



# How does exposure to overnutrition in utero lead to childhood adiposity? Testing the insulin hypersecretion hypothesis in the EPOCH cohort

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## Abstract

**Aims/hypothesis** Our aim was to explore metabolic pathways linking overnutrition in utero to development of adiposity in normal-weight children.

**Methods** We included 312 normal-weight youth exposed or unexposed to overnutrition in utero (maternal BMI  $\geq 25$  kg/m<sup>2</sup> or gestational diabetes). Fasting insulin, glucose and body composition were measured at age ~10 years (baseline) and ~16 years (follow-up). We examined associations of overnutrition in utero with baseline fasting insulin, followed by associations of baseline fasting insulin with adiposity (BMI *z* score [BMIZ], subcutaneous adipose tissue [SAT], visceral adipose tissue [VAT]), insulin resistance (HOMA-IR) and fasting glucose during follow-up.

**Results** >All participants were normal weight at baseline (BMIZ  $-0.32 \pm 0.88$ ), with no difference in BMIZ for exposed vs unexposed youth ( $p = 0.14$ ). Of the study population, 47.8% were female sex and 47.4% were of white ethnicity. Overnutrition in utero corresponded with 14% higher baseline fasting insulin (geometric mean ratio 1.14 [95% CI 1.01, 1.29]), even after controlling for VAT/SAT ratio. Higher baseline fasting insulin corresponded with higher BMIZ (0.41 [95% CI 0.26, 0.55]), SAT (13.9 [95% CI 2.4, 25.4] mm<sup>2</sup>), VAT (2.0 [95% CI 0.1, 3.8] mm<sup>2</sup>), HOMA-IR (0.87 [95% CI 0.68, 1.07]) and fasting glucose (0.23 [95% CI 0.09, 0.38] SD).

**Conclusions/interpretation** Overnutrition in utero may result in hyperinsulinaemia during childhood, preceding development of adiposity. However, our study started at age 10 years, so earlier metabolic changes in response to overnutrition were not taken into account. Longitudinal studies in normal-weight youth starting earlier in life, and with repeated measurements of body weight, fat distribution, insulin sensitivity, beta cell function and blood glucose levels, are needed to clarify the sequence of metabolic changes linking early-life exposures to adiposity and dysglycaemia.

**Keywords** Beta cell dysfunction · Beta cell hypersecretion · Fasting insulin · Longitudinal cohort · Type 2 diabetes · Youth

## Abbreviations

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EPOCH	Exploring Perinatal Outcomes among Children
GDM	Gestational diabetes mellitus
HAPO	Hyperglycemia and Adverse Pregnancy Outcomes
KPCO	Kaiser Permanente of Colorado
MET	Metabolic equivalent
SAT	Subcutaneous adipose tissue
VAT	Visceral adipose tissue

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## Introduction

Youth-onset type 2 diabetes is on the rise in the USA [1] and worldwide [2]. The occurrence of and rise in incidence of paediatric type 2 diabetes is related to the obesity epidemic given that

## Research in context

### What is already known about this subject?

- Current understanding of the sequence of metabolic changes that culminate in type 2 diabetes revolves around the notion that obesity-related insulin resistance is the primary metabolic abnormality, with beta cell dysfunction occurring as a later manifestation and type 2 diabetes occurring when beta cells are no longer able to compensate for worsening insulin resistance
- Type 2 diabetes is heterogeneous both phenotypically and in terms of pathophysiology
- One alternative proposition is that insulin hypersecretion, rather than insulin resistance, is the primary metabolic abnormality in the sequence of events leading to type 2 diabetes

### What is the key question?

- Does exposure to overnutrition in utero (overweight/obese mother or gestational diabetes) lead to higher fasting insulin levels in normal-weight youth and, in turn, set youth on a trajectory of higher adiposity across adolescence?

### What are the new findings?

- Overnutrition in utero was associated with hyperinsulinaemia during childhood in normal-weight children
- Higher insulin levels during childhood were in turn associated with higher adiposity, greater insulin resistance and higher fasting glucose levels from childhood through adolescence

### How might this impact on clinical practice in the foreseeable future?

- If it is the case that chronic hyperinsulinaemia precedes insulin resistance in the pathogenesis of youth-onset type 2 diabetes, then preventive strategies should focus on reducing insulin hypersecretion in addition to improving insulin sensitivity

excess adiposity (starting as early as birth [3]) is the leading risk factor for type 2 diabetes [4, 5] and the two conditions have shared in utero origins [6–9]. However, the specific sequence of metabolic changes leading to obesity, hyperglycaemia and, eventually, youth-onset type 2 diabetes is unclear.

