Newborn Adiposity and Cord Blood C-Peptide as Mediators of the Maternal Metabolic Environment and Childhood Adiposity

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OBJECTIVE

Excessive childhood adiposity is a risk factor for adverse metabolic health. The objective was to investigate associations of newborn body composition and cord C-peptide with childhood anthropometrics and explore whether these newborn measures mediate associations of maternal midpregnancy glucose and BMI with childhood adiposity.

RESEARCH DESIGN AND METHODS

Data on mother/offspring pairs ($N = 4,832$) from the epidemiological Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study and HAPO Follow-up Study (HAPO FUS) were analyzed. Linear regression was used to study associations between newborn and childhood anthropometrics. Structural equation modeling was used to explore newborn anthropometric measures as potential mediators of the associations of maternal BMI and glucose during pregnancy with childhood anthropometric outcomes.

RESULTS

In models including maternal glucose and BMI adjustments, newborn adiposity as measured by the sum of skinfolds was associated with child outcomes (adjusted mean difference, 95% CI, *P* value) BMI (0.26, 0.12–0.39, <0.001), BMI *z*-score (0.072, 0.033–0.11, <0.001), fat mass (kg) (0.51, 0.26–0.76, <0.001), percentage of body fat (0.61, 0.27–0.95, <0.001), and sum of skinfolds (mm) (1.14, 0.43–1.86, 0.0017). Structural equation models demonstrated significant mediation by newborn sum of skinfolds and cord C-peptide of maternal BMI effects on childhood BMI (proportion of total effect 2.5% and 1%, respectively), fat mass (3.1%, 1.2%), percentage of body fat (3.6%, 1.8%), and sum of skinfolds (2.9%, 1.8%), and significant mediation by newborn sum of skinfolds and cord C-peptide of maternal glucose effects on child fat mass (proportion of total association 22.0% and 21.0%, respectively), percentage of body fat (15.0%, 18.0%), and sum of skinfolds (15.0%, 20.0%).

CONCLUSIONS

Newborn adiposity is independently associated with childhood adiposity and, along with fetal hyperinsulinemia, mediates, in part, associations of maternal glucose and BMI with childhood adiposity.

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**A complete list of the HAPO Follow-up Study Cooperative Research Group can be found in the supplementary material online.*

Excessive childhood adiposity is a risk factor for adverse metabolic health. Whether the origins of excess childhood adiposity begin before birth or develop in childhood is not known. High birth weight is a risk factor for childhood obesity, yet most obese children had normal birth weight (1). Small studies have demonstrated associations between newborn and childhood adiposity, but no association between birth weight and childhood weight in the same participants (2,3). Importantly, adiposity reflects fat mass, whereas weight and BMI include both fat and lean mass (4,5). Deciphering associations between newborn and childhood body composition, which includes measures of weight and adiposity, depends on consideration of the nature of each anthropometric measurement.

The maternal metabolic milieu adds complexity to relationships between newborn and childhood adiposity. Independent associations of maternal hyperglycemia and high maternal BMI with both newborn and childhood adiposity are documented (6–10), but links between newborn and childhood adiposity in the context of maternal glucose and BMI are not clearly defined. Additionally, maternal glucose modulates fetal insulin (11), the predominant growth factor in utero, which in turn regulates fetal fat accretion (12). The extent to which fetal insulin contributes to childhood adiposity has not been described.

This study addresses the hypothesis that newborn adiposity reflects the maternal metabolic milieu and mediates complex associations between maternal glucose and BMI and childhood adiposity using data from the Hyperglycemia and Adverse Pregnancy Outcome Study (HAPO) (13) and HAPO Follow-up Study (HAPO FUS) (14). HAPO, conducted 2000–2006, and HAPO FUS, conducted 2013–2016, were international, epidemiological studies designed to determine whether hyperglycemia in pregnancy, less severe than overt diabetes, is associated with increased risk of adverse maternal, newborn, and childhood metabolic and anthropometric outcomes. HAPO FUS represents the largest prospective cohort with body composition measurements at birth and during peripubertal years, providing the opportunity to distinguish newborn adiposity and birth weight associations with comparable childhood measures and as reflections of maternal metabolism.

Previous HAPO and HAPO FUS reports demonstrated that maternal glucose and BMI during pregnancy are independently and additively associated with newborn and childhood weight and adiposity (15,16). The objectives of the current study are to investigate associations of newborn body composition and cord C-peptide with childhood anthropometrics, independent of maternal midpregnancy glucose and BMI, and explore whether newborn body composition and cord C-peptide mediate associations of maternal glucose and BMI during pregnancy with childhood adiposity. Critical to addressing these objectives is the distinction between purely adiposity measures versus those that reflect a composite of both fat and lean mass such as birth weight and BMI.

