Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study

Associations With Neonatal Anthropometrics

The HAPO Study Cooperative Research Group*

OBJECTIVE—To examine associations of neonatal adiposity with maternal glucose levels and cord serum C-peptide in a multicenter multinational study, the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study, thereby assessing the Pedersen hypothesis linking maternal glycemia and fetal hyperinsulinemia to neonatal adiposity.

RESEARCH DESIGN AND METHODS—Eligible pregnant women underwent a standard 75-g oral glucose tolerance test between 24 and 32 weeks gestation (as close to 28 weeks as possible). Neonatal anthropometrics and cord serum C-peptide were measured. Associations of maternal glucose and cord serum C-peptide with neonatal adiposity (sum of skin folds >90th percentile or percent body fat >90th percentile) were assessed using multiple logistic regression analyses, with adjustment for potential confounders, including maternal age, parity, BMI, mean arterial pressure, height, gestational age at delivery, and the baby's sex.

RESULTS—Among 23,316 HAPO Study participants with glucose levels blinded to caregivers, cord serum C-peptide results were available for 19,885 babies and skin fold measurements for 19,389. For measures of neonatal adiposity, there were strong statistically significant gradients across increasing levels of maternal glucose and cord serum C-peptide, which persisted after adjustment for potential confounders. In fully adjusted continuous variable models, odds ratios ranged from 1.35 to 1.44 for the two measures of adiposity for fasting, 1-h, and 2-h plasma glucose higher by 1 SD.

CONCLUSIONS—These findings confirm the link between maternal glucose and neonatal adiposity and suggest that the relationship is mediated by fetal insulin production and that the Pedersen hypothesis describes a basic biological relationship influencing fetal growth. ***Diabetes*** **58:453–459, 2009**

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A complete list of members of the HAPO Study Writing Group can be found in the APPENDIX.

The objective of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study was to clarify the risk of adverse outcome associated with degrees of glucose intolerance during pregnancy that are less severe than overt diabetes. Glucose tolerance was measured by a 75-g 2-h oral glucose tolerance test (OGTT) in a large, heterogeneous, multinational, ethnically diverse cohort of women at 24–32 (mean 28) weeks gestation with medical caregivers blinded to status of glucose tolerance (except when predefined thresholds were met) (1). Associations between maternal glycemia and increased size at birth, delivery by cesarean section, development of neonatal hypoglycemia, and the presence of fetal hyperinsulinemia were the predefined primary outcomes of the study. Results of the study showing continuous relationships of maternal glucose levels below those diagnostic of diabetes with each of the primary outcomes have been reported (2). Associations of maternal glucose and birth weight >90th percentile and fetal hyperinsulinemia [cord C-peptide concentration greater than the HAPO Study 90th percentile (1.7 μg/l)] were strong. Weaker associations were found with cesarean delivery and clinical neonatal hypoglycemia (2).

In 1952, Pedersen (3) postulated that maternal hyperglycemia was transmitted to the fetus, which, in turn, produced and released large amounts of insulin, with the resultant fetal hyperinsulinemia being the cause of various aspects of diabetic fetopathy, including deposition of large amounts of body fat, which gave the infant its characteristic appearance. Pedersen documented increased body weight in infants of diabetic mothers compared with control subjects. Fetal hyperinsulinemia, in the absence of maternal diabetes, has been demonstrated to cause "diabetes-like" fetopathy in rhesus monkey offspring (4). At least some of the increased fetal weight has been shown to be attributable to increased fat accretion (5).

In 1977, Whitelaw (6) reported an association between diabetic control and skin fold thickness in infants of diabetic mothers. Sparks reported that body fat more specifically represents effects of the in utero environment, whereas lean body mass represents more of the genetic component of growth (7). For example, male neonates have greater birth weight than females primarily because of increases in lean body mass (8). Therefore, we elected to estimate body composition, in particular fat mass and percent body fat, as specific outcomes in the HAPO Study cohort.

These reports and many others validate the basic tenets of the Pedersen hypothesis. However, efforts to define the strength of associations with hyperglycemia are confounded by treatment. Furthermore, the direct link between maternal glycemia, fetal insulin response, and

neonatal body composition has not yet been demonstrated in the subdiabetic glucose range. Goals of this report are *1*) to examine associations of maternal glycemia with newborn anthropometrics (skin folds, percent body fat); and *2*) to present data linking fetal hyperinsulinemia (assessed by cord serum C-peptide) to the development of larger and more obese babies.

RESEARCH DESIGN AND METHODS

The protocol was approved by the institutional review board at all 15 field centers. All participants gave written informed consent. An external data monitoring committee provided oversight. Study methods have been published previously (1,2,9). A brief overview is presented here.

All pregnant women at each field center were eligible to participate unless they had one or more exclusion criteria (2): age <18 years, delivery planned at another hospital, date of last menstrual period (LMP) not certain and no ultrasound estimation from 6–24 weeks of gestational age available, unable to complete the OGTT by 32 weeks gestation, multiple pregnancy, conception using gonadotropin ovulation induction or by in vitro fertilization, glucose testing before recruitment or a diagnosis of diabetes during this pregnancy, diabetes antedating pregnancy requiring treatment with medication, participation in another study that may interfere with HAPO, known to be HIV positive or to have hepatitis B or C, prior participation in HAPO, or inability to converse in the languages used in field center forms without the aid of an interpreter. If glucose measurements were made outside of HAPO after initial enrollment, the participant was excluded from further participation.