Current understanding of the sequence of metabolic changes that culminate in adult-onset type 2 diabetes revolves around the notion that obesity-related insulin resistance is the primary metabolic abnormality (potentially due to genetic predisposition), with beta cell dysfunction occurring as a later manifestation and type 2 diabetes occurring when beta cells are no longer able to compensate for worsening insulin resistance. However, type 2 diabetes is heterogeneous both phenotypically and in terms of pathophysiology [10]. There is debate regarding whether reduced insulin secretion occurs first, leading to diabetes, or whether chronic hyperinsulinaemia leads to development of excess adiposity and eventual beta cell failure [11]. The latter scenario is known as the ‘insulin hypersecretion’ hypothesis, which has recently gained traction: exposure to environmental insults that result in early-life overnutrition (e.g. chronic exposure to excess energy and/or nutrient intake [12] or obesogenic food additives [13]) may overstimulate beta cells and induce chronic insulin hypersecretion. Similar to the traditional hypothesis, the insulin hypersecretion hypothesis posits that in the long term, particularly among those with genetic susceptibility, beta cells may fail. However, unlike the traditional hypothesis, hyperinsulinaemia

rather than insulin resistance is the primary metabolic abnormality in the series of pathophysiological events leading to development of type 2 diabetes. Specifically, chronic hyperinsulinaemia may promote fat storage and development of insulin resistance as an adaptive response [11, 14]. This alternative pathway may be especially relevant for youth-onset type 2 diabetes, as demonstrated in the Restoring Insulin Secretion (RISE) study, in which youth with impaired glucose tolerance or recent-onset type 2 diabetes were more hyperinsulinaemic and insulin resistant than adults [15], a phenomenon that has also been reported in high-risk indigenous groups [16]. If chronic hyperinsulinaemia precedes insulin resistance in the pathogenesis of youth-onset type 2 diabetes (a possibility that remains yet to be determined), then preventive strategies should focus on reducing insulin hypersecretion, in addition to improving insulin sensitivity.

Current literature in support of the insulin hypersecretion hypothesis focuses on the link between hyperinsulinaemia and insulin resistance: insulinoma patients develop insulin resistance to downregulate the effect of excess insulin secretion from the tumour [17–19]; and normoglycaemic young adults developed iatrogenic insulin resistance within 3–5 days of chronic hyperinsulinaemia induced via insulin infusion balanced by glucose infusion to prevent hypoglycaemia in a small clinical trial [20]. However, definitive studies with human data derived from long-term longitudinal observational or experimental studies during early life are lacking.

In this study, we capitalise on an observational study of normal-weight youth for whom we have information on in utero exposures, and prospectively collected data on fasting insulin, glucose and adiposity measures at median age 10 years and approximately 6 years later. We used these data to test the hypothesis that exposure to overnutrition in utero resulting from the mother being overweight/obese or having gestational diabetes leads to higher fasting insulin levels in normal-weight youth, which in turn sets youth on a trajectory of higher adiposity across adolescence.

## Methods

### Study population

The Exploring Perinatal Outcomes among Children (EPOCH) study is a historical prospective cohort of youth with the original aim of characterising long-term consequences of in utero exposure to maternal diabetes and obesity. Details on recruitment are published [21]. Between 2006 and 2009, we recruited 604 children whose mothers were members of the Kaiser Permanente of Colorado (KPCO) Health plan. The mother–child pairs were invited to attend two research visits at a mean age of 10 years (baseline) and 16 years (follow-up). For this analysis we excluded children of seven women who had type 1 diabetes, and 142 for whom we did not have any information on gestational diabetes mellitus (GDM) or pre-pregnancy BMI. We further excluded 143 youth who were already overweight/obese at baseline, defined as age- and sex-specific  $z$  score  $\geq 1$  SD based on the WHO growth reference for children 5–19 years of age [22], in order to specifically explore the metabolic changes leading to development of excess adiposity during early life. The final analytic sample included 312 youth classified as normal weight at baseline. The study was approved by the Colorado Multiple Institutional Review Board (protocol no. 05-0623). All mothers provided written informed consent and children provided verbal assent.

### Overnutrition in utero

We defined overnutrition in utero as exposure to mother being overweight/obese pre-pregnancy or having GDM [23]. We calculated maternal pre-pregnancy BMI ( $\text{kg}/\text{m}^2$ ) using clinically recorded pre-pregnancy weight from KPCO medical records and height was measured at the mothers' baseline visit. We categorised the women's BMI as overweight/obese ( $\geq 25 \text{ kg}/\text{m}^2$ ) or non-overweight/obese ( $< 25 \text{ kg}/\text{m}^2$ ). Maternal GDM (yes vs no) was ascertained from the KPCO perinatal database, diagnosed using the standard two-step protocol [24].

