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# The importance of maternal insulin resistance throughout pregnancy on neonatal adiposity

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**Abstract**

**Background:** Although previous studies evaluated the association of maternal health parameters with neonatal adiposity, little is known regarding the complexity of the relationships among different maternal health parameters throughout pregnancy and its impact on neonatal adiposity.

**Objectives:** To evaluate the direct and indirect associations between maternal insulin resistance during pregnancy, in women with obesity, and neonatal adiposity. In addition, associations between maternal fasting glucose, triglycerides (TG), non-esterified fatty acids (NEFA), and neonatal adiposity were also assessed.

**Methods:** This is a longitudinal, secondary analysis of the DALI study, an international project conducted in nine European countries with pregnant women with obesity. Maternal insulin resistance (HOMA-IR), fasting glucose, TG, and NEFA were measured three times during pregnancy (<20, 24–28, and 35–37 weeks of gestation). Offspring neonatal adiposity was estimated by the sum of four skinfolds. Structural equation modelling was conducted to evaluate the direct and indirect relationships among the variables of interest.

**Results:** Data on 657 mother-infant pairs (50.7% boys) were analysed. Neonatal boys exhibited lower mean sum of skinfolds compared to girls (20.3 mm, 95% CI 19.7, 21.0 vs 21.5 mm, 95% CI 20.8, 22.2). In boys, maternal HOMA-IR at <20 weeks was directly associated with neonatal adiposity ( $\beta = 0.35$  mm, 95% CI 0.01, 0.70). In girls, maternal HOMA-IR at 24–28 weeks was only indirectly associated with neonatal adiposity, which implies that this association was mediated via maternal HOMA-IR, glucose, triglycerides, and NEFA during pregnancy ( $\beta = 0.26$  mm, 95% CI 0.08, 0.44).

**Conclusions:** The timing of the role of maternal insulin resistance on neonatal adiposity depends on fetal sex. Although the association was time-dependent, maternal insulin resistance was associated with neonatal adiposity in both sexes.

**KEYWORDS**

endocrinology, gynaecology, insulin, lipid metabolism, obesity

**1 | BACKGROUND**

Maternal obesity is associated with neonatal<sup>1–4</sup> and childhood adiposity, and childhood obesity<sup>2,5</sup> is not only associated with a higher chance of obesity, but also premature death and disability in adulthood.<sup>5</sup> While maternal insulin resistance and fasting glucose have a positive association with neonatal adiposity,<sup>6–13</sup> the role of maternal triglycerides and non-esterified fatty acids on neonatal adiposity remains controversial.<sup>6–8,11</sup> It is unclear whether such associations are causal, in the causal pathway or a consequence of the metabolic pathways involved.

Shapiro et al<sup>6</sup> reported that HOMA-IR, glucose, but not triglycerides and non-esterified fatty acids at 24–32 weeks of gestation mediated the association between maternal pre-pregnancy body mass index (BMI) and neonatal adiposity. Although this study<sup>6</sup> provided relevant insights regarding the possible underlying mechanisms in the relationship between maternal metabolic profile and

neonatal adiposity, some aspects still require further evaluation. First, it is crucial that the entire pregnancy period is taken into account. Fetal development depends on the maternal metabolic profile throughout the whole pregnancy period,<sup>6–13</sup> illustrated by the fact that obese women or women who develop gestational diabetes have larger fetuses already by 20 weeks of gestation.<sup>14,15</sup> Second, there is evidence that neonatal girls might be more insulin resistant than boys,<sup>16,17</sup> and mechanisms underlying the relationship between maternal metabolism and neonatal adiposity might differ depending on the neonatal sex.<sup>18</sup>

The aim of this investigation was to evaluate the direct and indirect associations between insulin resistance during pregnancy and neonatal adiposity, as measured by sum of skinfolds. In addition, the direct and indirect associations between fasting glucose, triglycerides, non-esterified fatty acids, and neonatal adiposity were also assessed. This is a novel approach because it assesses the complexity of the relationships among different maternal health



parameters throughout pregnancy and its impact on neonatal adiposity. Although the observational design does not allow to assert on causality, it is a valuable approach for generating hypothesis to be tested in clinical trials.

