence of macrosomia at 27% (26 of 98). Within this group, one-third of those infants delivered after labor (including four by cesarean section) were macrosomic, whereas one-fourth of those delivered by elective cesarean section were also macrosomic. The class F/R infants included 18% (3 of 17) macrosomics, but the class A/B (group III, combined labor, and cesarean section) had only 10% (4 of 40). In the total diabetic population of 155 subjects, 33 subjects were macrosomic, i.e., 21.3%. Infant BMI was greatest in the control LGA group. Otherwise, there were no differences in BMI among the groups. Furthermore, infant BMI did not correlate with maternal BMI.

Umbilical plasma glucose concentration at delivery for the five groups is shown in Table 3. Two-way ANOVA of log-transformed data indicated no difference between values obtained in the cesarean section–delivered groups with control AGA and LGA infants. However, the values obtained in the three diabetic groups were significantly elevated, with the highest being in group V, F/R, at a mean of 6.38 mM compared with AGA control subjects of 3.62 mM (Table 3). Slightly higher values were found in labor groups I and III compared with the respective cesarean section groups.

Umbilical plasma total and individual amino acid analyses were conducted for each subgroup on a total of 62 samples (data not shown). Specific attention was paid to those amino acids that are insulinogenic or respond metabolically to insulin action. A general linear model procedure detected no differences in the concentrations of total or specific

Table 2—*Infant data*

Group	White class	Gestational age (days)	Infant weight (SDU)	Infant BMI (kg/m^2)
I	Control subjects	273 ± 61	0.05 ± 0.94	14.0 ± 1.16
	AGA	253–290	1.75–1.98	11.2–18.1
				(55)‡
II	Control subjects	277 ± 13.2	2.82 ± 1.04	16.6 ± 0.48
	LGA	259–299	2.11–4.82	16.2–17.4
				(5)
III	A/B	268 ± 6.9	0.92 ± 1.39	14.8 ± 1.59
		255–283	1.05–+4.86	12.6–18.3
				(15)
IV	B,C,D	261 ± 8.6	1.38 ± 1.44	15.2 ± 1.52
		230–278	1.95–+6.13	12.1–19.4
				(37)
V	F/R	255 ± 10.6	0.62 ± 1.28	14.0 ± 1.91
		228–273	1.44–+2.4	11.0–16.5
				(7)

Data are means ± SD and range; (*n*), different from total *n*.

Table 3—*Chemical determinations on umbilical plasma (serum) at delivery*

Group	*n*	Glucose (mM)	Total insulin (pM)	Proinsulin components (%)	C-peptide (nM)
Cesarean section					
I	66	3.62 ± 0.51	81.2 ± 63.9	23.3 ± 11.6	0.32 ± 0.11
		2.28–5.39	14–337	3.1–39.5	0.13–0.62
		(41)	41	(18)	
II	6	3.43 ± 0.60	116.0 ± 54.8	34.8 ± 14.0	0.39 ± 0.21
		2.78–4.06	43–201	12.0–51.3	0.28–0.82
III	19	4.50 ± 0.82	203.9 ± 192.5	33.3 ± 16.7	0.68 ± 0.60
		3.44–6.44	43–718	5.8–54.1	0.26–3.0
			(12)	(9)	
IV	76	5.62 ± 2.19	1060 ± 1710	25.3 ± 9.6	1.24 ± 0.94
		2.06–14.1	29–7613	6.1–40.6	0.14–5.48
			(39)	(25)	
V	17	6.38 ± 3.16	854 ± 451	16.0 ± 12.7	1.20 ± 0.53
		2.11–14.0	265–1557	4.6–29.8	0.33–2.13
Labor			(7)	(3)	
I	23	5.12 ± 0.92	99.5 ± 67.3	39.8	0.34 ± 0.22
		3.61–7.06	36–144	(1)	0.12–1.11
II	—	—	—	—	—
III	21	6.20 ± 1.70	204.5 ± 149.7	—	0.84 ± 1.22
		2.89–11.06	22–387		0.07–6.00
			(4)		
IV	22	5.84 ± 2.46	800 ± 879	—	1.00 ± 0.66
		2.72–12.06	144–2490		0.23–3.14
			(6)		
V	—	—	—	—	—

Data are means ± SD and range; (*n*), different from total *n*.

amino acids among the groups. In particular, leucine, isoleucine, valine, and the sum of branch-chain amino acids were not different. Furthermore, no differences in the concentrations of arginine and alanine were observed. The remaining amino acids also were not different. Mean umbilical plasma concentrations for the cesarean section control AGA group were leucine 128 ± 29 μM, branch-chain amino acid (sum) 423 ± 85 μM, arginine 96 ± 31 μM, and alanine 341 ± 60 μM.