### Offspring fasting insulin and glucose

At in-person research visits, we collected a blood sample from the participants' antecubital vein after they had fasted for 8 h. All samples were refrigerated immediately, processed within 24 h and stored at  $-80^\circ\text{C}$  until time of analysis. Using these samples, we measured fasting insulin and glucose by RIA (Millipore, Darmstadt, Germany). Using fasting insulin and glucose, we calculated the HOMA-IR [25].

### Offspring adiposity

**Anthropometric assessment** At baseline and follow-up, we measured height (m) on a calibrated stadiometer, weight (kg) on a digital scale and waist circumference (cm) via a non-stretchable measuring tape just above the uppermost lateral border of the right ilium [26]. All anthropometric measures were assessed in duplicate and the mean was used for analysis.

**MRI imaging** A trained technician performed MRI of the abdominal region with a 3 T HDx Imager (General Electric, Waukegan, WI, USA) with the participant in the supine position. A series of T1-weighted coronal images were taken to locate the L4/L5 plane. One axial, 10 mm, T1-weighted image, at the umbilicus or L4/L5 vertebrae, was analysed to determine visceral adipose tissue (VAT;  $\text{mm}^2$ ) and subcutaneous adipose tissue (SAT;  $\text{mm}^2$ ).

**Ratios and indices** We used weight and height to calculate BMI ( $\text{kg}/\text{m}^2$ ) and derived an age- and sex-specific BMI  $z$  score using the WHO growth reference [22]. We also calculated the waist/height ratio as a metric of central adiposity, and the VAT/SAT ratio as a marker of visceral adiposity.

### Covariates and background characteristics

At baseline, mothers reported prenatal smoking habits (smoked while pregnant with index child, yes vs no) and education level via a self-administered questionnaire. We categorised maternal education as a three-level variable ( $<$ high school, high school diploma or equivalent, and  $>$ high school). At this visit, the mothers reported any treatment they received for GDM (diet and/or exercise only, diet and/or exercise with insulin, or none) [27].

Participants self-reported their race/ethnicity at baseline as non-Hispanic White, non-Hispanic Black, Hispanic and non-Hispanic other. At both visits, participants reported their pubertal development based on pictorial diagrams of the Tanner stages [28, 29]. We based pubertal status on pubic hair development in boys and breast development in girls. At both visits, we obtained information on physical activity levels using the 3-Day Physical Activity Recall (3DPAR) Questionnaire [30] to

derive mean daily energy expenditure (metabolic equivalents [METs]), and used the Block Kid's Food Frequency Questionnaire [31] to derive estimates of total energy intake (kJ/day) as a proxy for overall nutrient intake.

## Data analysis

We conducted the main analyses in two steps. First, we examined associations of exposure to overnutrition in utero with fasting insulin levels at baseline (age ~10 years). Because fasting insulin was skewed, we  $\log_e$ -transformed this variable for regression analysis and back-transformed for interpretation as a % change. In models, the independent variable was an indicator for exposure to overnutrition in utero (yes vs no) and  $\log_e$ -transformed fasting insulin was the dependent variable. We then conducted a series of multivariable models, accounting for known determinants of in utero overnutrition and childhood metabolic risk. In Model 1, we adjusted for the child's age, sex and race/ethnicity. Model 2 further accounted for maternal education and smoking habits during pregnancy. Model 3 included Model 2 covariates plus the child's physical activity levels and total energy intake to account for concurrent lifestyle. Model 4 adjusted for Model 2 covariates plus VAT-to-SAT ratio, an indicator of central visceral adiposity that is a strong determinant of cardiometabolic risk [32]. For completeness, we also assessed the impact of adjustment for alternate metrics of adiposity (VAT, SAT, waist/height ratio).

Next, we examined the association of  $\log_e$ -transformed fasting insulin at baseline with markers of adiposity and blood glucose across follow-up using mixed-effects linear regression. In these models, the independent variables included baseline  $\log_e$ -transformed fasting insulin and longitudinal age, and the dependent variable was repeated measurements of each marker of adiposity or blood glucose over follow-up (i.e. the baseline and the follow-up values for each outcome, with a maximum of two measurements per individual) both in native units and as internally standardised  $z$  scores (with the exception of BMI  $z$  score, which was externally standardised using the WHO growth reference) to facilitate comparability of effect estimates. We also included a random effect for the intercept and an unstructured correlation matrix to account for within-individual correlations. The association of interest is the  $\beta$  estimate for overnutrition in utero, interpreted as the difference in mean levels of a given outcome over 6 years of follow-up for exposed vs unexposed youth.

In multivariable analysis, we accounted for the covariates in Models 1–3 described above. In models where fasting glucose or HOMA-IR was the dependent variable, we assessed the impact of adjustment for concurrent adiposity via longitudinal measures of VAT/SAT ratio in Model 4. In all models, we tested for an interaction with sex and found no evidence of effect modification; therefore, data for boys and

girls were analysed together. For all estimates, we considered  $\alpha = 0.05$  as the threshold for statistical significance.