## 2 | METHODS

### 2.1 | Design and participants

This is a longitudinal, secondary analysis of the DALI study which was a multicentre parallel randomised trial conducted in nine European countries (Austria, Belgium, Denmark, Ireland, Italy, Netherlands, Poland, Spain, and United Kingdom) during 2012-2015. Pregnant women with a pre-pregnancy body mass index (BMI) of  $\geq 29$  kg/m<sup>2</sup>, before 20 weeks of gestation, with a singleton pregnancy, and aged  $\geq 18$  years were invited to participate.<sup>19</sup> Exclusions included diagnosis with early gestational diabetes mellitus (GDM), pre-existing diabetes, and chronic medical conditions. The study was prospectively registered as a randomised controlled trial (RCT) on 21 November 2011 (ISRCTN70595832).

### 2.2 | Randomisation, masking, and interventions

Using a computerised random number generator, women were randomised to one of the following groups, pre-stratified for site: (a) healthy eating; (b) physical activity; (c) healthy eating + physical activity; (d) healthy eating + physical activity + vitamin D; (e) healthy eating + physical activity + placebo; (f) vitamin D; (g) placebo; and (h) control. Staff involved with measurements, but not participants, were blinded to the intervention. Since methodology has been extensively described elsewhere,<sup>19</sup> only variables of interest will be detailed in this manuscript.

### 2.3 | Measurements

#### 2.3.1 | Neonatal adiposity

Triceps, subscapular, supra-iliac, and quadriceps skinfolds were measured with a Harpenden skinfold calliper, and values summed. Each skinfold measurement was repeated once, and if a difference of more than 0.2 mm was registered, a third measurement was performed and the average of the three was taken. Time between birth and measurements was registered in hours—neonatal age at the measurement.

#### 2.3.2 | Maternal measurements

Maternal height was determined at baseline with a stadiometer (SECA 206; SECA, Birmingham United Kingdom). Women were weighed on calibrated electronic scales (SECA 888 and 877) at

### Synopsis

#### Study question

How is the synergic relationship of maternal insulin resistance, fasting glucose, triglycerides, and non-esterified fatty acids with neonatal adiposity in pregnant women with obesity?

#### What's already known

Maternal insulin resistance and fasting glucose have a positive association with neonatal adiposity, and the role of maternal triglycerides and non-esterified fatty acids on neonatal adiposity remains controversial.

#### What this study adds

The association between maternal insulin resistance and neonatal adiposity differed depending on the infant sex. While adiposity in boys was associated directly with maternal insulin resistance in the beginning of pregnancy, adiposity in girls was associated with maternal insulin resistance at 24-28 weeks of gestation via a chain of relationships through fasting glucose, triglycerides, and free fatty acids.

baseline (<20 weeks), 24-28 weeks, and at 35-37 weeks of gestation. Body mass index (BMI) was calculated as weight in kg divided by the square of height in metres.

After fasting for 10 hours, blood was collected in three different periods during pregnancy (<20, 24-28, and 35-37 weeks of gestation). The procedures were identical in all pregnancy periods. All the samples were centrifuged and separated aliquots (1000 or 250  $\mu$ L) placed in microrack tubes and stored at  $-20$  or  $-80^{\circ}\text{C}$  in the central trial laboratory, prior to analysis, in Graz, Austria. The maternal plasma concentrations of fasting glucose, triglycerides, and non-esterified fatty acids were quantified. For insulin and leptin, commercially available enzyme-linked immunosorbent assays (ELISAs) were used. Insulin resistance was derived from homeostasis model assessment (HOMA-IR).<sup>20</sup>

#### 2.3.3 | Covariates

Information on possible covariates was collected in the baseline questionnaire or from medical files: intervention groups, maternal age, gestational age during pregnancy (<20, 24-28 and 35-37 weeks), maternal ethnicity (European descent and non-European descent), maternal education (low [high school], medium [completed high school] and high [higher education]), smoking status at 35-37 weeks of gestation (yes and no), gestational age at birth (weeks of gestation), and neonatal age (hours).