RESEARCH DESIGN AND METHODS

HAPO was conducted at 15 international field centers with >25,000 participants; HAPO FUS was conducted at 10 of those centers with >4,800 participating mother-offspring pairs. Children eligible for HAPO FUS included only those whose mothers remained blinded to the HAPO oral glucose tolerance test (OGTT) results and who were born ≥37 weeks' gestation and had no major malformations. Methods for both studies have been published (13,14). Institutional review boards at each field center approved both studies. Participants provided written informed consent and, where required, assent.

Participants

In HAPO, pregnant women underwent a study visit at ~28 weeks' gestation that included measurements of height, weight, blood pressure, and a 75-g OGTT with blood sampled at fasting, 1 h, and 2 h (13). Participants and providers were blinded to the results unless specific glucose thresholds were exceeded, then unblinding occurred. Participants who were unblinded were excluded from HAPO analyses and not eligible for participation in the HAPO FUS. Blinded participants were not treated. Demographic data, including self-identified race/ethnicity, smoking, and alcohol use, were collected. Prenatal care and delivery were determined by clinical practice at each field center. Medical records were abstracted for data regarding prenatal, labor and delivery, postpartum, and newborn course.

Newborn Measurements

Cord blood was collected at delivery and serum C-peptide was measured as previously described (17). Newborn weight, length, and flank, triceps, and subscapular skinfolds were measured within 72 h of delivery; measurements were obtained in duplicate and if results differed by more than a prespecified amount (>10 g for weight, >0.5 cm for length, and >0.5 mm for skinfolds), a third measurement was performed. For statistical analysis, the average of the measurements was used. If a third measurement was taken and two of the three 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 using a calibrated electronic scale. Length was measured on a hard-surface standardized board constructed for the HAPO Study. Skinfold thicknesses were measured with calibrated calipers (Harpenden, Baty, U.K.) on the newborn's left side. Flank skinfold thickness was measured just above the iliac crest on a diagonal fold on the midaxillary line, subscapular just below the lower angle of the scapula at ~45° angle to the spine, and triceps by taking the vertical fold over the triceps muscle half the distance between the acromion process and olecranon (6).

Child Anthropometrics

For the HAPO FUS, centralized training and maintenance of certification was conducted by the Coordinating Center (14). The child's weight was measured twice to the nearest 0.1 kg using a calibrated scale. If results differed by >0.5 kg, a third measurement was obtained. Height was measured twice with a stadiometer to the nearest 0.5 cm. If results differed by >1.0 cm, a third measurement was obtained. Calibrated calipers (Harpenden) were used to measure skinfolds twice at three sites: triceps, subscapular, and suprailiac to the nearest 0.1 mm. If results differed by >1.0 mm,

a third measurement was obtained (14). Body composition was measured using air displacement plethysmography (Bod Pod, Cosmed, Italy) which provided fat mass and percentage of body fat.

Outcomes and Predictors

Childhood outcomes included BMI, BMI *z*-score, fat mass, percentage of body fat, and sum of skinfolds. BMI *z*-scores were calculated using sex- and age-specific lambda-mu-sigma (LMS) curves (18). Sum of skinfolds (mm) was calculated by summing the three skinfolds.

Newborn anthropometric measures were analyzed both as independent predictors of childhood anthropometrics and as potential mediators of associations between maternal pregnancy variables and childhood anthropometrics. Newborn sum of skinfolds (mm) was calculated by summing the flank, triceps, and subscapular skinfold measurements. Newborn fat mass was estimated using measurements of birth weight, length, and flank skinfold as previously described (19). Birth weight-for-length was calculated by dividing birth weight by length. The following newborn and childhood anthropometrics were included to specifically measure adiposity: newborn sum of skinfolds, newborn fat mass, childhood fat mass, childhood percentage of body fat, and childhood sum of skinfolds.

For statistical analyses, newborn measures were standardized into *z*-scores by subtracting the mean and dividing by the SD from the original HAPO data set. In mediation models, maternal predictors included maternal BMI and sum of glucose *z*-scores from the pregnancy OGTT. Sum of glucose *z*-scores is an integrated measure of maternal glycemia that gives equal weight to each of the three glucose values during the OGTT. It was calculated by subtracting the mean glucose level from all HAPO values at that time point, dividing by the SD of the glucose values at that time point, and summing the three individual *z*-scores (20).