Indicators of insulin secretion (Table 3) include total insulin concentration, which was 81.2 ± 64 (range 14–337) pM in the cesarean section AGA group I compared with the highest values in group IV (B, C, and D) at 1,060 ± 1,710 (range 29–7,613) pM. The other groups were intermediate. Significant differences were observed among groups; thus, groups III, IV, and V differed from I and II. In addition, groups IV and V differed from groups II and III, but groups IV and V were not different.

Insulin antibody binding was found in a significant number of subjects in groups IV and V, i.e., IDDM patients. Considering the percent insulin binding >2 SD for the control subjects, 29% of group IV and 40% of group V were elevated. A significant number (8 of 67 and 2 of 15, respectively) had binding exceeding 10%. Nonetheless, free insulin concentrations (not shown) were similar to the total insulin values in each group, indicating that the effect of insulin antibodies in the overall sample was minimal. No differences between the labor and cesarean section groups were found. The infants with elevated insulin antibodies had similar body size to those without antibodies.

Umbilical plasma C-peptide concentrations (Table 3) follow the same statistical significance pattern as does total insulin in the cesarean section groups. The cesarean section AGA control group I had values of 0.32 ± 0.11 nM, and group IV (B, C, and D) had values of 1.24 ± 0.94. The other diabetic groups were intermediate. The LGA control group was similar to the AGA control subjects at 0.39 ± 0.21 nM. The upper limit of AGA control values (0.62 nM) was exceeded by the mean levels of all diabetic groups. The percentage of abnormal values (>0.62 nM) for the cesarean section–delivered diabetic groups were as follows: group III was 31.6%, group IV was 76.3%, and group V was 82.4%.

The percent of insulin immunore-

activity accounted for by proinsulin and proinsulin conversion intermediates was not different from the control subjects or each other for the diabetic groups (Fig. 1). Analysis of purified proinsulin and proinsulin conversion intermediates showed that the partially cleaved conversion intermediates eluted at a higher apparent molecular weight than intact proinsulin. The PLC of umbilical plasma samples eluted from the column in a position corresponding to the partially cleaved intermediates. A representative chromatogram is shown in Fig. 1.

To determine which factors contributed to infant size (SDU), a multivariate analysis was conducted with maternal prepregnancy weight, BMI, and HbA_{1c}. An initial analysis was performed to define the contribution of hypertension. Each group was subcategorized accordingly. No differences could be ascertained in the parameters studied relative to maternal hypertension so that all subsequent analyses were performed on combined data.

SDU was weakly correlated with maternal prepregnancy weight, BMI, and 3rd trimester HbA_{1c} ($r^2 = 0.11$, SDU = $-1.70 + 0.43$ HbA_{1c}, $P = 0.001$) (Fig. 2). Omitting group V from the analysis because of the potential for intrauterine growth retardation did not make any difference. Omitting the control subjects weakened the relationship further (SDU = $0.778 + 0.301$ HbA_{1c}, $r^2 = 0.047$). Glucose concentration was a poor predictor of SDU in a subset of 68 patients; thus, SDU = $5.19 - 0.06$ (total blood glucose) at 32 weeks ($r^2 = 0.06$, $P < 0.05$). One-hour postprandial glucose at 26 ± 2 weeks was a stronger predictor of SDU in a subset of 66 diabetic subjects. Thus, SDU = $-0.06 + 0.16$ (1-h postprandial glucose at 26 weeks), $r^2 = 0.13$, $P < 0.005$. At 30 ± 2 weeks, $r^2 = 0.01$, $P < 0.001$. In contrast, there was a positive, significant correlation of SDU with fetal insulin concentration (Fig. 3*A*), i.e., total insulin in umbilical plasma ($r^2 = 0.409$, $P = 0.001$), and with C-peptide (Fig. 3*B*) ($r^2 = 0.254$, $P = 0.001$). No significant relationship of total insulin and HbA_{1c} ($r^2 = 0.20$) was found.