## 2.4 | Statistical analyses

Prevalence, means, standard deviations (SD), medians, and interquartile ranges (IQR) were used to describe the sample. Descriptive mean differences are presented with respective 95% confidence intervals.

Structural equation modelling was performed to evaluate the direct and indirect associations among maternal HOMA index, glucose, triglycerides, non-esterified fatty acids, and neonatal sum of skinfolds. Direct association refers to the association between two variables, whereas indirect association refers to the association between two variables that is mediated via a third variable. All the standard errors in the analyses were adjusted for the cluster structure of the data (individuals nested within site of recruitment). Table 1 describes the role of each variable in the diagram in detail, including the variables of interest and the adjustments. We have more than 80% statistical power to detect associations with small effect sizes ( $f^2 = 0.06$ ) considering the number of neonatal boys ( $n = 333$ ) and girls ( $n = 324$ ) evaluated, accounting for the number of exposures and confounding variables in our analysis. All analyses were performed in STATA version 15 for windows (StataCorp LP).

## 2.5 | Missing data

We used the maximum likelihood for missing values method, which does not exclude a participant in the analysis because of a missing value in one of the variables. Thus, we avoided selection bias in our analysis.<sup>21</sup> Therefore, all the 657 neonates and their mothers in the study were included in the main analysis.

## 2.6 | Ethics approval

We obtained the local ethics committee approvals in all the nine countries, in which the study was conducted. Moreover, written informed consent of all women was acquired.

## 3 | RESULTS

In total, 657 neonates (50.7% boys) and their mothers with obesity were included in the study. On average, the mothers were 32.0 ( $\pm 5.4$ ) years of age. Median gestational age at delivery was 39.7 (IQR: 38.7; 40.9) weeks of gestation. Most mothers perceived themselves as European descent (86.4%). Among the mothers, 55.9% reported a high educational level and 31.8% a medium educational level. Median neonatal age at measurement was 5.0 (IQR: 2.0; 24.0) hours after birth. Boys exhibited a lower sum of skinfolds (mean sum of skinfolds 20.3 mm, 95% CI 19.7, 21.0) compared to girls (mean sum of skinfolds 21.5 mm, 95% CI 20.8, 22.2). Table 2 presents descriptive data on the mothers' metabolic variables at <20 weeks of gestation according to the sex of the fetus.

## 3.1 | Maternal insulin resistance in relation to neonatal adiposity

In boys, maternal HOMA-IR at <20 weeks of gestation was directly associated with neonatal sum of skinfolds ( $\beta = 0.35$  mm, 95% CI 0.01, 0.70). No other associations of maternal HOMA-IR with neonatal sum of skinfolds in boys were found (see Table 3). In girls, maternal HOMA-IR was not directly associated with neonatal sum of skinfolds at any time during pregnancy. However, maternal HOMA-IR at 24-28 weeks of gestation was indirectly associated with neonatal sum of skinfolds ( $\beta = 0.26$  mm, 95% CI 0.08, 0.44).

Figure 1 illustrates the indirect association of maternal HOMA-IR at 24-28 weeks of gestation with neonatal sum of skinfolds only in girls because we did not observe an indirect association between maternal HOMA-IR and neonatal sum of skinfolds in boys. Note that not all the arrows were drawn for clarity. We only drew the most relevant arrows related to the primary and secondary outcomes of the present study. For more details regarding all the relationships tested in our diagram, see Table 1. The indirect association of maternal HOMA-IR at 24-28 weeks of gestation with neonatal sum of skinfolds indicates that a higher maternal HOMA-IR at 24-28 weeks initiates a chain of relationships through maternal fasting glucose, triglycerides, and NEFA at 24-28 weeks and HOMA-IR, fasting glucose, triglycerides, and NEFA at 35-37 weeks that culminates in a higher neonatal sum of skinfolds (Figure 1). The overall indirect association between maternal HOMA-IR at 24-28 weeks and neonatal sum of skinfolds was 0.26 mm (95% CI 0.06, 0.36), and the indirect association between maternal HOMA-IR at 24-28 weeks and neonatal sum of skinfolds only via maternal fasting glucose at 24-28 weeks was 0.21 mm (95% CI 0.06, 0.36), indicating that 81.0% of the indirect relationship between maternal HOMA-IR at 24-28 weeks and neonatal sum of skinfolds was mediated via fasting glucose at 24-28 weeks.