Finally, we conducted sensitivity analyses. We examined the effect of a mother being overweight/obese and having GDM as separate exposures to assess for potentially different programming effects of the two. We also assessed the impact of adjusting for Tanner stage, a mediator that may influence both glucose–insulin homeostasis and body composition, and treatment for GDM. We assessed jack-knifed Studentised residuals for all models to confirm assumptions of normality. We carried out all analyses using SAS software (version 9.3; SAS Institute, Cary, NC, USA).

## Results

In this sample, 46.2% (144) of the 312 participants were exposed to overnutrition in utero, either by mothers being overweight/obese or mothers having GDM. The relatively high prevalence is due to the fact that, by design, the EPOCH study oversampled for exposure to GDM and/or maternal obesity. Of the 144 participants exposed to overnutrition in utero, 86 had mothers who were overweight/obese only, 21 were exposed to GDM only, 30 had both types of exposure and seven were exposed to at least GDM (i.e. maternal BMI was missing).

The median age of participants was 10.5 years at baseline and 16.6 years at follow-up. Approximately half of the participants were female (47.8%) and white (47.4%). At both visits, exposed children were slightly younger than their unexposed counterparts, with no differences between the two groups with respect to sex or race/ethnicity. Table 1 shows additional characteristics of the sample at baseline and follow-up, stratified by exposure status. At baseline, all children were normal weight (mean  $\pm$  SD BMI  $z$  score  $-0.32 \pm 0.88$ ) as per our selection criteria. BMI  $z$  score was not different for exposed vs unexposed youth ( $p = 0.14$ ). Fasting insulin (+7 pmol/l), HOMA-IR (+0.2) and some adiposity markers were somewhat higher among exposed youth at baseline (i.e. small differences in waist circumference [+1.4 cm], waist/height ratio [+0.009], SAT [+10.7 mm<sup>2</sup>]), whereas fasting glucose, VAT and VAT/SAT ratio were not different between the two groups (Table 1). At follow-up, all measures of adiposity and blood glucose increased in both groups except for VAT/SAT ratio. It is noteworthy that a greater proportion of exposed participants crossed the threshold into overweight/obese status (13.9% of exposed group vs 5.4% of unexposed group;  $p = 0.03$ ) and that at the follow-up visit, exposed youth had higher fasting insulin, HOMA-IR, BMI  $z$  score and SAT than their unexposed counterparts.

Table 2 shows associations of exposure to overnutrition in utero with fasting insulin levels at baseline in multiple models that adjusted for potential confounders. We observed

**Table 1** Background characteristics of 312 normal-weight youth stratified by exposure to overnutrition in utero in the EPOCH cohort

	Exposed ( <i>n</i> =144) <sup>a</sup>	Unexposed ( <i>n</i> =168) <sup>a</sup>	<i>p</i> value
Perinatal and background characteristics			
Pre-pregnancy BMI, kg/m <sup>2</sup>	28.3±4.8	21.4±2.1	<0.0001
GDM, % ( <i>n</i> )	40.3 (58)	0 (0)	<0.0001
Mother graduated college, % ( <i>n</i> )	79.2 (114)	77.4 (130)	0.70
Mother smoked during pregnancy, % ( <i>n</i> )	8.3 (12)	8.9 (15)	0.85
Female sex, % ( <i>n</i> )	44.4 (64)	50.6 (85)	0.28
Race/ethnicity, % ( <i>n</i> )			0.55
Non-Hispanic White	45.8 (66)	48.8 (82)	
Hispanic	39.6 (57)	41.7 (70)	
Non-Hispanic Black	6.3 (9)	4.8 (8)	
Non-Hispanic Other	8.3 (12)	4.8 (8)	
Characteristics of youth at baseline			
Age, years	10.0±1.7	10.7±1.4	<0.0001
Fasting insulin, pmol/l <sup>b</sup>	61.4±2.9	54.4±2.7	0.08
Fasting glucose, mmol/l	4.4±0.5	4.4±0.4	0.77
HOMA-IR	1.76±0.08	1.56±0.08	0.10
BMI <i>z</i> score	−0.24±0.90	−0.39±0.86	0.14
Waist circumference, cm	60.8±0.4	59.4±0.4	0.007
Waist/height ratio	0.430±0.003	0.421±0.002	0.02
SAT, mm <sup>2</sup>	73.7±3.3	63.0±3.1	0.02
VAT, mm <sup>2</sup>	15.8±0.6	14.8±0.5	0.21
VAT/SAT ratio	0.257±0.011	0.276±0.010	0.20
Characteristics of youth at follow-up			
Age, years	16.1±1.3	16.6±1.2	0.003
Fasting insulin, pmol/l	106.7±4.2	91.6±4.2	0.01
Fasting glucose, mmol/l	4.9±0.1	4.9±0.1	0.63
HOMA-IR	3.38±0.14	2.87±0.14	0.01
BMI <i>z</i> score	0.11±0.97	−0.14±0.86	0.04
Overweight/obese, % ( <i>n</i> ) <sup>b</sup>	13.9 (20)	5.4 (9)	0.03
Waist circumference, cm	75.6±0.8	75.4±0.8	0.87
Waist/height ratio	0.449±0.05	0.450±0.005	0.92
SAT, mm <sup>2</sup>	150.4±8.3	128.2±8.5	0.06
VAT, mm <sup>2</sup>	27.4±1.4	25.6±1.4	0.40
VAT/SAT ratio	0.213±0.012	0.238±0.012	0.66