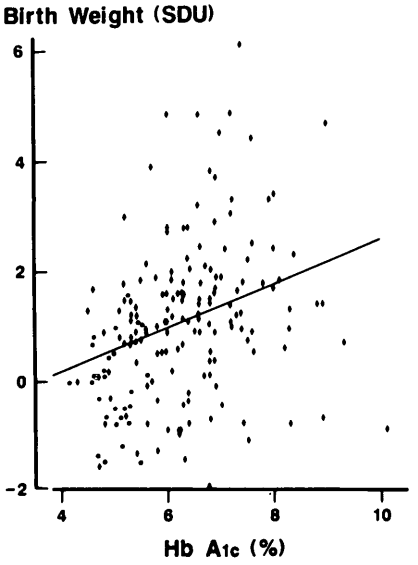

Figure 2—*Relationship of infant birth weight in SDUs, corrected for gestational age and sex, to mean maternal 3rd trimester HbA_{1c} (%). n = 179, y = −1.70 + 0.43 x; $r^2 = 0.11$, P < 0.0001. Limit of HbA_{1c} at 6.6% is mean + 2 SD of controls; limits of SDU are >2. ●, Control subjects; ♦, diabetic subjects.*

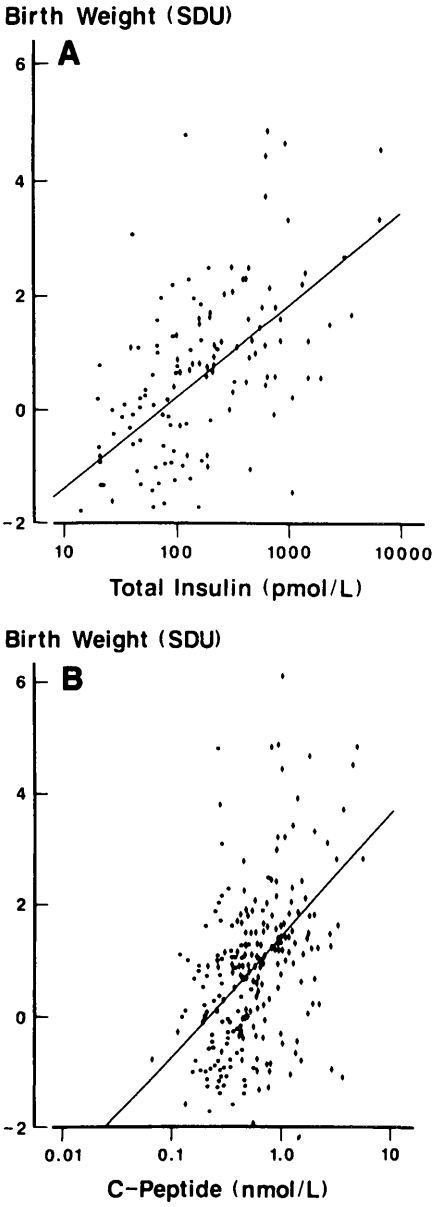

Figure 3—A: *the relationship of infant birth weight (SDUs relative to a large Helsinki reference population) compared with umbilical plasma total insulin (pM). ●, Control subjects; ♦, diabetic subjects. The regression line refers to all data except LGA control subjects, group II.* n = *123*; y = *−2.87 + 1.53* x; $r^2 = 0.41$, P = *0.001*, r = *0.64*. B: *a similar relationship for infant birth weight (SDU) to umbilical plasma C-peptide concentration (nM).* n = *244*; y = *1.23 + 2.09* x; $r^2 = 0.25$, P = *0.001*, r = *0.5*.

CONCLUSIONS— Functional hyperinsulinism in the fetus of the diabetic mother has been inferred from the physiological events that are a consequence of altered maternal/fetal metabolism and that may be expressed in neonatal hypoglycemia and suppressed plasma free fatty acid levels (1,28). Elevations of fetal plasma C-peptide concentration have been observed in umbilical plasma at delivery by cesarean section as well as in cordocentesis (1,6). This study confirms these earlier observations on C-peptide.