## 3.2 | Maternal fasting glucose, triglycerides, and non-esterified fatty acids in relation to neonatal adiposity

In boys, maternal triglycerides at <20 weeks were indirectly and negatively associated with neonatal sum of skinfolds ( $\beta = -0.49$  mm, 95% CI  $-0.97, -0.01$ ). Moreover, triglycerides at 24-28 weeks of gestation were directly and negatively associated with neonatal sum of skinfolds ( $\beta = -1.39$  mm, 95% CI  $-2.58, -0.21$ ). Maternal fasting glucose and NEFA were not associated with neonatal sum of skinfolds in boys at any time during pregnancy (Table 4).

In girls, maternal fasting glucose at <20 weeks was inversely and directly associated with neonatal sum of skinfolds ( $\beta = -2.52$  mm, 95% CI  $-4.86, -0.19$ ), whereas the indirect association was positive ( $\beta = 1.82$  mm, 95% CI 0.85, 2.79). Thus, the total association between maternal fasting glucose at <20 weeks and neonatal sum of skinfolds was not significant ( $\beta = -0.70$  95% CI  $-2.94, 1.54$ ).

**TABLE 1** Pathways between all the variables in the analysis

	Outcomes
<b>Exposures</b>	
HOMA-IR at 13-20 wk	HOMA-Index at 24-28 and 35-37 wk; Glucose at 13-20, 24-28 and 35-37 wk; Triglycerides at 13-20, 24-28 and 35-37 wk; Non-esterified fatty acids at 13-20, 24-28 and 35-37 wk; Neonatal fatness
HOMA-IR at 24-28 wk	HOMA-Index at 35-37 wk; Glucose at 24-28 and 35-37 wk; Triglycerides at 24-28 and 35-37 wk; Non-esterified fatty acids at 24-28 and 35-37 wk; Neonatal fatness
HOMA-IR at 35-37 wk	Glucose at 35-37 wk; Triglycerides at 35-37 wk; Non-esterified fatty acids at 35-37 wk; Neonatal fatness
Fasting glucose at 13-20 wk	Glucose at 24-28 and 35-37 wk; Neonatal fatness
Fasting glucose at 24-28 wk	Glucose at 35-37 wk; Neonatal fatness
Fasting glucose at 35-37 wk	Neonatal fatness
Triglycerides at 13-20 wk	Triglycerides at 24-28, 35-37 wk; Neonatal fatness
Triglycerides at 24-28 wk	Triglycerides at 35-37 wk; Neonatal fatness
Triglycerides at 35-37 wk	Neonatal fatness
Non-esterified fatty acids at 13-20 wk	Non-esterified fatty acids at 24-28, 35-37 wk; Neonatal fatness
Non-esterified fatty acids at 24-28 wk	Non-esterified fatty acids at 35-37 wk; Neonatal fatness
Non-esterified fatty acids at 35-37 wk	Neonatal fatness
<b>Confounders</b>	
Intervention groups	BMI at 13-20, 24-28 and 35-37 wk;
Gestational age	HOMA-Index at 13-20, 24-28 and 35-37 wk;
Maternal ethnicity	Glucose at 13-20, 24-28 and 35-37 wk;
Maternal education	Triglycerides at 13-20, 24-28 and 35-37 wk;
Maternal age	Non-esterified fatty acids at 13-20, 24-28 and 35-37 wk;
Smoking status	Neonatal fatness
BMI at 13-20 wk	BMI at 24-28 and 35-37 wk; HOMA-Index at 13-20, 24-28 and 35-37 wk; Glucose at 13-20, 24-28 and 35-37 wk; Triglycerides at 13-20, 24-28 and 35-37 wk; Non-esterified fatty acids at 13-20, 24-28 and 35-37 wk; Neonatal fatness
BMI at 24-28 wk	BMI at 35-37 wk; HOMA-Index at 24-28 and 35-37 wk; Glucose at 24-28 and 35-37 wk; Triglycerides at 24-28 and 35-37 wk; Non-esterified fatty acids at 24-28 and 35-37 wk; Neonatal fatness
BMI at 35-37 wk	HOMA-Index at 35-37 wk; Glucose at 35-37 wk; Triglycerides at 35-37 wk; Non-esterified fatty acids at 35-37 wk; Neonatal fatness
Neonatal age	Neonatal fatness
Gestational age at birth	