Data are mean ± SE, mean ± SD, or % (*n*)

<sup>a</sup> Values for continuous variables are age adjusted due to older age of unexposed participants and are presented as mean ± SE, except for BMI *z* score (based on the WHO age- and sex-specific growth reference) which is mean ± SD

<sup>b</sup> WHO age- and sex-specific BMI *z* score ≥ 1

*p* values from an independent *t* test for continuous variables or from a Wald  $\chi^2$  test for categorical and dichotomous variables

consistently higher insulin (13–14% higher fasting insulin, or  $\beta$  of 1.13–1.14 units of  $\log_e$  fasting insulin) among exposed vs unexposed youth in Models 1–3. This relationship was not attenuated even after accounting for concurrent adiposity via VAT/SAT ratio in Model 4 (1.15 [95% CI: 1.01, 1.30] units  $\log_e$ -transformed fasting insulin for exposed vs unexposed,  $p = 0.03$ ). We noted similar magnitudes of effect for exposure

status after accounting for alternative adiposity indicators (i.e. VAT, SAT, waist/height ratio), with  $\beta$  ranging from 1.11 to 1.13, and  $p$  values ranging from 0.05 to 0.11.

Table 3 shows associations of baseline fasting insulin with adiposity markers, fasting glucose and HOMA-IR during follow-up. Higher fasting insulin at baseline was associated with higher adiposity, fasting glucose and HOMA-IR across

**Table 2** Associations of overnutrition in utero (exposed vs unexposed) with fasting insulin levels at baseline (age ~10 years) among 312 normal-weight youth in the EPOCH cohort

	N	Mean±SE fasting insulin (pmol/l) <sup>a</sup>	β (95% CI) for geometric mean <sup>b</sup>	p value
Model 1 <sup>c</sup>				0.04
Unexposed	168	44.7 ± 1.1	1.00 (Reference)	
Exposed	144	50.9 ± 1.1	1.14 (1.01, 1.29)	
Model 2 <sup>d</sup>				0.04
Unexposed	168	44.7 ± 1.1	1.00 (Reference)	
Exposed	144	50.9 ± 1.1	1.14 (1.01, 1.29)	
Model 3 <sup>e</sup>				0.05
Unexposed	164	44.3 ± 1.1	1.00 (Reference)	
Exposed	138	50.4 ± 1.1	1.13 (1.00, 1.28)	
Model 4 <sup>f</sup>				0.03
Unexposed	158	46.5 ± 1.1	1.00 (Reference)	
Exposed	131	53.3 ± 1.1	1.15 (1.01, 1.30)	

<sup>a</sup> Fasting insulin was  $\log_e$ -transformed prior to regression analysis, then the values were back-transformed via exponentiation

<sup>b</sup> Fasting insulin was natural  $\log_e$ -transformed prior to regression analysis and the  $\beta$  estimate was exponentiated. Estimates may be interpreted as a percentage change

<sup>c</sup> Model 1: Adjusted for child's age, sex, and race/ethnicity

<sup>d</sup> Model 2: Model 1 + mother's education and prenatal smoking habits

<sup>e</sup> Model 3: Model 2 + physical activity levels (METs over a period of 3 days) and total energy intake (kJ/day)