Although the presence of hyperinsulinemia has been established through measurement of plasma insulin (total or free) and C-peptide in fetal plasma (5,29), data on in vivo insulinogenic stimuli are inconclusive (1,30). Alterations of fetal glucose substrate administered via the mother indicate that the early (2nd trimester) fetus does not respond to hyper-

glycemia with elevated insulin levels (31). Studies in preterm infants also indicate that the prompt, adult response to glucose administration does not occur, whereas infusion of insulinogenic amino acids produces a significant insulin elevation (12). The most direct physiological evidence for fetal insulin control comes from studies of the chronically catheterized fetal lamb (11). Chronic infusion of dextrose produces inconsistent hyperinsulinemia. Other more direct studies have involved perifused human fetal islets (10). The most effective stimuli were arginine and leucine, but the effect varied with gestational age.

Insulin biosynthesis is expressed by insulin and C-peptide, which are secreted by the β-cell in equimolar amounts. Processing of precursor proinsulin is not perfect, and proinsulin and a variety of intermediates may also be secreted into the circulation. These are referred to in aggregate as PLCs (32). Gel filtration has been considered the preferred technique for this measurement. Other techniques have included affinity chromatography with insulin antibodies bound to Sepharose (33), and most recently by direct RIA (34).

Unlike Heding et al. (33) who used affinity chromatography, we did not find disproportionate elevation of PLCs in umbilical plasma of infants of diabetic mothers compared with infants of nondiabetic mothers. The differences between these two reports are most likely due to the methodology because both studies included a spectrum of well-managed IDDM mothers. The implications of the absolute increase in PLC may well be limited. Because insulin-like growth factor 1 (IGF-1) and proinsulin share similarities in both primary and tertiary structure, Gruppuso et al. (35) examined the binding of proinsulin and proinsulin conversion intermediates to human placental IGF-1 receptors. Insulin was ~0.5% as potent as IGF-1, but proinsulin was only 2% as potent as insulin. There is no evidence that proinsulin or six of the conversion intermediates could be physiologically significant by way of IGF-1 receptor binding.

This large study details the presence of increased fetal umbilical plasma total and free insulin, in addition to C-peptide. The highest values were found in groups IV and V, i.e., White's classes B, C, and D, and F/R; however, the gestational diabetic group also had significant elevations of these parameters. The mechanisms (secretagogues) responsible for hypersecretion of insulin are less clear. Maternal hyperglycemia, i.e., HbA_{1c}, did not correlate with fetal insulin or C-peptide concentrations. Similarly, umbilical plasma glucose concentration in fetuses delivered by cesarean section (thus avoiding the stress of labor) was not related. Because abnormalities in mixed nutrients were proposed by Freinkel and Metzger (36) as the basis for hyperinsulinemia, total and individual amino acids were determined in umbilical plasma. These were remarkably stable in this population and did not differ among the groups studied.

Among the explanations for the failure to identify an insulin secretagogue mechanism are *1*) concentrations of substrate do not represent flux, thus it is possible that the β-cell has transporters for glucose and/or specific amino acids that are overly active; *2*) postprandial substrate elevations may be necessary to stimulate fetal insulin secretion; *3*) possible increase in sympathetic-parasympathetic activity in the β-cell; and *4*) autonomous endogenous insulin secretion as occurs in neonatal hyperinsulinemic hypoglycemia. The latter is unlikely because the hyperinsulinemia is present in utero, but subsides promptly after delivery. Recently, postprandial maternal glucose concentration has been found by others to better correlate with infant size than fasting glucose or HbA_{1c} (3,13).

If fetal hyperinsulinemia secondary to disturbed metabolic control of the diabetic mother is the basis for fetal macrosomia, then rigid control of diabetes, as has occurred in the past decade, should have reduced its frequency, as with respiratory distress syndrome. This has not been found. Thus, a continued frequency of macrosomia of 25–35% has been reported (2,3). This present study continues to have 10–27% depending on White's class, despite improved maternal control evident in 3rd trimester HbA_{1c}. Although a significant difference was observed in maternal HbA_{1c} between the groups with normal vs. macrosomic infants, the correlation between SDU and HbA_{1c} was very weak. No relationship was previously found for a similar, less well-controlled population (37). In addition, data from glucose profiles obtained during hospitalization demonstrated only a weak relationship with fetal size at birth.