Maternal fasting glucose at 24-28 weeks was directly and positively associated with neonatal sum of skinfolds ( $\beta = 2.51$  mm, 95% CI 0.80, 4.23). Maternal NEFA at 35-37 weeks were directly and negatively associated with neonatal sum of skinfolds ( $\beta = -3.26$  mm, 95% CI  $-5.91$ ,  $-0.61$ ). Maternal triglycerides were not associated with neonatal sum of skinfolds in girls at any time during pregnancy (Table 4).

### 3.3 | Comment

#### 3.3.1 | Principal findings

The primary aim of this investigation was to evaluate the potential pathophysiological pathways underlying the association of maternal insulin resistance with neonatal adiposity in pregnant women

Maternal variables	<20 wk of gestation			
	Boys		Girls	
	n	Mean (95% CI)	n	Mean (95% CI)
HOMA-IR ( $[\mu\text{U}/\text{mL}] * [\text{mmol}/\text{L}]$ )	327	3.0 (2.7, 3.4)	315	3.3 (2.9, 3.6)
Fasting Glucose (mmol/L)	331	4.6 (4.6, 4.6)	320	4.6 (4.6, 4.7)
Triglycerides (mmol/L)	320	1.3 (1.3, 1.4)	307	1.4 (1.3, 1.4)
Non-esterified fatty acids (mmol/L)	320	0.6 (0.6, 0.7)	307	0.64 (0.6, 0.7)
BMI ( $\text{kg}/\text{m}^2$ )	333	34.4 (34.0, 34.8)	324	34.5 (34.0, 35.0)

**TABLE 2** Mean and 95% confidence intervals of the mothers' anthropometric and metabolic variables at <20 wk of gestation according to the sex of the fetus

Exposures	Neonatal sex	Neonatal sum of skinfolds (mm)		
		Direct association	Indirect association	Total association
HOMA-IR at < 20 wk	Boys	0.35 (0.01, 0.70)	-0.02 (-0.17, 0.14)	0.34 (0.01, 0.66)
	Girls	0.20 (-0.02, 0.43)	-0.05 (-0.18, 0.08)	0.15 (-0.08, 0.38)
HOMA-IR at 24-28 wk	Boys	-0.43 (-0.88, 0.03)	0.09 (-0.05, 0.22)	-0.34 (-0.83, 0.15)
	Girls	0.01 (-0.41, 0.41)	0.26 (0.08, 0.44)	0.26 (-0.09, 0.61)
HOMA-IR at 35-37 wk	Boys	0.05 (-0.12, 0.22)	0.05 (-0.03, 0.14)	0.10 (-0.11, 0.31)
	Girls	-0.01 (-0.12, 0.10)	0.07 (-0.01, 0.15)	0.06 (-0.05, 0.17)

Note: Indirect association refers to the association between the exposure (maternal HOMA-IR at different periods during pregnancy) and the outcome (neonatal sum of skinfolds) which is mediated by all the variables in the pathway (see Table 1 for detailed description).

with obesity. Since this is a longitudinal study, it is not possible to infer causality in our conclusions. Our results showed that the association between maternal insulin resistance and neonatal adiposity differed depending on the infant sex. While adiposity in boys was associated directly with maternal insulin resistance in the beginning of pregnancy, adiposity in girls was associated with maternal insulin resistance at 24-28 weeks of gestation via a chain of relationships through fasting glucose, triglycerides, and free fatty acids.