Statistical Analyses

Continuous variables are summarized using means and SDs and categorical variables are summarized using tables of frequencies and counts. Associations between newborn anthropometric measures and continuous childhood anthropometric outcomes were examined using linear regression. Covariate adjustments were examined as follows based on previous HAPO analyses and known potential confounders: model 1: field center (each with a high level of racial/ethnic homogeneity), child's sex, gestational age at delivery, maternal age, mean arterial pressure, height, parity, smoking, drinking, and family history of diabetes at pregnancy OGTT; model 2: model 1 covariates + maternal sum of glucose *z*-scores and BMI at pregnancy OGTT.

Linear regression model fit was assessed by scatterplots of residuals versus fitted values, histograms, and qqplots of residuals, and DFbeta statistics. Quadratic terms and restricted cubic splines estimated with the *rms* R package (21) were used to assess linearity between the continuous predictor and continuous outcomes for linear regression models. Statistical significance was determined according to $P < 0.05$. Analyses presented here are considered secondary for the HAPO FUS and are not corrected for multiple comparisons. All analyses were conducted in R 3.3.1 software (22).

Structural equation modeling was used to explore models treating newborn anthropometric measures as potential mediators of the associations of maternal BMI and glucose during pregnancy with childhood anthropometric outcomes. Structural equation models treated both maternal BMI and glucose sum of *z*-scores at pregnancy OGTT as exposure variables. Separate models were evaluated for childhood outcomes: BMI, BMI *z*-score, fat mass, percentage of body fat, and sum of skinfolds. Mediation effects for newborn sum of skinfolds, fat mass, birth weight, birth weight-for-length, and log cord C-peptide *z*-scores were examined separately. All structural equation models included adjustment for model 1 covariates.

RESULTS

Characteristics of the 4,832 HAPO FUS mothers and their offspring are reported in Table 1. As reported previously, characteristics were similar for HAPO mothers who did and did not participate in HAPO FUS (14). The HAPO study visit occurred at mean gestational age of 27.7 weeks, and delivery of the newborns occurred at mean gestational age 39.8 weeks. HAPO FUS study visits occurred when children were mean age of 11.4 years.

Associations Between Newborn and Childhood Anthropometric Measures

Associations between measures of newborn and childhood anthropometrics are reported in Table 2. Newborn sum of skinfolds, fat mass, birth weight, birth weight-for-length and cord blood C-peptide were significantly and positively associated with childhood BMI, BMI *z*-score, fat mass, percentage of body fat, and sum of skinfolds in model 1, with the exception of birth weight-for-length, which was not associated with child fat mass, percentage of body fat, or sum of skinfolds. The association of newborn sum of skinfolds with childhood anthropometrics remained statistically significant after adjustment for maternal glucose and BMI (model 2), whereas newborn fat mass, birth weight, and birth weight-for-length were associated with childhood BMI and BMI *z*-score. Newborn fat mass and birth weight were associated with child fat mass but not with child percentage of body fat or sum of skinfolds. Newborn cord C-peptide was associated with childhood anthropometrics, except for BMI *z*-score in model 2. Adjusted mean differences for these associations in model 2 were attenuated to approximately half of the model 1 estimates.

Sex-specific adjusted mean difference associations of newborn measures with childhood outcomes are provided in Supplementary Table 1. Statistical power was limited for subgroup analyses, but in exploratory sex-specific analyses, associations were comparable for boys and girls.

Mediation Effects of Newborn Adiposity Measures

As previously reported for HAPO and HAPO FUS, maternal glucose and BMI are associated with both newborn and childhood weight/BMI and adiposity outcomes (15,16). Mediation analysis was performed to evaluate the extent to which the associations of maternal glucose and BMI with childhood anthropometrics are mediated by newborn anthropometrics. Table 3 reports the portion of the association of maternal glucose and BMI with childhood