Gestational age and expected date of delivery (EDD) were determined from the date of the LMP, if the participant was certain of her dates. If uncertain, EDD was determined from an ultrasound performed between 6 and 24 weeks gestation. Final EDD was also determined from ultrasound if *1*) gestational dating from LMP differed from ultrasound dating by >5 days, when the ultrasound was performed between 6 and 13 weeks, or *2*) if dating differed by >10 days when the ultrasound was done between 14 and 24 weeks.

OGTT. Participants underwent a standard 75-g OGTT between 24 and 32 weeks gestation (as close to 28 weeks as possible). Height, weight, and blood pressure were measured at the OGTT visit using standardized procedures and calibrated equipment. Data concerning smoking and alcohol use, first-degree family history of diabetes, and demographic data were collected using standardized questionnaires. Race/ethnicity was self-identified by participants. A sample for random plasma glucose (RPG) was collected at 34–37 weeks gestation as a safety measure to identify cases with hyperglycemia above a predefined threshold.

Glucose analysis and unblinding. Aliquots of fasting and 2-h OGTT and RPG samples were analyzed at field center laboratories. Values were unblinded if fasting plasma glucose (FPG) exceeded 5.8 mmol/l, if 2-h OGTT plasma glucose exceeded 11.1 mmol/l, if RPG was ≥8.9 mmol/l, or if any plasma glucose value was <2.5 mmol/l. Otherwise, women, caregivers, and HAPO Study staff (except for laboratory personnel) remained blinded to glucose values. To avoid confounding effects of center to center analytical variation, aliquots of all OGTT specimens were analyzed at the HAPO Central Laboratory, and these results are used here. Only women whose results remained blinded, with no additional glucose testing outside the HAPO protocol, are included in these analyses.

Cord serum C-peptide sample. Cord blood was collected at delivery, and serum was analyzed for C-peptide on an Autodelfia instrument at the Central Laboratory (9). Cord serum C-peptide (secreted in equimolar concentrations with insulin) was used as the index of fetal β-cell function rather than insulin because insulin degradation is increased in the presence of even small amounts of hemolysis, which occurs in ~15% of cord samples, and because C-peptide concentration is not altered by hemolysis (10). Functional sensitivity of the assay was 0.2 μg/l (9).

Prenatal care and delivery. Prenatal care and timing of delivery were determined by standard field center practice. No field center arbitrarily delivered patients before full term or routinely performed cesarean delivery at a specified maternal or gestational age.

Neonatal care and anthropometrics. After delivery, infants received customary routine care. Medical records were abstracted to obtain data regarding prenatal, labor and delivery, postpartum, and newborn course.

Neonatal anthropometrics were obtained within 72 h of delivery. To ensure accuracy and reliability of anthropometric data and consistency across field centers, a rigorous training and certification procedure was established for study research nurses and midwives. Personnel were trained during regional training sessions run by Clinical and Data Coordinating Center staff. A training videotape providing instruction in anthropometric measurements was viewed. Research personnel observed measurements and then performed measurements on five infants. After training and before recruitment, research personnel continued to perform measurements locally on two infants per week and demonstrated their proficiency with measuring neonatal anthropometrics during a dry-run site visit. To maintain quality control of skin fold measures, all research personnel underwent annual recertification, which included reviewing the videotape and providing data in tandem with another certified individual on three to five babies.

Anthropometric measurements included weight, length, head circumference, and skin fold thickness at three sites (flank, subscapular, and triceps). Two measurements were made, and if results differed by more than a prespecified amount (>10 g for weight, >0.5 cm for length and head circumference, or >0.5 mm for skin folds, respectively), a third was done. For these analyses, the average of the two measurements was used, unless a third measurement was taken. In that case, if two of three measurements differed by less than the prespecified amount, the average of those two was used; otherwise the average of all three was used.

Birth weight was obtained without diaper using a calibrated electronic scale. Length was measured on a standardized plastic length board constructed for use in the HAPO Study. Head circumference was measured across the occipital fontanel (standard plastic measuring tape). Skin fold thickness was measured with skin fold calipers (Harpenden, Baty, U.K.). Flank skin fold was measured on the left side just above the iliac crest on a diagonal fold on the mid axillary line, triceps by taking the vertical fold over the triceps muscle half the distance between the acromion process and olecranon, and subscapular just below the lower angle of the scapula at ~45° angle to the spine.

Mean coefficients of variation for anthropometric measurements were: birth weight 0.04%, length 0.17%, head circumference 0.16%, flank skin fold 2.91%, subscapular skin fold 2.57%, and triceps skin fold 2.73%.