### 3.3.2 | Strengths of the study

Strengths of our study are that we have measures of maternal metabolic parameters at three time points in pregnancy, enabling us to look at longitudinal associations. Data were collected in nine European countries, increasing the external validity of our findings. Importantly, the mechanistic path analysis adds substantial insights on how some of the metabolic parameters during pregnancy in women with obesity could affect neonatal adiposity, although we cannot assert on causality and our model should be tested in clinical trials.

### 3.3.3 | Limitations of the data

Our findings should be interpreted with caution, since women were exposed to different interventions that could influence

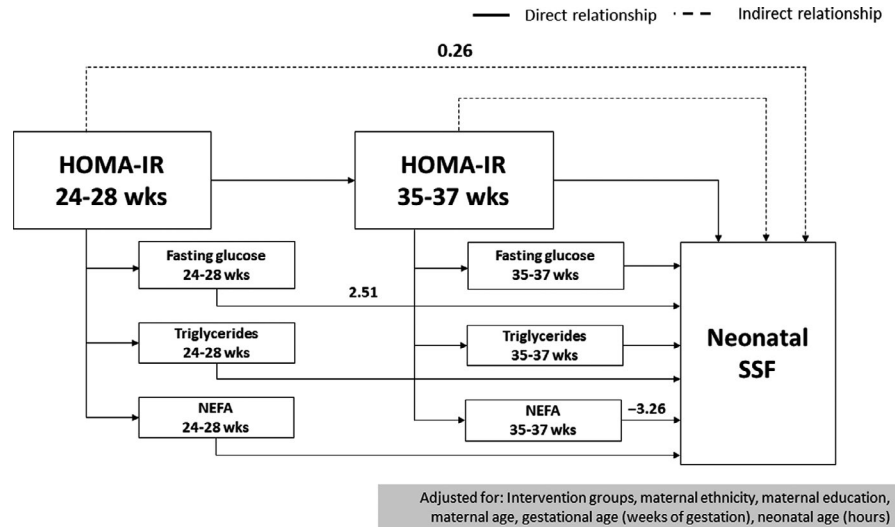
our results. However, the analyses were adjusted for the intervention component. Another limitation is the lack of normal or overweight women in the study; thus, we can only generalise our findings to Caucasian obese women. However, the prevalence and the increasing rate of obesity in women in reproductive age are alarming and so the complication related to obesity during pregnancy.<sup>22,23</sup> Furthermore, more direct measures of neonatal adiposity would have been preferred, such as from air displacement plethysmography, although sum of skinfolds provide valid estimates of adiposity in neonates.<sup>24,25</sup>

### 3.3.4 | Interpretation

Regarding the pathophysiological pathways on the relationship between maternal metabolism and neonatal adiposity, Shapiro et al<sup>7</sup> observed that 21.0% of the association between maternal pre-pregnancy BMI and neonatal adiposity was mediated by HOMA-IR and fasting glucose levels, but not by triglycerides or non-esterified fatty acids.<sup>7</sup> This is partly in line with our results, although Shapiro et al<sup>7</sup> included pregnant women with and without obesity whereas we only included pregnant women with obesity. We observed that fasting glucose mediated 81.0% of the association between maternal HOMA-IR and neonatal adiposity, but only in girls.