Table 1—Characteristics of HAPO FUS mother and child participants

Maternal characteristics during HAPO	$N = 4,832$
	Mean (SD)
Age (years)	29.9 (5.7)
Height (cm)	161.6 (6.5)
Weight (kg)	70.8 (17.1)
BMI (kg/m^2)	27.1 (6.2)
Gestational age at OGTT (weeks)	27.7 (1.7)
Mean arterial pressure (mmHg)	80.5 (8.0)
Fasting plasma glucose (mg/dL)	81.0 (6.6)
1-h plasma glucose (mg/dL)	133.1 (30.2)
2-h plasma glucose (mg/dL)	110.4 (23.0)
Glucose sum of *z*-scores	−0.04 (2.3)
Race/ethnicity	*n* (%)
White, non-Hispanic	2,287 (47.3)
Black, non-Hispanic	775 (16.0)
Hispanic	507 (10.5)
Asian	1,176 (24.3)
Other	87 (1.8)
Any prenatal smoking	245 (5.1)
Any prenatal alcohol use	406 (8.4)
Parity (any prior delivery >20 weeks)	2,485 (51.4)
Family history of diabetes mellitus	1,077 (22.3)
Newborn characteristics at delivery	Mean (SD)
Gestational age at delivery (weeks)	39.8 (1.2)
Birth weight (g)	3,383.2 (477.0)
Length (cm)	50.2 (2.3)
Birth weight-for-length *z*-score	−0.1 (1.1)
Sum of skinfolds (mm)	12.3 (2.6)
Percentage of body fat (ref. Catalano formula) (%)	11.3 (3.6)
Cord C-peptide (μg/L)	1.0 (0.6)
	n (%)
Sex (% female)	2,367 (49.0)
Childhood characteristics during HAPO FUS	Mean (SD)
Age (years)	11.4 (1.2)
Height (cm)	148.6 (10.2)
Weight (kg)	43.2 (13.3)
BMI (kg/m^2)	19.3 (4.3)
BMI *z*-score	0.5 (1.2)
Fat mass (kg)	10.2 (7.8)
Percentage of body fat (%)	21.2 (10.5)
Sum of skinfolds (mm)	39.2 (21.4)

anthropometric outcomes that is mediated by newborn sum of skinfolds. The direct effect reflects the portion of the association between maternal glucose or BMI and each child outcome that is not mediated by newborn sum of skinfolds; the indirect effect represents the portion of the association mediated by newborn sum of skinfolds, while the total effect represents the sum of the direct and indirect effects (Fig. 1). Joint estimation of direct, indirect, and total effects for maternal glucose and BMI on child BMI and BMI *z*-score with newborn sum of skinfolds as the mediator indicated significant mediation for maternal BMI (proportion of total effect 2.5%) but not for maternal glucose. For childhood fat mass, percentage of body fat, and sum of skinfolds outcomes, direct, indirect, and total effects for both maternal BMI and glucose were statistically significant when newborn sum of skinfolds was modeled as a mediator variable. The proportion of the total effect of maternal glucose mediated by newborn sum of skinfolds was 22.0% for child fat mass and 15.0% for both childhood percentage of body fat and sum of skinfolds. The proportion of the maternal BMI total effect mediated by newborn sum of skinfolds was 3.1%, 3.6%, and 2.9% for childhood fat mass, percentage of body fat, and sum of skinfolds outcomes, respectively.

For newborn cord C-peptide, significant indirect effects were observed for all outcomes (Table 3) except BMI *z*-score. Joint estimation of direct and total effects for maternal glucose and BMI on child BMI indicated significant effects for maternal BMI but not for maternal glucose, with 1.0% of the total effect mediated by cord C-peptide. For childhood fat mass, percentage of body fat, and sum of skinfolds, direct and total effects for both maternal glucose and BMI were statistically significant, as were indirect effects when cord C-peptide was modeled as a mediator variable. Approximately 1.2–1.8% of the total effect of maternal BMI for these outcomes was mediated by newborn cord C-peptide. The proportion of total effect of maternal glucose mediated by cord C-peptide was 21.0% for child fat mass, 18.0% for child percentage of body fat, and 20.0% for child sum of skinfolds.

In analyses for newborn fat mass as a mediator of the associations of maternal glucose and BMI with childhood outcomes (Supplementary Table 2), direct and indirect effects for child BMI and BMI *z*-score were significant for maternal BMI but not for glucose. The proportion of the maternal BMI total effect mediated by newborn fat mass was 3.5% for child BMI and 4.7% for child BMI *z*-score. For the child fat mass outcome, direct, indirect, and total effects for both maternal BMI and glucose were statistically significant. Approximately 12.0% of the total effect of maternal glucose and 2.4% of the total effect of maternal BMI for child fat mass were mediated by newborn fat mass. For childhood percentage of body fat and sum of skinfolds, indirect effects of maternal glucose and BMI mediated by newborn fat mass were not significant.

In models examining birth weight and birth weight-for-length as mediators, statistically significant indirect effects were observed for childhood BMI and BMI *z*-score. In joint models, direct and total effects for maternal BMI, but not for maternal glucose, were significant for these outcomes with proportion mediated by birth weight and birth weight-