Outcomes

Sum of skin folds >90th percentile. For gestational age (36–44 weeks only), 90th percentiles were determined using eight newborn gender-ethnic groups (Caucasian or other, Black, Hispanic, or Asian), with adjustment for gestational age, field center, and parity (0, 1, and 2+). A newborn was

TABLE 1
Characteristics of HAPO participants*

Maternal characteristics	
Age (years)	19,885 (29.2 ± 5.8)
Education (years)	18,223 (12.9 ± 3.4)
Pre-pregnant BMI	18,200 (23.8 ± 5.0)
BMI (kg/m^2)†	19,885 (27.5 ± 5.0)
MAP (mmHg)†	19,885 (80.7 ± 8.2)
FPG (mmol/l)†	19,885 (4.5 ± 0.4)
1-h Plasma glucose (mmol/l)†	19,885 (7.5 ± 1.7)
2-h Plasma glucose (mmol/l)†	19,885 (6.2 ± 1.3)
Gestational age (weeks)†	19,885 (27.8 ± 1.8)
Ethnicity	
White, non-Hispanic	9,189 (46.2)
Black, non-Hispanic	2,279 (11.5)
Hispanic	1,774 (8.9)
Asian	6,181 (31.1)
Other	462 (2.3)
Prenatal smoking (any)	1,217 (6.1)
Prenatal alcohol use (any)	1,272 (6.4)
Family history of diabetes	4,411 (22.2)
Parity (prior delivery ≥20 weeks)	10,408 (52.3)
Newborn characteristics	
Gestational age (weeks)	19,885 (39.4 ± 1.6)
Birth weight (g)	19,884 (3,308 ± 505)
Length (cm)	19,496 (49.8 ± 2.4)
Head circumference (cm)	19,723 (34.2 ± 1.5)
Flank skin fold (mm)‡	17,159 (3.9 ± 1.0)
Triceps skin fold (mm)‡	17,136 (4.1 ± 0.9)
Subscapular skin fold (mm)‡	17,115 (4.2 ± 1.0)
Sum of skin folds (mm)‡	17,100 (12.3 ± 2.6)
Body fat (%)‡	17,050 (11.3 ± 3.7)
Cord serum C-peptide (μg/l)	19,885 (1.0 ± 0.6)
Male	10,235 (51.5)

Data are *n* (mean ± SD) or *n* (%). *Participants with a cord serum C-peptide measurement. †Measured at the OGTT. ‡Babies with gestational age 36–44 weeks at birth.

TABLE 2
Relationship between maternal glucose and sum of skin folds >90th percentile*

	n	>90th percentile†	Model I	Model II
FPG (mmol/l)				
<4.2	3,340	177 (5.3)	1.00	1.00
4.2–4.4	6,270	480 (7.7)	1.48 (1.24–1.77)	1.39 (1.16–1.66)
4.5–4.7	5,186	504 (9.7)	1.92 (1.61–2.30)	1.66 (1.38–1.99)
4.8–4.9	2,287	278 (12.2)	2.47 (2.03–3.01)	2.00 (1.64–2.45)
5.0–5.2	1,556	259 (16.6)	3.57 (2.92–4.37)	2.72 (2.20–3.36)
5.3–5.5	576	119 (20.7)	4.65 (3.62–5.99)	3.37 (2.59–4.38)
≥5.6	174	46 (26.4)	6.42 (4.44–9.29)	4.71 (3.22–6.89)
Continuous‡	19,389	1,863 (9.6)	1.52 (1.45–1.59)	1.39 (1.33–1.47)
1-h Plasma glucose (mmol/l)				
≤5.8	3,482	212 (6.1)	1.00	1.00
5.9–7.3	6,258	483 (7.7)	1.29 (1.09–1.52)	1.22 (1.03–1.45)
7.4–8.6	5,007	468 (9.3)	1.59 (1.34–1.88)	1.50 (1.26–1.78)
8.7–9.5	2,324	310 (13.3)	2.37 (1.98–2.85)	2.22 (1.84–2.69)
9.6–10.7	1,570	245 (15.6)	2.85 (2.35–3.46)	2.63 (2.14–3.22)
10.8–11.7	536	103 (19.2)	3.67 (2.84–4.74)	3.38 (2.59–4.41)
≥11.8	212	42 (19.8)	3.81 (2.64–5.49)	3.57 (2.46–5.20)
Continuous‡	19,389	1,863 (9.6)	1.44 (1.37–1.51)	1.42 (1.35–1.49)
2-h Plasma glucose (mmol/l)				
≤5.0	3,537	209 (5.9)	1.00	1.00
5.1–6.0	6,135	496 (8.1)	1.40 (1.18–1.66)	1.32 (1.11–1.56)
6.1–6.9	4,948	481 (9.7)	1.71 (1.45–2.03)	1.60 (1.35–1.90)
7.0–7.7	2,556	352 (13.8)	2.54 (2.13–3.04)	2.38 (1.98–2.86)
7.8–8.7	1,444	198 (13.7)	2.53 (2.06–3.11)	2.39 (1.93–2.95)
8.8–9.8	576	90 (15.6)	2.95 (2.26–3.84)	2.80 (2.13–3.69)
≥9.9	193	37 (19.2)	3.78 (2.57–5.55)	3.59 (2.42–5.33)
Continuous‡	19,389	1,863 (9.6)	1.37 (1.31–1.44)	1.36 (1.30–1.43)