**TABLE 3** Path coefficients of the direct, indirect, and total association between maternal HOMA-IR and neonatal sum of skinfolds

**FIGURE 1** Paths in the association between maternal HOMA-IR at 24–28 wk and neonatal sum of skinfolds in girls. Fasting glucose, triglycerides, and non-esterified fatty acids (NEFA) at 24–28 wk were directly associated with fasting glucose, triglycerides, and NEFA at 35–37 wk, respectively. SSF refers to the neonatal sum of skinfolds (mm). Solid lines refer to direct relationships; dashed lines refer to indirect relationships



**TABLE 4** Path coefficients of the direct, indirect, and total association between maternal fasting glucose, triglycerides, non-esterified fatty acids, and neonatal sum of skinfolds

		Outcome: Sum of skinfolds (mm)		
		Boys		
Exposures, in mmol/l	Gestational age during pregnancy	Total association	Direct association	Indirect association
		$\beta$ (95% CI)	$\beta$ (95% CI)	$\beta$ (95% CI)
Fasting glucose	<20 wk	0.44 (-1.10, 1.98)	-0.31 (-2.06, 1.43)	0.75 (-0.60, 2.11)
	24-28 wk	0.68 (-1.36, 2.72)	0.09 (-1.86, 2.04)	0.59 (-0.38, 1.57)
	35-37 wk	1.22 (-0.75, 3.19)	1.22 (-0.75, 3.19)	
Triglycerides	<20 wk	1.12 (-0.59, 2.84)	1.61 (-0.31, 3.54)	-0.49 (-0.97, -0.01)
	24-28 wk	-0.87 (-1.58, -0.15)	-1.39 (-2.58, -0.21)	0.53 (-0.23, 1.28)
	35-37 wk	0.78 (-0.26, 1.81)	0.78 (-0.26, 1.81)	
Non-esterified fatty acids	<20 wk	1.01(-1.27, 3.28)	1.19 (-0.33, 2.71)	-0.18 (-1.92, 1.55)
	24-28 wk	-1.58 (-5.90, 2.75)	-1.77 (-6.17, 2.62)	0.20 (-0.38, 0.77)
	35-37 wk	1.32 (-2.56, 5.20)	1.32 (-2.56, 5.20)	
		Outcome: Sum of skinfolds (mm)		
		Girls		
Exposures, in mmol/l	Gestational age during pregnancy	Total association	Direct association	Indirect association
		$\beta$ (95% CI)	$\beta$ (95% CI)	$\beta$ (95% CI)
Fasting glucose	<20 wk	-0.70 (-2.94, 1.54)	-2.52 (-4.86, -0.19)	1.82 (0.85, 2.79)
	24-28 wk	2.84 (1.38, 4.30)	2.51 (0.80, 4.23)	0.33 (-0.24, 0.90)
	35-37 wk	0.71 (-0.49, 1.91)	0.71 (-0.49, 1.91)	
Triglycerides	<20 wk	0.80 (-0.30, 1.90)	0.43 (-1.31, 2.17)	0.37 (-0.99, 1.72)
	24-28 wk	0.01 (-1.47, 1.50)	-0.64 (-2.46, 1.19)	0.65 (-0.44, 1.74)
	35-37 wk	0.93 (-0.63, 2.49)	0.93 (-0.63, 2.49)	
Non-esterified fatty acids	<20 wk	1.73 (-0.88, 4.34)	1.47 (-0.38, 3.31)	0.26 (-1.07, 1.60)
	24-28 wk	2.23 (-1.92, 6.38)	3.02 (-0.99, 7.03)	-0.79 (-1.74, 0.16)
	35-37 wk	-3.26 (-5.91, -0.61)	-3.26 (-5.91, -0.61)	

Note: Indirect association refers to the association between the exposure and the outcome (neonatal sum of skinfolds) which is mediated by all the variables in the pathway (see Table 1 for detailed description). The empty cells on the indirect relationships between fasting glucose, triglycerides, non-esterified fatty acids at 35–37 wk of gestation and neonatal sum of skinfolds are due to the lack of possible indirect relationships to be estimated (there are no intermediate variable between these exposures and the outcome).