Table 2—Associations of newborn measures with childhood anthropometric measures

Childhood outcome	Model 1: Adjusted mean difference (95% CI, *P*)	Model 2: Adjusted mean difference (95% CI, *P*)
	Newborn sum of skinfolds *z*-score	
BMI (kg/m^2)	0.52 (0.39–0.66, <0.001)	0.26 (0.12–0.39, <0.001)
BMI *z*-score	0.15 (0.11–0.18, <0.001)	0.072 (0.033–0.11, <0.001)
Fat mass (kg)	0.96 (0.71–1.22, <0.001)	0.51 (0.26–0.76, <0.001)
Percentage of body fat (%)	1.16 (0.83–1.50, <0.001)	0.61 (0.27–0.95, <0.001)
Sum of skinfolds (mm)	2.35 (1.64–3.060, <0.001)	1.14 (0.43–1.86, 0.0017)
	Newborn fat mass *z*-score	
BMI (kg/m^2)	0.59 (0.45–0.73, <0.001)	0.29 (0.15–0.43, <0.001)
BMI *z*-score	0.19 (0.15–0.23, <0.001)	0.11 (0.073–0.15, <0.001)
Fat mass (kg)	0.86 (0.59–1.12, <0.001)	0.34 (0.085–0.60, 0.009)
Percentage of body fat (%)	0.90 (0.56–1.25, <0.001)	0.29 (−0.057–0.64, 0.10)
Sum of skinfolds (mm)	1.50 (0.77–2.23, <0.001)	0.14 (−0.58–0.87, 0.70)
	Newborn birth weight *z*-score	
BMI (kg/m^2)	0.67 (0.52–0.82, <0.001)	0.32 (0.17–0.47, <0.001)
BMI *z*-score	0.23 (0.18–0.27, <0.001)	0.13 (0.088–0.17, <0.001)
Fat mass (kg)	1.070 (0.79–1.35, <0.001)	0.48 (0.20–0.76, <0.001)
Percentage of body fat (%)	1.080 (0.71–1.46, <0.001)	0.34 (−0.037–0.72, 0.077)
Sum of skinfolds (mm)	1.74 (0.96–2.52, <0.001)	0.12 (−0.67–0.90, 0.77)
	Newborn birth weight-for-length *z*-score	
BMI (kg/m^2)	0.26 (0.15–0.36, <0.001)	0.14 (0.036–0.24, 0.0077)
BMI *z*-score	0.095 (0.065–0.13, <0.001)	0.061 (0.032–0.091, <0.001)
Fat mass (kg)	0.13 (−0.068–0.32, 0.20)	−0.077 (−0.27–0.11, 0.43)
Percentage of body fat (%)	0.097 (−0.16–0.36, 0.47)	−0.15 (−0.40–0.11, 0.25)
Sum of skinfolds (mm)	0.35 (−0.19–0.89, 0.21)	−0.16 (−0.69–0.37, 0.55)
	Newborn log cord C-peptide *z*-score	
BMI (kg/m^2)	0.33 (0.20–0.46, <0.001)	0.14 (0.011–0.26, 0.033)
BMI *z*-score	0.088 (0.051–0.12, <0.001)	0.035 (−0.0019–0.071, 0.063)
Fat mass (kg)	0.58 (0.35–0.81, <0.001)	0.25 (0.021–0.48, 0.033)
Percentage of body fat (%)	0.82 (0.50–1.13, <0.001)	0.41 (0.093–0.72, 0.011)
Sum of skinfolds (mm)	1.72 (1.060–2.37, <0.001)	0.85 (0.19–1.51, 0.011)

All adjusted mean differences in childhood anthropometric measures are reported for each newborn measure higher by 1 SD. Model 1: field center, child's sex, maternal gestational age at delivery, maternal age, mean arterial pressure, height, parity, smoking, drinking, and family history of diabetes at pregnancy OGTT. Model 2: Model 1 + maternal glucose sum of *z*-scores and BMI at pregnancy OGTT.

for-length ranging from 1.1% to 4.8% (Supplementary Table 3). In the newborn birth weight mediation analyses, statistically significant indirect effects were also observed for child fat mass. In joint models, direct and total effects for both maternal glucose and BMI were significant, and the proportion mediated was 18.0% for maternal glucose and 3.2% for maternal BMI. Indirect effects for child fat mass were not statistically significant for newborn birth weight-for-length as a mediator. For the outcomes of childhood percentage of body fat and sum of skinfolds, indirect effects of newborn birth weight and birth weight-for-length as mediators were not significant.

CONCLUSIONS

In the multiethnic HAPO and HAPO FUS cohorts, newborn sum of skinfolds, as a measure of newborn adiposity, was independently associated with childhood adiposity outcomes, including fat mass, percentage of body fat, and sum of skinfolds, as well as child BMI and BMI *z*-score, which reflect both fat and lean mass, independent of maternal midpregnancy BMI and glucose. These associations of adiposity measures between the newborn and peripubertal years, independent of maternal factors, suggest that adiposity at birth is a marker of future metabolic health. These findings add to the existing literature (23,24) and also demonstrate that newborn adiposity, as measured by sum of skinfolds, partly mediated the association of maternal BMI and glucose with measures of childhood adiposity. Together, these findings indicate complex interactions between the in utero environment and newborn anthropometrics in relation to child anthropometrics.