Menon et al. (38) observed antibody-mediated transfer of animal insulin from mother to fetus. They proposed that macrosomia was related to this additional insulin. This required insulin antibodies for the transfer. Our present study does not support this hypothesis, because no difference in infant size was found between those with or without antibodies. Indeed, no simple direct relationship between total insulin or C-peptide and body size (SDU) was found. Using a multiple regression analysis technique, we could account for 54% (r^2) of the relationship. Two factors, maternal prepregnancy BMI and diabetes class, were additional contributors. The other variables were indirect (HbA_{1c}) or direct (total insulin and C-peptide) contributors. Apparently, this clinical study does not have a unifying explanation for fetal macrosomia in the diabetic pregnancy. It is puzzling still that insufficient metabolic evidence has been produced for the hyperinsulinemia. Either our tools for assessing metabolic control are inadequate, which is likely, or the insulinogenic stimuli transferred from mother to fetus are inadequately understood.

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References

1. Cornblath M, Schwartz R: Infant of the diabetic mother. In *Disorders of Carbohydrate Metabolism in Infancy*. 3rd ed. Cornblath M, Schwartz R, Eds. Boston, MA, Blackwell Scientific Publications, 1991, p. 125–174
2. Landon MB, Gabbe SG, Piana R, Mennuti MT, Main EK: Neonatal morbidity in pregnancy complicated by diabetes mellitus: predictive value of maternal glycemic profiles. *Am J Obstet Gynecol* 156:1089–1095, 1987
3. Combs CA, Gavin LA, Gunderson E, Main EK, Kitzmiller JL: Relationship of fetal macrosomia to maternal postprandial glucose control during pregnancy. *Diabetes Care* 15:1251–1257, 1992
4. Susa JB, McCormick KL, Widness JA, Singer DB, Oh W, Adamsons K, Schwartz R: Chronic hyperinsulinemia in the fetal rhesus monkey: effects on fetal growth and composition. *Diabetes* 28:1058–1063, 1979
5. Kuhl C, Andersen GE, Hertel J, Molsted-Pedersen L: Metabolic events in infants of diabetic mothers during the first 24 hours after birth. I. Changes in plasma glucose, insulin, and glucagon. *Acta Paediatr Scand* 71:19–25, 1982
6. Block MB, Pildes RS, Mossabhoy NA, Steiner DF, Rubenstein AH: C-peptide immunoreactivity (CPR): a new method for studying infants of insulin-treated diabetic mothers. *Pediatrics* 53:923–928, 1974
7. Heding LG, Persson B, Stangenberg M: β-cell function in newborn infants of diabetic mothers. *Diabetologia* 19:427–432, 1980
8. Bradley RJ: cited in Diabetic Pregnancy. In *Current Reviews in Obstetrics and Gynecology*. Brudenell M, Doddridge MC, Lind T, Eds. Edinburgh, Scotland, Churchill Livingstone, 1989, p. 142
9. Persson B: Amniotic fluid levels of insulin and C-peptide in pregnancies complicated by diabetes mellitus. In *Carbohydrate Metabolism in Pregnancy and the Newborn*. Vol. IV. Sutherland HW, Stowers JM, Pearson DWM, Eds. London, Springer-Verlag, 1989, p. 119–128
10. Milner RDG, Ashworth MA, Barson HJ: Insulin release from human fetal pancreas in response to glucose, leucine, and arginine. *J Endocrinol* 52:497–505, 1972
11. Philipps AF, Porte PJ, Stabinsky S, Rosenkrantz, TS, Raye JR: Effects of chronic fetal hyperglycemia upon oxygen consumption in the ovine uterus and conceptus. *J Clin Invest* 74:279–286, 1984
12. Grasso S, Saporito N, Messina A, Reitano G: Serum-insulin response to glucose and amino acids in the premature infant. *Lancet* 2:755–757, 1968
13. Jovanovic-Peterson L, Peterson CM, Reed GF, Metzger BE, Mills JL, Knopp RH, Aarons JH, the National Institute of Child Health and Human Development: Maternal postprandial glucose levels and infant birth weight: the Diabetes in Early Pregnancy Study. *Am J Obstet Gynecol* 164: 103–111, 1991
14. Widness JA, Teramo KA, Clemons GK, Voutilainen P, Stenman U- H, McKinlay SM, Schwartz R: Direct relationship of antepartum glucose control and fetal erythropoietin in human type 1 (insulin-dependent) diabetic pregnancy. *Diabetologia* 33:378–383, 1990