Data are *n* (%) or OR (95% CI). *n* = total number in the glucose category (excluding births with gestational age <30 weeks and fetal deaths). Model I, adjusted for the variables used in estimating 90th percentiles; model II, adjusted for age, BMI, BMI^2, height, mean arterial blood pressure, gestational age at the OGTT, smoking, alcohol use, hospitalization prior to delivery, and any family history of diabetes. *Defined based on sex, ethnicity, field center, gestational age (36–44 weeks), and parity. †Number in the glucose category with the sum of skin folds >90th percentile (% proportion in the glucose category with sum of skin folds >90th percentile). ‡Glucose higher by 1 SD (0.4 mmol/l for FPG, 1.7 mmol/l for 1-h plasma glucose, 1.3 mmol/l for 2-h plasma glucose).

considered to have a sum of skin folds >90th percentile if the sum was greater than the estimated 90th percentile for the baby's sex, gestational age, ethnicity, field center, and maternal parity. Otherwise, the newborn was considered to have a sum ≤90th percentile.

Individual skin fold (flank, triceps, and subscapular) >90th percentile. An individual skin fold >90th percentile was defined using the same methods as for sum of skin folds >90th percentile.

Percent body fat >90th percentile. Fat mass was calculated from birth weight, length, and flank skin fold according to the equation given by Catalano et al. (11) that was based on measurements of total body electrical conductivity (TOBEC). The derived formula was also prospectively validated with estimates of fat mass by TOBEC. Percent body fat was then calculated as 100 × fat mass/birth weight. Percent body fat >90th percentile was defined using the same methods as for sum of skin folds >90th percentile.

Birth weight >90th percentile. Birth weight >90th percentile was also defined using the same methods as for sum of skin folds >90th percentile, with gestational ages of 30–44 weeks included.

Statistical analyses. Descriptive statistics include means and SDs for continuous variables and numbers and percentages for categorical variables. For analyses of associations of glycemia and cord serum C-peptide with fetal adiposity (sum of skin folds >90th percentile or percent body fat >90th percentile), each glucose measurement and cord C-peptide were considered as both categorical and continuous variables in multiple logistic regression analyses. For individual skin folds, only continuous variable results are presented. In categorical analyses, each measure of glycemia was divided into seven categories with ~50% of all values in the two lowest categories and 3 and 1% in the two highest categories, respectively (2). To make categorical analyses of outcomes with cord C-peptide comparable with those for maternal glycemia, we also divided cord C-peptide into seven categories so that ~50% of values were in the two lowest categories and ~3 and 1% were in the two highest categories, respectively. For glucose as a continuous variable, odds ratios (ORs) were calculated for each measure (fasting, 1-, and 2-h plasma glucose) higher by 1 SD. To assess whether the log of the odds of fetal adiposity was linearly related to glucose, we added squared terms in each glucose measure. We also tested for interactions of glucose with field center, BMI, age, height, and mean arterial pressure (MAP) for each glucose measure for each outcome. Because of the large sample size in HAPO and the generally large number of women with each outcome, squared terms as well as interaction terms were considered statistically significant only for $P < 0.001$. We also examined cord C-peptide as a continuous variable with both linear and squared terms for each outcome. However, because the squared term was significant ($P < 0.001$) for each outcome and the categorical analysis appeared to provide a better fit to the data, only the categorical analyses are reported here for cord C-peptide.

For each outcome, two logistic models (I and II) were fit, with model I including adjustment for the variables used to define the 90th percentile for the neonatal anthropometric measures and model II including additional adjustment for multiple potential confounders that had been prespecified. Potential confounders included in model II were maternal age, BMI, height, gestational age, and MAP at the OGTT; family history of diabetes; hospitalization before delivery; smoking status; and alcohol use. Squared terms for age, BMI, and MAP were prescreened for possible inclusion in model II adjustment in models that included only linear and squared terms for these variables. Squared terms were included if $P < 0.001$. All analyses were conducted in SAS version 9.1 or Stata 10.0.

RESULTS

A cord C-peptide result was available for 19,885 babies of the 23,316 blinded HAPO Study participants who were included in the first report of HAPO Study results (2). Results shown in Table 1 are from those 19,885 babies and their mothers. Mean maternal BMI at the OGTT was 27.5, and the correlation with prepregnant BMI, which was based on self-reported prepregnant weight, is 0.92. Mean glucose levels among this group were 4.5, 7.5, and 6.2 mmol/l for fasting, 1-h, and 2-h plasma glucose, respectively. Mean gestational age at delivery was 39.4 weeks,