While newborn adiposity had significant, independent associations with all childhood adiposity outcomes, birth weight and birth weight-for-length were associated with child BMI and BMI *z*-score but not with all of the childhood adiposity measures. Birth weight and BMI reflect both fat and lean mass, whereas adiposity specifically reflects fat mass. These findings are consistent

Table 3—Mediation models for newborn sum of skinfolds *z*-score and cord blood C-peptide *z*-score

Maternal exposure	Newborn outcome	Direct effect: β (95% CI, *P* value)	Indirect effect: β (95% CI, *P* value)	Total effect: β (95% CI, *P* value)	Proportion mediated
Outcome: Childhood BMI (kg/m^2)					
Glucose sum of *z*-scores	Sum of skinfolds	0.027 (−0.031 to 0.084, 0.36)	**0.019** (0.0087–0.030, <0.001)	0.046 (−0.011 to 0.10, 0.11)	*
	Cord C-peptide	0.018 (−0.040 to 0.076, 0.55)	0.013 (0.00096–0.026, 0.035)	0.031 (−0.026 to 0.088, 0.29)	*
BMI (kg/m^2)	Sum of skinfolds	**0.30** (0.27–0.33, <0.001)	**0.0079** (0.0035–0.012, <0.001)	**0.31** (0.28–0.34, <0.001)	**0.025**
	Cord C-peptide	**0.30** (0.27–0.33, <0.001)	**0.0032** (0.00012–0.0063, 0.042)	**0.31** (0.28–0.34, <0.001)	**0.01**
Childhood BMI *z*-score					
Glucose sum of *z*-scores	Sum of skinfolds	0.0043 (−0.012 to 0.021, 0.61)	0.0054 (0.0023–0.0084, <0.001)	0.0097 (−0.0067 to 0.026, 0.25)	*
	Cord C-peptide	0.00086 (−0.016 to 0.018, 0.92)	0.0034 (−0.00021 to 0.0070, 0.065)	0.0042 (−0.012 to 0.021, 0.62)	*^
BMI (kg/m^2)	Sum of skinfolds	**0.085** (0.077–0.093, <0.001)	**0.0022** (0.00093–0.0035, <0.001)	**0.087** (0.079–0.095, <0.001)	**0.025**
	Cord C-peptide	0.086 (0.078–0.095, <0.001)	0.00082 (−0.000077 to 0.0017, 0.073)	0.087 (0.079–0.096, <0.001)	^
Childhood fat mass (kg)					
Glucose sum of *z*-scores	Sum of skinfolds	**0.13** (0.022–0.24, 0.019)	**0.037** (0.018–0.057, <0.001)	**0.17** (0.061–0.27, 0.0021)	**0.22**
	Cord C-peptide	0.097 (−0.012 to 0.21, 0.080)	**0.025** (0.0019–0.048, 0.034)	**0.12** (0.016–0.23, 0.024)	**0.21**
BMI (kg/m^2)	Sum of skinfolds	**0.47** (0.42–0.53, <0.001)	**0.015** (0.0071–0.024, <0.001)	**0.49** (0.44–0.54, <0.001)	**0.031**
	Cord C-peptide	**0.47** (0.41–0.52, <0.001)	**0.0058** (0.00020–0.011, 0.042)	**0.47** (0.42–0.53, <0.001)	**0.012**
Childhood percentage body fat (%)					
Glucose sum of *z*-scores	Sum of skinfolds	**0.26** (0.11–0.40, <0.001)	**0.045** (0.019–0.071, <0.001)	**0.30** (0.16–0.45, <0.001)	**0.15**
	Cord C-peptide	**0.19** (0.047–0.34, 0.010)	**0.041** (0.0088–0.072, 0.012)	**0.23** (0.090–0.38, 0.0015)	**0.18**
BMI (kg/m^2)	Sum of skinfolds	**0.51** (0.44–0.59, <0.001)	**0.019** (0.0076–0.030, <0.001)	**0.53** (0.46–0.60, <0.001)	**0.036**
	Cord C-peptide	**0.52** (0.44–0.59, <0.001)	**0.0094** (0.0016–0.017, 0.018)	**0.53** (0.45–0.60, <0.001)	**0.018**
Childhood sum of skinfolds (mm)					
Glucose sum of *z*-scores	Sum of skinfolds	**0.47** (0.17–0.78, 0.0023)	**0.085** (0.030–0.14, 0.0024)	**0.56** (0.26–0.86, <0.001)	**0.15**
	Cord C-peptide	**0.34** (0.028–0.64, 0.033)	**0.082** (0.018–0.15, 0.012)	**0.42** (0.12–0.72, 0.0066)	**0.2**
BMI (kg/m^2)	Sum of skinfolds	**1.18** (1.030–1.34, <0.001)	**0.035** (0.012–0.058, 0.0029)	**1.22** (1.070–1.37, <0.001)	**0.029**
	Cord C-peptide	**1.12** (0.97–1.28, <0.001)	**0.021** (0.0038–0.039, 0.017)	**1.14** (0.99–1.30, <0.001)	**0.018**

Values in boldface type are statistically significant. *Total effect not significant. ^Indirect effect not significant.