15. Ylinen K, Hekali R, Teramo K: HbA_{1c} during pregnancy of insulin-dependent diabetics and healthy controls. *Obstet Gynecol* 1:223–228, 1981
16. Pihkala J, Hakala T, Voutilainen P, Raivio K: Uudet suomalaiset sikion kasuukayrat. *Duodecim* 105:1540–1546, 1989
17. Schumacher LB, Pawson IG, Silliman K, Kretchmer N: Ethnic variation in newborn anthropometry: the macrosomic infant. In *Body Composition Measurements in Infants and Children*. Klish WJ, Kretchmer N, Eds. Columbus, OH, Report of the 98th Ross Conference on Pediatric Research, 1989, p. 95–103
18. Stenman U-H, Pesonen K, Ylinen K, Huhtala M-L, Teramo K: Rapid chromatographic quantification of HbA_{1c}. *J Chromatogr* 297:327–332, 1984
19. Gorden P, Roth J: Plasma insulin: fluctuations in the "big" insulin component in man after glucose and other stimuli. *J Clin Invest* 48:2225–2234, 1969
20. Kuzuya H, Blix P, Horwitz D, Steiner D, Rubenstein A: Determination of free and total insulin and C-peptide in insulin-treated diabetics. *Diabetes* 26:22–29, 1977
21. Rudkowski R, Antony G: The effect of immediate polyethylene glycol precipitation on free insulin measurements in diabetic patients with insulin antibodies. *Diabetes* 35:253–257, 1986
22. Dixon K: Measurement of antibodies to insulin in serum. *Clin Chem* 20/10:1275–1281, 1974
23. Graybill FA: *Theory and Application of the Linear Model*. North Scituate, MA, Duxbury Press, 1976
24. Searle SR: *Linear Models for Unbalanced Data*. New York, Wiley, 1987
25. SAS Institute: *SAS/STAT User's Guide*. Release 6.03 ed. Cary, NC, 1988
26. Green JR, Schumacher LB, Pawson IG, Partridge JC, Kretchmer N: Influence of maternal body habitus and glucose tolerance on birth weight. *Obstet Gynecol* 78: 235–240, 1991
27. Bray GA: Treatment for obesity. In *Diabetes Mellitus and Obesity*. Brodoff BN, Bleicher SJ, Eds. Baltimore, MD, Williams & Wilkins, 1982, p. 322–332
28. Pedersen J: The Fetus and Newborn Infant. In *The Pregnant Diabetic and Her Newborn*. Pedersen J, Ed. Baltimore, MD, Williams & Wilkins, 1967, p. 60–107
29. Knip M, Lautala P, Leppaluoto J, Akerblom HK, Kouvalainen K: Relation of enteroinsulinar hormones at birth to macrosomia and neonatal hypoglycemia in infants of diabetic mothers. *J Pediatr* 103: 603–611, 1983
30. Ashworth MA, Leach FN, Milner RDG: Development of insulin secretion in the human fetus. *Arch Dis Child* 48:151–152, 1973
31. Obenshain SS, Adam PAJ, King KC, Teramo K, Raivio KO, Raiha N, Schwartz R: Human fetal insulin response to sustained maternal hyperglycemia. *N Engl J Med* 283:566–570, 1970
32. DeHaen C, Little SA, May JM, Williams RH: Characterization of proinsulin-insulin intermediates in human plasma. *J Clin Invest* 62:727–737, 1978
33. Heding LG: Specific and direct radioim-

munoassay for human proinsulin in serum. *Diabetologia* 13:467–474, 1977
34. Bowsher RR, Wolny JD, Frank BH: A rapid and sensitive radioimmunoassay for the measurement of proinsulin in human serum. *Diabetes* 41:1084–1090, 1992
35. Gruppuso PA, Frank BH, Schwartz R: Binding of proinsulin and proinsulin conversion intermediates to human placental insulin-like growth factor I receptors. *J Clin Endocrinol Metab* 67:194–197, 1988
36. Freinkel N, Metzger BE: Pregnancy as a tissue culture experience: the critical implications of maternal metabolism for fetal development. *Ciba Found Symp* 63:3–23, 1979
37. Schwartz R, Teramo KA: Primary hyperinsulinemia and macrosomia in infants of diabetic mothers. In *Infants of Diabetic Mothers*. Gabbe SG, Oh W, Eds. Columbus, OH, Report of the 93rd Ross Conference on Pediatric Research, 1987, p. 40–50
38. Menon RK, Cohen RM, Sperling MA, Cutfield WS, Mimouni F, Khoury JC: Transplacental passage of insulin in pregnant women with insulin-dependent-diabetes mellitus: its role in fetal macrosomia. *N Engl J Med* 323:309–315, 1990