TABLE 3
Relationship between maternal glucose and percent body fat >90th percentile*

	n	>90th percentile†	Model I	Model II
FPG (mmol/l)				
<4.2	3,336	208 (6.2)	1.00	1.00
4.2–4.4	6,255	478 (7.6)	1.24 (1.05–1.47)	1.16 (0.98–1.38)
4.5–4.7	5,174	499 (9.6)	1.61 (1.36–1.90)	1.39 (1.17–1.65)
4.8–4.9	2,274	295 (13.0)	2.24 (1.86–2.70)	1.83 (1.51–2.21)
5.0–5.2	1,545	263 (17.0)	3.09 (2.54–3.74)	2.36 (1.92–2.89)
5.3–5.5	570	101 (17.7)	3.24 (2.51–4.19)	2.34 (1.79–3.05)
≥5.6	173	48 (27.7)	5.77 (4.02–8.29)	4.29 (2.95–6.24)
Continuous‡	19,327	1,892 (9.8)	1.47 (1.41–1.54)	1.35 (1.28–1.42)
1-h Plasma glucose (mmol/l)				
≤5.8	3,466	219 (6.3)	1.00	1.00
5.9–7.3	6,230	495 (7.9)	1.28 (1.09–1.51)	1.24 (1.05–1.47)
7.4–8.6	5,003	495 (9.9)	1.63 (1.38–1.92)	1.63 (1.37–1.93)
8.7–9.5	2,317	308 (13.3)	2.27 (1.89–2.73)	2.30 (1.91–2.78)
9.6–10.7	1,563	221 (14.1)	2.44 (2.00–2.97)	2.45 (1.99–3.02)
10.8–11.7	535	105 (19.6)	3.62 (2.81–4.66)	3.75 (2.87–4.88)
≥11.8	213	49 (23.0)	4.43 (3.13–6.27)	4.77 (3.33–6.83)
Continuous‡	19,327	1,892 (9.8)	1.42 (1.35–1.48)	1.44 (1.37–1.52)
2-h Plasma glucose (mmol/l)				
≤5.0	3,521	226 (6.4)	1.00	1.00
5.1–6.0	6,114	522 (8.5)	1.36 (1.16–1.60)	1.30 (1.10–1.53)
6.1–6.9	4,934	483 (9.8)	1.58 (1.34–1.86)	1.54 (1.30–1.83)
7.0–7.7	2,551	334 (13.1)	2.20 (1.84–2.62)	2.20 (1.83–2.64)
7.8–8.7	1,439	197 (13.7)	2.31 (1.89–2.83)	2.38 (1.93–2.93)
8.8–9.8	575	88 (15.3)	2.63 (2.02–3.43)	2.79 (2.13–3.68)
≥9.9	193	42 (21.8)	4.06 (2.81–5.86)	4.42 (3.03–6.47)
Continuous‡	19,327	1,892 (9.8)	1.33 (1.27–1.39)	1.35 (1.29–1.42)

Data are *n* (%) or OR (95% CI). *n* = total number in the glucose category (excluding births with gestational age <30 weeks and fetal deaths). Model I, adjusted for the variables used in estimating 90th percentiles; model II, adjusted for age, BMI, BMI^2, height, mean arterial blood pressure, gestational age at the OGTT, smoking, alcohol use, hospitalization prior to delivery, and any family history of diabetes. *Defined based on sex, ethnicity, field center, gestational age (36–44 weeks), and parity. †Number in the glucose category with percent body fat >90th percentile (% proportion in the glucose category with percent body fat >90th percentile). ‡Glucose higher by 1 SD (0.4 mmol/l for FPG, 1.7 mmol/l for 1-h plasma glucose, 1.3 mmol/l for 2-h plasma glucose).

and the mean birth weight was 3,308 g. Skin fold measurements were available for 19,389 babies overall and for ~17,100 babies with a cord C-peptide result.

Tables 2 and 3 show associations of maternal glucose with sum of skin folds >90th percentile and percent body fat >90th percentile, including ORs and 95% CIs for each category compared with the lowest or referent category. Overall, 1,863 (9.6%) babies had a sum of skin folds >90th percentile and 9.8% a percent body fat >90th percentile. With higher levels of maternal fasting, 1-, and 2-h plasma glucose concentrations, the proportion of babies with sum of skin folds or percent body fat >90th percentile rose, for example from 5.3 to 26.4% across FPG categories for sum of skin folds and from 6.2 to 27.7% for percent body fat. In model I, the OR was 6.42 in the highest category of FPG for sum of skin folds and 5.77 for percent body fat. For sum of skin folds, there was modest attenuation of the ORs with adjustment for model II confounders for all three glucose measures. For percent body fat >90th percentile, the ORs for FPG were modestly attenuated but became larger for 1- and 2-h plasma glucose. For both measures of neonatal adiposity, there was a strong graded association across increasing levels of maternal glycemia. In continuous variable models for sum of skin folds >90th percentile, ORs ranged from 1.37 to 1.52 in model I and 1.36 to 1.42 in model II for each measure higher by 1 SD. In addition, for percent body fat >90th percentile ORs ranged from 1.33 to 1.47 for model I and 1.35 to 1.44 for model II. There were no significant nonlinear associations for glucose or significant interactions with field center, BMI, height, or MAP. There was, however, a significant interaction for 1-h plasma glucose and age in relation to sum of skin folds >90th percentile, suggesting a stronger association of 1-h plasma glucose with this outcome with increasing maternal age.