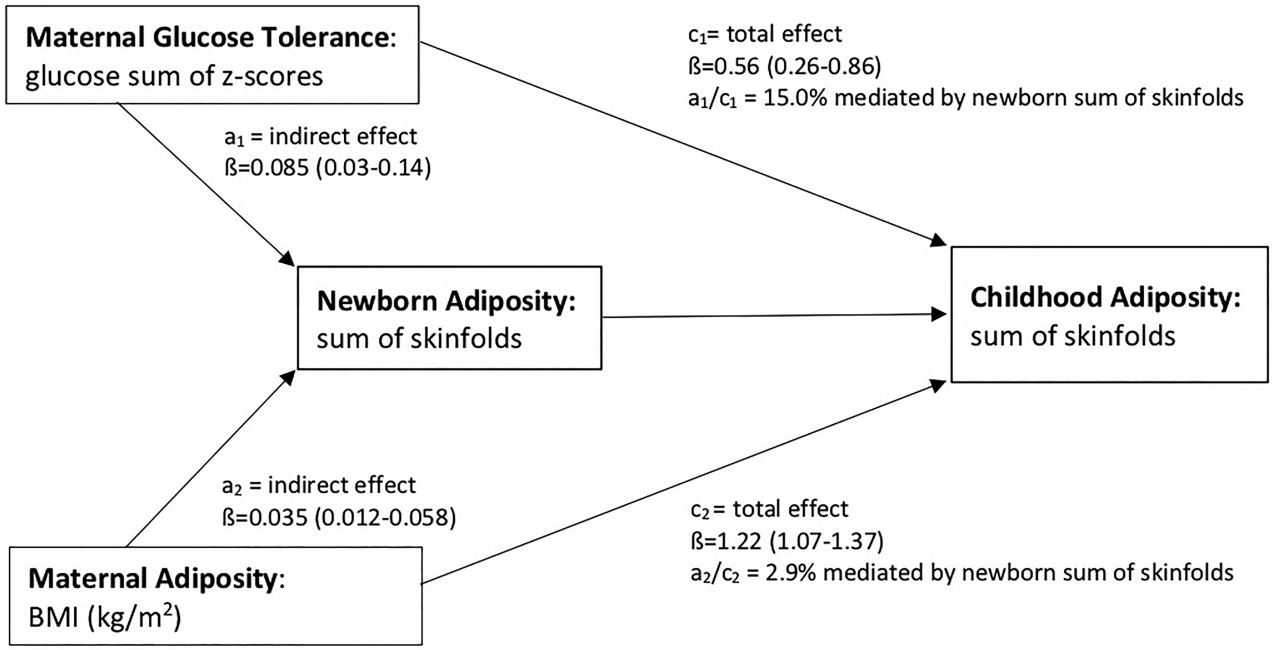

Figure 1—Mediation model of maternal glucose and BMI with childhood adiposity with newborn adiposity as the mediator. Diagram of a mediation analysis displaying maternal predictors glucose sum of *z*-scores and BMI (measured at mean 27.7 weeks' gestation), childhood sum of skinfolds outcome (measured at mean 11.4 years of age), and newborn sum of skinfolds (measured at delivery) as the mediator. Regression coefficients and 95% CIs are reported for total effects of maternal glucose and BMI on childhood sum of skinfolds (c_1 and c_2) and indirect effects of maternal glucose and BMI on the newborn sum of skinfolds mediator (a_1 and a_2). The proportion of the total effect of maternal glucose sum of *z*-scores on childhood sum of skinfolds mediated by newborn sum of skinfolds is 15.0%. The proportion of the total effect of maternal BMI on childhood sum of skinfolds mediated by newborn sum of skinfolds is 2.9%.

with other cohorts that demonstrated birth weight is not a reliable proxy for newborn and childhood adiposity (2,3).