The direct association between maternal glucose and neonatal adiposity is well established in the literature.<sup>6,8-10</sup> Results from the Healthy Heart Study<sup>6,7</sup> and the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study<sup>26</sup> demonstrated the importance of maternal glucose levels for neonatal adiposity. In our sample only including pregnant women with obesity, we did not observe any relationship between maternal fasting glucose and neonatal adiposity in boys. In girls, maternal fasting glucose at 24-28 weeks was associated with neonatal adiposity. No relationship was observed between maternal fasting glucose at <20 weeks or at 35-37 weeks and neonatal adiposity. We encourage future investigations to evaluate this matter in more depth, with assessment of maternal glucose levels at multiple time points during pregnancy.

The importance of triglycerides and non-esterified fatty acids levels during pregnancy on neonatal adiposity is not well established.<sup>6,8,11</sup> When associations are found they are usually positive.<sup>27</sup> In our sample with pregnant women with obesity, maternal triglycerides were negatively associated with neonatal adiposity in boys at <20 weeks (indirectly) and at 24-28 weeks (directly). In girls, maternal non-esterified fatty acids at 35-37 weeks showed a negative association with neonatal adiposity. Pregnant women with obesity from DALI exposed to motivational sessions on healthy eating presented higher levels of NEFA, and higher levels of NEFA were also found in the cord blood of the offspring when compared to controls.<sup>28</sup> In addition, maternal diet has been shown to influence triglyceride and free fatty acid levels, even after a period of fasting.<sup>29</sup> Whether this influenced the association between lipids and neonatal adiposity is unknown. However, there seems to be a complex interaction between maternal glycaemia and lipids in their influence on fetal growth, since maternal lipids were only associated with fetal growth in women with hyperglycaemia but not in those with normal glycaemia.<sup>30</sup> Whether this is due to differences in insulin resistance and therefore maternal lipolysis needs further study.

Sex differences in the associations between maternal metabolism and neonatal adiposity have been reported previously, but are inconsistent.<sup>16,17,31</sup> Inconsistencies might be due to study population (ie women with obesity versus with normal weight, with normal glycaemia versus with hyperglycaemia) or due to differences in the timing in pregnancy. Theoretically, male and female fetuses seem to experience different growth strategies in utero.<sup>32,33</sup> It is not well established yet, but male embryos tend to have more rapid cell divisions compared to females,<sup>34</sup> generating a more rapid growth.<sup>32,33</sup> This higher growth rate might explain the higher response to changes in nutrient supply reported in male fetuses.<sup>32,33</sup> These different growth strategies might result in differences in nutritional needs, at different times in pregnancy and our findings support this concept. However, more systematic assessment of sex differences in the relationship of maternal metabolic parameters with neonatal adiposity is needed.

We evaluated the pathophysiological pathway from maternal insulin resistance, through maternal glucose, triglycerides, and non-esterified fatty acids to neonatal adiposity in pregnant women with obesity. It indicates again the relevance of the early pregnancy

period for neonatal outcomes in boys and in the middle of pregnancy for girls. Although our model explained most of the pathophysiological pathways from insulin resistance to neonatal adiposity in girls, the pathways in boys were still uncertain. A next step would be to investigate the associations of maternal metabolism with cord blood parameters, to confirm the Pedersen-Freinkel hypothesis that maternal glucose and lipids increase fetal insulin levels and thereby increase fetal fat accretion.<sup>35</sup>

## 4 | CONCLUSIONS

Maternal insulin resistance in women with obesity was associated with adiposity in neonates. More specifically, higher insulin resistance in the beginning of pregnancy was related to higher adiposity in boys, whereas this association in girls was observed with insulin resistance in the middle of the pregnancy period. Obstetricians, midwives, and health professionals should be aware of the deleterious impact of maternal insulin resistance on the offspring health and support pregnant women with life style changes (nutrition and physical activity) which could lower insulin resistance during pregnancy and consecutively benefit neonatal body composition.<sup>36</sup>

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