Associations of glucose measures in continuous variable models for fat-free mass >90th percentile showed similar associations to those for percent fat >90th percentile, with ORs in model II ranging from 1.30 to 1.44 (data not shown). When birth weight, sum of skin folds, percent fat, and fat free mass were modeled as continuous variables in multiple regression analyses with adjustment for the same confounders (model II), mean differences between the highest and lowest categories for the glucose measures ranged from 242 to 305 g for birth weight, 1.4 to 2.0 mm for sum of skin folds, 1.5 to 2.5% for percent fat, and 157 to 168 g for fat free mass (all $P < 0.001$).

Table 4 shows relationships between maternal glucose and individual skin folds >90th percentile in continuous variable analyses. Each individual skin fold measurement was positively related to maternal glycemia. Strongest associations were with subscapular skin fold where ORs for each glucose measure higher by 1 SD ranged from 1.40 to 1.56 in model I and from 1.37 to 1.47 in model II. For triceps skin fold, ORs ranged from 1.38 to 1.50 in model I and from 1.38 to 1.40 in model II; whereas for flank skin fold, ORs ranged from 1.29 to 1.44 in model I and from 1.28 to 1.35 in model II. Associations did not vary significantly

TABLE 4
Relationship* between maternal glucose and individual skin folds >90th percentile†

Outcome	Model I	Model II
Flank skin fold >90th percentile		
FPG	1.44 (1.38–1.51)	1.34 (1.27–1.41)
1-h Plasma glucose	1.37 (1.31–1.44)	1.35 (1.28–1.42)
2-h Plasma glucose	1.29 (1.23–1.35)	1.28 (1.21–1.34)
Triceps skin fold >90th percentile		
FPG	1.50 (1.43–1.57)	1.40 (1.34–1.48)
1-h Plasma glucose	1.39 (1.33–1.46)	1.39 (1.32–1.46)
2-h Plasma glucose	1.38 (1.32–1.44)	1.38 (1.31–1.45)
Subscapular skin fold >90th percentile		
FPG	1.56 (1.49–1.64)	1.43 (1.36–1.51)
1-h Plasma glucose	1.50 (1.44–1.58)	1.47 (1.39–1.54)
2-h Plasma glucose	1.40 (1.34–1.46)	1.37 (1.30–1.43)

Data are OR (95% CI). Model I, adjusted for the variables used in estimating 90th percentiles; model II, adjusted for field center, age, BMI, height, parity, smoking, alcohol use, hospitalization prior to delivery, any family history of diabetes, mean arterial blood pressure, and gestational age at OGTT. *Continuous variable analysis, glucose higher by 1 SD (0.4 mmol/l for FPG, 1.7 mmol/l for 1-h plasma glucose, and 1.3 mmol/l for 2-h plasma glucose). †Defined based on sex, ethnicity, field center, gestational age (36–44 weeks only), and parity.

by field center, BMI, height, or MAP for any of these outcomes. There was, however, a significant interaction of 1-h plasma glucose with age in the model II analysis for triceps skin fold, which suggested a stronger association of 1-h plasma glucose with triceps skin fold with increasing maternal age.

When model II ORs for birth weight >90th percentile, percent body fat >90th percentile, or sum of skin folds >90th percentile associations with glucose as a continuous variable were adjusted for C-peptide (using linear and squared terms in C-peptide because of its nonlinear association with the outcomes) we found 23–38% reductions in the ORs for associations with individual glucose measures (data not shown).

Associations between categories of cord C-peptide and neonatal anthropometrics are shown in Table 5. With higher levels of cord C-peptide, frequency of each measure of size and adiposity rose. For example, the frequency of birth weight >90th percentile ranged from 4.5 to 25.6% across categories of cord C-peptide. In model I, ORs for the three measures ranged from 5.97 to 7.31 in the highest category of cord C-peptide. In model II, ORs were modestly attenuated, but strong graded associations remained.

When these outcomes and fat free mass were modeled as continuous variables in multiple regression analyses with adjustment for the same confounders (model II), mean differences between the highest and lowest categories for cord C-peptide were 345 g for birth weight, 2.0 mm for sum of skin folds, 2.7% for percent fat, and 221 g for fat free mass (all $P < 0.001$) (data not shown).