Newborn adiposity, as measured by sum of skinfolds and fat mass, mediated a portion of the known associations between maternal glucose and BMI and greater childhood adiposity. The findings from the mediation analyses allow, to some extent, for evaluation of potential causal pathways underlying the well-described association studies between the maternal in utero environment and childhood size. These findings are an important addition to the existing literature examining critical periods of development, such as fetal exposures, newborn size, and childhood size, and the relationship to adverse metabolic health (8,9). The limited proportion of the association of maternal BMI with childhood adiposity mediated by newborn adiposity implies that pathways largely independent of newborn adiposity contribute to this association (e.g., genetics, epigenetic programming, and hypothalamic-appetite regulation) (25–27). Another explanation may be common genetics between maternal and childhood obesity that is not reflected in size at birth. For example, a recent study demonstrated that a polygenic risk score based on adult obesity demonstrated little association with birth weight but a strong association with weight at age 8 (28).

The current study suggests several contributors to offspring adiposity. Independent associations of maternal glucose and BMI with newborn and childhood adiposity have been documented by HAPO (6), HAPO FUS (16), and others (8–10,29). The current study demonstrates that a portion of the latter association is mediated by newborn adiposity. Additionally, this study demonstrates an association of newborn adiposity with childhood adiposity, independent of maternal glucose and BMI. The modest magnitude of this independent association suggests that further understanding of adiposity developmental pathways will be necessary to determine the timing and nature of interventions to prevent excessive adiposity during childhood.

Of interest, newborn adiposity and cord C-peptide mediated a higher proportion of the association of maternal glucose with measures of childhood adiposity (15–22%) than the association of maternal BMI with childhood adiposity (1–3.6%). This larger portion of mediation by newborn adiposity is consistent with the fetal overgrowth hypothesis, first proposed by Pedersen (11) and extended by Freinkel (12). Fetal insulin production in utero promotes fetal growth and fat accretion. Cord C-peptide is a crude measure of fetal insulin secretion (12), suggesting that excessive fetal insulin secretion is an important contributor to the developmental origins of adiposity.

The findings that newborn adiposity and cord C-peptide mediate a significant proportion of the association between maternal glycemia and childhood adiposity support the use of these newborn measures as shorter-term outcome measures when investigating treatment strategies for gestational diabetes mellitus, with the ultimate goal of reducing childhood adiposity. A number of interventional studies in women with gestational diabetes mellitus have reported reductions in birth weight after treatment (30–32), while one reported reduced neonatal adiposity among treated mothers (33). However, the effect of maternal treatment on childhood adiposity is not clear. In trials where longitudinal childhood follow-up

was evaluated, researchers failed to detect differences in offspring obesity (31,34). These negative trials may reflect initiation of treatment for maternal hyperglycemia too late in pregnancy (35), the small number and young age of the children at follow-up, and/or limiting childhood anthropometrics to BMI. The interrelationships of maternal glycemia and newborn and childhood adiposity demonstrated in the current study suggest that reducing maternal hyperglycemia could decrease newborn and childhood adiposity, but because HAPO FUS was an observational study, a long-term interventional study would be necessary to address this question.

A major strength of this study is that HAPO FUS represents the largest, multiethnic, international cohort of children with research measures of in utero maternal metabolic measures, adiposity at birth and at age 10–14 years, including multiple direct adiposity measures as opposed to only measures of birth weight and BMI that reflect both fat and lean mass. The findings of this report thus are applicable to the general population. Another strength is the common protocol across all field centers and the detailed research measures obtained during pregnancy, at birth, and at age 10–14 years. This allowed for the ability to account for multiple confounders.

The study is limited by lack of diet and physical activity data. A recent report from the Vitamin D And Lifestyle Intervention for GDM Prevention (DALI) randomized trial of lifestyle interventions in pregnancy demonstrated reduced adiposity in neonates born to mothers in the combined healthy eating and physical activity intervention group, suggesting the importance of pregnancy lifestyle factors on offspring adiposity (36). Another limitation is lack of data on paternal anthropometrics, which also contribute to childhood overweight risk (25). Lastly, total offspring fat was measured rather than fat distribution (8,9), and sum of skinfolds was the only measure of adiposity obtained in both newborns and children.

In this multiethnic longitudinal study, newborn adiposity was associated with childhood BMI and adiposity independent of maternal BMI and glycemia, whereas birth weight was associated with childhood BMI but not all measures of childhood adiposity. Newborn adiposity as well as cord C-peptide mediated a portion of associations of maternal glucose and BMI with childhood adiposity, yet the proportion of maternal glucose effects on childhood adiposity mediated by newborn adiposity and cord C-peptide was substantially higher than the proportion of maternal BMI effects mediated by these newborn measures. In summary, newborn adiposity reflects the maternal metabolic milieu and mediates, in part, the complex relationships between maternal glucose and BMI and childhood adiposity.

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