<sup>f</sup> Model 4: Model 2 + VAT/SAT ratio

the follow-up. In Model 1, which accounted for child's age, sex, and race/ethnicity, each unit of  $\log_e$ -transformed fasting insulin corresponded with 0.40 (95% CI 0.25, 0.54) higher BMI z score, 14.1 (95% CI 3.1, 25.2) mm<sup>2</sup> (or 0.48 [95% CI 0.31, 0.65] z score) higher SAT, 1.9 (95% CI 0.1, 3.8) mm<sup>2</sup> (or 0.42 [95% CI 0.25, 0.60] z score) higher VAT and 0.88 (95% CI 0.69, 1.07) units (or 0.94 [95% CI 0.82, 1.07] z score) higher HOMA-IR. These estimates were materially unchanged after accounting for maternal education and prenatal smoking (Model 2), and child's physical activity and diet (Model 3) (Table 3). Adjustment for longitudinal measures of concurrent adiposity did not attenuate the effect estimate for baseline insulin in relation to HOMA-IR during follow-up. For example, after accounting for VAT/SAT ratio in Model 4, each unit of  $\log_e$ -transformed fasting insulin at baseline was associated with 0.78 (95% CI 0.57, 0.99) units higher HOMA-IR. We observed similar estimates when adjusting for other adiposity indicators. While baseline insulin was only marginally positively associated with waist/height ratio or fasting glucose assessed in native units, the association was statistically significant when these outcomes were assessed as z scores (Table 3).

### Sensitivity analyses

When we assessed exposure to overnutrition in utero separately for mothers being overweight/obese and mothers having

GDM, we noted a similar direction of associations with fasting insulin at baseline, though the estimates were only marginally significant due to reduced sample sizes (electronic supplementary material [ESM] Table 1).

Inclusion of Tanner stage as a covariate did not change the direction or magnitude of results, so we do not include this variable in final models given that it is likely a mediator and therefore adjusting for it may introduce bias [33]. Adjustment for GDM treatment somewhat attenuated the effect of in utero overnutrition on baseline fasting insulin (geometric mean ratio 1.09 [95% CI 0.95, 1.25]), although the overall direction and precision of the estimate remained consistent with estimates in Table 2.

## Discussion

### Summary

Among 312 diverse normal-weight youth, exposure to overnutrition in utero due to mothers being overweight/obese or having GDM was associated with 13–14% higher fasting insulin levels at approximately 10 years of age even after adjustment for differences in markers of adiposity and body composition. Higher fasting insulin at age 10 years was in turn associated with higher prevalence of overweight/obese individuals at follow-up and greater adiposity accrual across

**Table 3** Associations of fasting insulin levels at baseline (age ~10 years) with adiposity and blood glucose across 6 years of follow-up among 312 normal-weight youth in the EPOCH cohort

Adiposity or blood glucose marker across 6 years of follow-up	Outcomes in native units per 1 unit log <sub>e</sub> -transformed fasting insulin at baseline		Outcomes in z scores per 1 unit log <sub>e</sub> -transformed fasting insulin at baseline	
	β (95% CI)	p value	β (95% CI)	p value
<b>Model 1 (n=312)<sup>a</sup></b>				
BMI z score	0.40 (0.25, 0.54)	<0.0001	–	–
Waist circumference, cm	1.5 (0.4, 2.5)	0.007	0.59 (0.44, 0.74)	<0.0001
Waist/height ratio	0.005 (–0.001, 0.012)	0.09	0.24 (0.08, 0.40)	0.004
SAT, mm <sup>2</sup>	14.1 (3.1, 25.2)	0.01	0.48 (0.31, 0.65)	<0.0001
VAT, mm <sup>2</sup>	1.9 (0.1, 3.8)	0.04	0.42 (0.25, 0.60)	<0.0001
Fasting glucose, mmol/l	0.05 (–0.02, 0.12)	0.15	0.24 (0.09, 0.39)	0.002
HOMA-IR	0.88 (0.69, 1.07)	<0.0001	0.94 (0.82, 1.07)	<0.0001
<b>Model 2 (n=312)<sup>b</sup></b>				
BMI z score	0.41 (0.26, 0.55)	<0.0001	–	–
Waist circumference, cm	1.5 (0.4, 2.6)	0.006	0.59 (0.44, 0.74)	<0.0001
Waist/height ratio	0.006 (–0.001, 0.012)	0.09	0.24 (0.08, 0.40)	0.004
SAT, mm <sup>2</sup>	13.9 (2.4, 25.4)	0.02	0.47 (0.30, 0.65)	<0.0001
VAT, mm <sup>2</sup>	2.0 (0.1, 3.8)	0.04	0.42 (0.25, 0.59)	<0.0001
Fasting glucose, mmol/l	0.05 (–0.02, 0.12)	0.16	0.23 (0.09, 0.38)	0.002
HOMA-IR	0.87 (0.68, 1.07)	0.0001	0.94 (0.81, 1.07)	<0.0001
<b>Model 3 (n=300)<sup>c</sup></b>				
BMI z score	0.41 (0.26, 0.55)	<0.0001	–	–
Waist circumference, cm	1.4 (0.3, 2.5)	0.01	0.58 (0.43, 0.73)	<0.0001
Waist/height ratio	0.005 (–0.002, 0.012)	0.13	0.23 (0.07, 0.40)	0.006
SAT, mm <sup>2</sup>	12.1 (0.4, 23.8)	0.04	0.45 (0.28, 0.62)	<0.0001
VAT, mm <sup>2</sup>	2.0 (0.1, 3.9)	0.04	0.43 (0.26, 0.61)	<0.0001
Fasting glucose, mmol/l	0.04 (–0.03, 0.11)	0.23	0.23 (0.07, 0.38)	0.004
HOMA-IR	0.85 (0.64, 1.05)	<0.0001	0.92 (0.79, 1.06)	<0.0001
<b>Model 4 (n=286)<sup>d</sup></b>				
Fasting glucose, mmol/l	0.04 (–0.03, 0.11)	0.24	0.22 (0.07, 0.37)	0.005
HOMA-IR	0.78 (0.57, 0.99)	<0.0001	0.87 (0.73, 1.01)	<0.0001