TABLE 5
Relationship between cord serum C-peptide and neonatal anthropometrics

Cord serum C-peptide (μg/l)	*n*	No. with outcome	Model I	Model II
Birth weight >90th percentile*				
≤0.5	2,911	131 (4.5)	1.00	1.00
0.6–0.8	6,530	392 (6.0)	1.36 (1.11–1.66)	1.26 (1.03–1.55)
0.9–1.2	5,899	614 (10.4)	2.47 (2.03–2.99)	2.21 (1.82–2.70)
1.3–1.5	2,077	283 (13.6)	3.35 (2.70–4.15)	2.89 (2.32–3.60)
1.6–2.1	1,639	333 (20.3)	5.41 (4.37–6.69)	4.68 (3.77–5.82)
2.2–3.0	571	140 (24.5)	6.89 (5.32–8.93)	5.62 (4.31–7.33)
≥3.1	242	62 (25.6)	7.31 (5.21–10.25)	6.72 (4.75–9.51)
Total	19,869	1,955 (9.8)		
Sum of skin folds >90th percentile*				
≤0.5	2,412	117 (4.9)	1.00	1.00
0.6–0.8	5,647	369 (6.5)	1.37 (1.11–1.70)	1.27 (1.03–1.58)
0.9–1.2	5,145	513 (10.0)	2.17 (1.77–2.67)	1.92 (1.56–2.37)
1.3–1.5	1,821	267 (14.7)	3.37 (2.69–4.23)	2.84 (2.26–3.57)
1.6–2.1	1,409	265 (18.8)	4.54 (3.61–5.71)	3.74 (2.97–4.72)
2.2–3.0	485	101 (20.8)	5.16 (3.87–6.88)	4.00 (2.99–5.36)
≥3.1	181	46 (25.4)	6.68 (4.56–9.80)	5.57 (3.78–8.21)
Total	17,100	1,678 (9.8)		
Percent body fat >90th percentile*				
≤0.5	2,399	119 (5.0)	1.00	1.00
0.6–0.8	5,630	370 (6.6)	1.35 (1.09–1.67)	1.24 (1.00–1.54)
0.9–1.2	5,140	513 (10.0)	2.12 (1.73–2.61)	1.87 (1.52–2.31)
1.3–1.5	1,817	276 (15.2)	3.43 (2.74–4.30)	2.88 (2.30–3.62)
1.6–2.1	1,403	269 (19.2)	4.54 (3.62–5.70)	3.77 (2.99–4.75)
2.2–3.0	485	121 (25.2)	6.46 (4.90–8.51)	5.02 (3.79–6.66)
≥3.1	181	43 (23.8)	5.97 (4.05–8.81)	5.06 (3.41–7.52)
Total	17,050	1,726 (10.0)		

Data are *n* (%) or OR (95% CI). *n* = total number in the cord C-peptide category (excluding births with gestational age <30 weeks and fetal deaths); No. with outcome = number in the cord C-peptide category with the outcome (% proportion in the cord C-peptide category with the outcome). Model I, adjusted for the variables used in estimating 90th percentiles; model II, adjusted for age, BMI, BMI^2, height, mean arterial blood pressure, gestational age at the OGTT, smoking, alcohol use, hospitalization prior to delivery, and any family history of diabetes. *Defined based on sex, ethnicity, field center, gestational age (36–44 weeks for skin folds and body fat), and parity.

TABLE 6
Relationship between cord serum C-peptide and individual skin folds

Cord serum C-peptide (μg/l)	*n*	No. with outcome	Model I	Model II
Flank skin fold >90th percentile*				
≤0.5	2,421	135 (5.6)	1.00	1.00
0.6–0.8	5,664	374 (6.6)	1.20 (0.98–1.47)	1.12 (0.91–1.37)
0.9–1.2	5,167	496 (9.6)	1.80 (1.48–2.19)	1.61 (1.32–1.96)
1.3–1.5	1,826	267 (14.6)	2.90 (2.33–3.60)	2.48 (1.99–3.09)
1.6–2.1	1,413	227 (16.1)	3.24 (2.59–4.06)	2.70 (2.15–3.39)
2.2–3.0	487	98 (20.1)	4.27 (3.22–5.65)	3.39 (2.55–4.51)
≥3.1	181	33 (18.2)	3.78 (2.49–5.72)	3.17 (2.08–4.82)
Total	17,159	1,630 (9.5)		
Triceps skin fold >90th percentile*				
≤0.5	2,422	123 (5.1)	1.00	1.00
0.6–0.8	5,655	405 (7.2)	1.44 (1.17–1.77)	1.35 (1.10–1.66)
0.9–1.2	5,157	537 (10.4)	2.17 (1.77–2.66)	1.94 (1.58–2.38)
1.3–1.5	1,825	269 (14.7)	3.23 (2.59–4.04)	2.77 (2.21–3.47)
1.6–2.1	1,411	237 (16.8)	3.77 (3.00–4.74)	3.17 (2.51–3.99)
2.2–3.0	485	96 (19.8)	4.61 (3.46–6.15)	3.68 (2.75–4.93)
≥3.1	181	44 (24.3)	6.00 (4.08–8.82)	5.12 (3.47–7.56)
Total	17,136	1,711 (10.0)		
Subscapular skin fold >90th percentile*				
≤0.5	2,415	109 (4.5)	1.00	1.00
0.6–0.8	5,648	366 (6.5)	1.47 (1.18–1.83)	1.35 (1.09–1.69)
0.9–1.2	5,151	498 (9.7)	2.26 (1.83–2.80)	1.99 (1.60–2.47)
1.3–1.5	1,823	269 (14.8)	3.66 (2.90–4.62)	3.05 (2.41–3.86)
1.6–2.1	1,412	245 (17.4)	4.44 (3.51–5.63)	3.60 (2.83–4.58)
2.2–3.0	485	101 (20.8)	5.56 (4.16–7.45)	4.23 (3.14–5.69)
≥3.1	181	44 (24.3)	6.79 (4.60–10.03)	5.54 (3.73–8.23)
Total	17,115	1,632 (9.5)		

Data are *n* (%) or OR (95% CI). *n* = total number in the cord C-peptide category (excluding births with gestational age <30 weeks and fetal deaths), No. with outcome = number in the cord C-peptide category with the outcome (% proportion in the cord C-peptide category with the outcome). Model I, adjusted for the variables used in estimating 90th percentiles; model II, adjusted for age, BMI, BMI^2, height, mean arterial blood pressure, gestational age at the OGTT, smoking, alcohol use, hospitalization prior to delivery, and any family history of diabetes. *Defined based on sex, ethnicity, field center, gestational age (36–44 weeks), and parity.