<sup>a</sup> Model 1: Adjusted for child's age, sex, and race/ethnicity

<sup>b</sup> Model 2: Model 1 + mother's education and prenatal smoking habits

<sup>c</sup> Model 3: Model 2 + physical activity levels (METs over a period of 3 days) and total energy intake (kJ/day)

<sup>d</sup> Model 4: Model 2 + VAT/SAT ratio

6 years of follow-up, as indicated by higher BMI z score (+0.4 SD), waist circumference (+1.5 cm) and subcutaneous (+14 mm<sup>2</sup>) and visceral fat depots (+2 mm<sup>2</sup>), as well as with higher insulin resistance (+0.9 units of HOMA-IR) and internally standardised glucose (+0.23 SD). We noted similar but more statistically significant associations when assessing the outcomes as internally standardised z scores, likely because the standardisation improved normality. Taken together, these findings raise the possibility that in utero exposure to overnutrition may result in increased beta cell insulin release, which may subsequently promote adiposity accrual, insulin resistance and dysglycaemia.

### Overnutrition in utero is associated with higher fasting insulin levels in normal-weight children

We found that in utero exposure to overnutrition corresponds with higher fasting insulin levels during childhood in the absence of overweight/obesity. While higher fasting insulin may be a reflection of insulin resistance, this association persisted after adjustment for indicators of adiposity and fat distribution. Regardless of the adiposity marker used (e.g. VAT/SAT ratio, VAT or SAT alone, waist/height ratio), we observed a similar association of overnutrition in utero with fasting insulin at 10 years of age, suggesting that overnutrition

in utero is consistently associated with higher fasting insulin levels in normal-weight children and, more importantly, that this association is not completely explained by concurrent markers of adiposity. These results provide some support for the first part of the insulin hypersecretion hypothesis, that an environmental insult during early life (overnutrition in utero) may overstimulate the beta cells, potentially leading to chronic hyperinsulinaemia.

Current literature comprises limited and inconsistent data from other human cohorts. Two recent follow-up studies from the Hyperglycemia and Adverse Pregnancy Outcomes (HAPO) study found evidence for reduced stimulated beta cell function in offspring during childhood with respect to maternal glucose levels during pregnancy. In 2017, Tam et al. [34] reported that children (age ~7 years,  $n=970$ ) born to women with GDM exhibited some evidence of reduced stimulated beta cell function based on lower  $AUC_{\text{insulin}}/AUC_{\text{glucose}}$  from 0 to 120 min in an OGTT and lower HOMA-B, though neither of the differences were statistically significant. The authors also detected marginally lower values for the insulinogenic index at 30 min post-OGTT, indicative of lower early insulin response. Subsequently, Scholtens et al. found that among 4160 HAPO mother–child dyads maternal glucose levels at 1 h post-OGTT were inversely related to the child’s insulinogenic index even after adjusting for the child’s BMI [9]. While such findings point towards an attenuated stimulated insulin response among youth exposed to overnutrition in utero, a recent HAPO study analysis found that fetal hyperinsulinaemia (as indicated by cord blood C-peptide) partially mediated the effect of maternal glucose on childhood adiposity, suggesting that hyperinsulinaemia may have occurred even earlier in life, prior to development of adiposity [9].

In an analysis using data from both normal-weight and overweight/obese participants in EPOCH, Sauder et al. reported that maternal diabetes was associated with increased offspring insulin resistance (HOMA-IR) across childhood and adolescence independent of current BMI [35]. Both the HAPO studies and our prior findings in the full EPOCH cohort provide evidence for a long-term programming effect of the in utero metabolic milieu on glucose–insulin homeostasis during childhood and adolescence. However, none of the above studies address the question of whether the initial metabolic abnormality in response to an early-life insult is hyperinsulinaemia or insulin resistance. Although findings from the present analysis provide some support that the first metabolic abnormality may be hyperinsulinaemia, we cannot conclude that this is the case for certain as we had only one measure of fasting insulin, a crude estimate of insulin hypersecretion, obtained at ~10 years of age. Thus, earlier metabolic changes related to overnutrition in utero, such as subtle effects on whole-body or tissue-specific insulin sensitivity are not captured or accounted for. Moreover, although both the exposed and the unexposed groups were normal weight, there

were subtle differences in body composition between the two groups (e.g. slightly higher waist circumference, weight/height ratio and SAT in the exposed group), raising the possibility that higher fasting insulin at baseline (~10 years of age) in the exposed group reflects some degree of pre-existing insulin resistance. However, and importantly, differences in fasting insulin levels at age 10 years with respect to overnutrition in utero remained apparent even after accounting for minimal differences in concurrent adiposity and body composition, suggesting that these differences more likely reflect a direct effect of exposure to overnutrition in utero on insulin secretion, rather than increased insulin secretion in response to underlying insulin resistance in the exposed group. Longitudinal studies starting at or even before birth, with repeated measures of insulin secretion, insulin resistance and adiposity, are needed to definitively answer this question.

### **Higher fasting insulin is associated with greater adiposity, insulin resistance and higher fasting glucose across 6 years of follow-up**

We found a consistent positive association of fasting insulin at age 10 years with multiple indicators of adiposity (BMI  $z$  score, waist circumference, MRI-assessed SAT and VAT), insulin resistance (HOMA-IR) and internally standardised fasting glucose across 6 years of follow-up from age 10 years to 16 years. Similarly, the Da Qing Children Cohort Study showed that fasting insulin at age 5 years was associated with greater subsequent weight gain from age 5 years to 10 years, as well as with higher systolic BP, fasting glucose, triacylglycerols and HOMA-IR at age 10 years [36].

Our findings indicate that higher fasting insulin measured during childhood, following in utero exposure to overnutrition, is associated with greater subsequent adiposity, more insulin resistance and higher fasting glucose from 10 to 16 years of age. Additional follow-up of participants in the present analysis into young adulthood is needed to definitively address the question of whether higher fasting insulin in normal-weight children in response to in utero exposure to overnutrition is associated with markers of dysglycaemia later in life.

### **Strengths and limitations**

This study has the following strengths: (1) prospectively collected data on metabolic biomarkers and body composition across the adolescent transition, a sensitive period for development of cardiometabolic diseases [37]; (2) rich covariate information; (3) a longitudinal modelling strategy to efficiently leverage data collected across 6 years of follow-up; and (4) a focus on initially normal-weight youth, allowing us to isolate the potential effect of the in utero environment on fasting insulin, without confounding by insulin resistance.



A major limitation of this analysis is that we only have fasting insulin measurements at a single point in time (age ~10 years) as a proxy for chronic hyperinsulinaemia. As previously mentioned, we cannot provide a definitive answer to the question of whether early-life overnutrition leads to hyperinsulinaemia or insulin resistance first. However, we were able to distil that exposure to overnutrition in utero is associated with higher fasting insulin in normal-weight youth, even after accounting for small differences in body composition via MRI-assessed VAT and SAT depots, waist circumference and waist/height ratio. We were also able to provide evidence that higher fasting insulin levels preceded development of overweight/obese states and subsequent elevations in markers of adiposity and insulin resistance as the children mature. Another limitation is that the EPOCH cohort was enriched for in utero exposure to GDM, so our findings may not be directly translatable to other populations with lower prevalence of maternal GDM. Finally, we did not collect data on Tanner staging for testicular volume; thus, there may be residual confounding by tempo of sexual maturation in boys. Finally, there was a small amount of missingness (3–8%) for covariates but given the consistent estimates of association across the multivariable model, it is unlikely that missing covariate data introduced concerning bias into the results.

## Conclusions

Findings from this longitudinal study of initially normal-weight youth provide support for the hypothesis that exposure to overnutrition in utero results in insulin hypersecretion in the absence of an overweight/obese status, even after adjustment for differences in adiposity and body composition. Higher fasting insulin was in turn associated with adiposity accrual, insulin resistance and dysglycaemia during 6 years of follow-up. Large longitudinal studies with repeated measures of weight, body fat distribution, insulin secretion, insulin sensitivity and blood glucose among initially normal-weight children, starting very early in life and continuing beyond puberty are needed to better understand the pathophysiology of youth-onset type 2 diabetes.

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**Data availability** Data are available upon request from the authors.

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**Contribution statement** WP made substantial contributions to design of the study, interpretation of the data, drafted the first draft of the article and revised it critically for important intellectual content. MMK and KAS made substantial contributions to analysis and interpretation of the data, and provided critical and important intellectual feedback on the article. DD made substantial contributions to the conception and design of the study, analysis and interpretation of the data, and provided critical feedback on the paper. All authors gave final approval of the version to be published. WP is the guarantor of this work.

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