Table 6 shows associations between cord C-peptide and individual skin folds >90th percentile. Results for both model I and model II show that subscapular skin fold has the strongest and flank skin fold the weakest association with cord C-peptide level, with ORs for the highest versus lowest category of 6.79 and 5.54 in models I and II, respectively, for subscapular skin fold and 3.78 and 3.17 for flank skin fold.

DISCUSSION

These results support our hypothesis that increasing glucose concentration less severe than diabetes is associated with fetal overgrowth, specifically adiposity. Data presented here show a strong and continuous association between neonatal fat content and maternal glycemia and with fetal insulin levels as measured by cord C-peptide concentrations. These relationships were present for each maternal glucose measurement and cord C-peptide. Relationships persisted even when potential confounding variables such as field center, BMI, height, MAP, gestational age, smoking status, and alcohol use were taken into account. This pattern is similar to that reported for maternal glucose and birth weight >90th percentile (2) and was also seen for the association with fat free mass, a parameter derived by subtracting fat mass from total body weight. Significant interactions for 1-h plasma glucose and age in relation to sum of skin folds >90th percentile and triceps skin fold >90th percentile, which indicated stronger associations with increasing age, may be a chance finding due to the large number of interactions examined.

The findings reported here, however, are not proof of causality. Fetal insulin, stimulated by maternal glucose transport from mother to fetus across the placenta, may act on a variety of nutrients in addition to glucose, resulting in fetal overgrowth and adiposity. DiCianni et al. (12) reported that maternal triglyceride levels were also strongly correlated with fetal growth, a finding that is consistent with the fuel mediated teratogenesis hypothesis of Freinkel (13).

These findings confirm the link between maternal glycemia and neonatal fat deposition and suggest that the relationship is mediated by fetal insulin production. Furthermore, continuous relationships across the full range of maternal glycemia suggest that the Pedersen hypothesis is not limited to the high end of maternal glycemia but rather describes a basic biological relationship that influences maternal-fetal interactions around fetal growth. Each component of the OGTT and level of cord C-peptide was independently related to skin fold thickness with adjustment for multiple potential confounders. On average, maternal glucose was measured 11 weeks before collection of the cord blood serum C-peptide and measurements of neonatal anthropometrics. Thus, it is not surprising that associations of each glucose measure with anthropometric outcomes were significantly reduced but not eliminated when adjusted for cord C-peptide. Correlations among the glucose measures were modest (2), and an index of their integrated associations with these anthropometric outcomes is not available. No one glucose measurement was clearly superior to the others, suggesting that fluxes of

maternal nutrients whether in the fasting or postprandial state are related to fetal growth.

There is increasing evidence that increased size at birth is associated with an increased likelihood of adiposity in later life and with alterations in glucose metabolism and β-cell function (14). Increasing hyperglycemia in pregnancy, whether associated with high birth weight or not, is associated with obesity and glucose intolerance later in life (15). The finding of a continuous relationship between maternal glycemia and neonatal adiposity offers the potential to better understand, and possibly to influence, the development of obesity, a problem that is rapidly becoming epidemic around the world (16). To date, published studies of relationships between size at birth and risk of obesity in childhood and later life (14) are based primarily on birth weight for gestational age without information on degree of adiposity at birth. Thus, follow-up studies of the HAPO cohort or other populations that have information on both maternal metabolic factors and obesity and neonatal body composition and insulin or C-peptide can be very informative.

Limitations of the data include the fact that a cord C-peptide level was not available for 15% of neonates. Additionally, 14% of neonates who had a cord C-peptide measurement did not have the full set of skin fold measurements. Skin fold thickness is an indirect measure of adiposity. However, the formula for calculation of body fat that is based on baby length, weight, gestational age, and flank skin fold (11) was validated by measurements of TOBEC, and the three skin fold measures are strongly intercorrelated. Maternal body weight was only measured at the time of the OGTT. Prepregnancy body weight was self-reported and incomplete, and weight was not measured at delivery, making maternal weight gain unavailable in a large number of cases. These limitations preclude our ability to evaluate the differential effects of pre-existing obesity and maternal weight gain in contributing to the outcomes measured. However, there was a strong correlation between recalled prepregnancy weight and weight measured at the OGTT.

In summary, our data provide the "missing link" between maternal glycemia, fetal insulin response, and neonatal growth, specifically neonatal adiposity, confirming that the Pederson hypothesis first proposed >50 years ago (3) is not limited to overt diabetes, but extends across the full range of maternal glycemia. The multicenter nature of the cohort and the consistency of the results across several measures of growth and adiposity make the results particularly robust. The fact that the relationships extend across the entire range of glycemia is also striking. Whether the observed associations of the maternal metabolic environment with fetal growth are indicative of long-term effects on the increasing prevalence of obesity and diabetes in both adolescents and adults remains to be investigated.

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