Supplementary File

S1 Sample size calculation

We powered the study for the potentially least associated maternal metabolic risk factor (triglycerides) for birthweight. Knopp et al. reported a correlation between maternal triglycerides and birthweight of r=0.09 ($p<0.05$) in non-GDM women.(1) We conservatively assumed an effect size of 0.08. STATA 14.0 was used to calculate the sample size. After using 'Fisher's z tests comparing one correlation to a reference value' tool, a sample of 1225 will give 80% power to detect a correlation of 0.08 at 5% significance level (two sided). We conservatively assumed 20% attrition rate due to missing data and loss to follow up, thus giving a sample size of 1,531.

Wang J, *et al. BMJ Open* 2022; 12:e064122. doi: 10.1136/bmjopen-2022-064122

S2 Testing for degradation for cord blood insulin

No prior literature reported the impact of long-term -80 °C storage on plasma insulin. We therefore fitted a regression model to detect potential degradation for cord blood insulin. The median storage duration of cord blood sample is 488 (IQR 394 to 707) days. Cord blood insulin was found to be slightly degraded over time (r=-0.07, p=0.01). In the multivariate regression model, we included sample storage time as a covariate. In the additive Bayesian Network analysis, adjustments were made to account for any degradation by correcting the initial value using linear regression methods (adjusted cord blood insulin = initial value of cord blood insulin + (mean value of sample storage time – sample storage time) * β, β=-0.0044446).

S3 Adjusting gestational age at sampling for maternal lipid profile

The average sampling time for maternal overnight fasting blood sample at the second trimester was 20.46 gestation weeks. The table below shows the estimates of associations between maternal plasma lipid levels and gestational age when blood sampling was carried out.

lipids	Regression coefficient (β)
Total cholesterol	0.098
HDL-C	0.015
LDL-C	0.075
Triglycerides	0.059

Linear regression model

Adjustment Equation: Adjusted lipids = initial value + (20.46 – sampling time) * β

Wang J, *et al. BMJ Open* 2022; 12:e064122. doi: 10.1136/bmjopen-2022-064122

S4 Additive Bayesian Network methodologies

Introduction to Additive Bayesian Network analysis

A Bayesian network is a probabilistic graphical model that represents a set of variables and their conditional dependencies via a directed acyclic graphs (DAGs).(2) It is a well-established unsupervised machine learning methodology that is typically referred to as structure discovery model for dealing with multidimensional data.(3) Unlike other widely used multivariate approaches, such as principal component analysis, propensity score matching analysis and multivariable regression model, graphical modelling does not involve any dimension reduction. Most graphical models, including path analysis and structural equation modelling, rely on a pre-specified structure, whereas Bayesian network is entirely data-driven.

Unlike the contingency table parameterization in standard Bayesian network models, Additive Bayesian networks (ABN) allow us to obtain interpretable DAGs where each node in graph comprises a generalized linear model (GLM) or a generalized linear mixed model (GLMM, if binary variable involved).(4, 5) There are two mutually dependent parts in ABN model: a network structure (i.e. the DAG) and a set of parameters. Each node (corresponding to the variables in the dataset) in the DAG is the equivalent of a potential dependent variable in a Bayesian GLM or GLMM regression model. While other DAG nodes where relevant as identified by the unsupervised learning act as covariates, having a role of corresponding parameters. Therefore, an ABN model is ideally suited to analysing highly complex epidemiological data comprising many inter-dependent variables.

The technical process of ABN

After an initial data preparation phase we used a three-step procedure to determine an optimal DAGs for our data.

Step 0 Data pre-processing

Ten variables were chosen for ABN based on our knowledge gained from prior literature and findings of the classical statistical analyses. These included maternal age, maternal pre-pregnancy BMI, maternal fasting glucose concentration in OGTT, early gestational weight gain (GWG, adjusted for gestational age at weight measurement), maternal fasting plasma high-density lipoprotein cholesterol (HDL-C, adjusted for gestational age at blood sampling) in 2nd trimester, maternal fasting plasma triglycerides in 2nd trimester (adjusted for gestational age at blood sampling), birthweight Z-Score (adjusted for gestational age at delivery and neonatal gender), cord blood insulin (CBI, adjusted for sample storage duration) concentration, gestational age at delivery, and neonatal gender. All continuous variables were standardized to Z-Scores to eliminate the influence of different measurement units (maternal triglycerides and cord blood insulin were log-transformed before standardization). Participants with data missing for at least one of these ten variables (6% of participants) were excluded from the analysis. The number of mother-child pairs that was finally included in ABN analysis is 1,429.

Step 1 Identification of the optimal model

The identification of the single optimal model is referred to as structure discovery. The purpose of this step is to combine all individual GLMs into a single, probabilistically cohesive model describing all the inter-dependent relationships via a DAG. We blocked all directions of arcs between variables that are biologically impossible to occur. This was done using the adjacency matrix in figure S1.

```
ban <- matrix( c(
  # 01 02 03 04 05 06 07 08 09 10
     0, 1, 1, 1, 1, 1, 1, 1, 1, 1, # 01 mage
     0, 0, 1, 1, 1, 1, 1, 1, 1, 1, # 02 prebmi
     0, 0, 0, 0, 0, 0, 1, 1, 1, 1, # 03 gwg
     0, 0, 0, 0, 0, 0, 1, 1, 1, 1, # 04 glu
     0, 0, 0, 0, 0, 0, 1, 1, 1, 1, # 05 hdl
     0, 0, 0, 0, 0, 0, 1, 1, 1, 1, # 06 tg
     0, 0, 0, 0, 0, 0, 0, 0, 0, 0, # 07 bwz
     0, 0, 0, 0, 0, 0, 0, 0, 0, 0, # 08 ins
     1, 1, 1, 1, 1, 1, 1, 1, 0, 1, # 09 sex
     0, 0, 0, 0, 0, 0, 0, 0, 0, 0 # 10 gaw
), byrow=TRUE, ncol=10)
```

Variable labels explanation: 01 mage, maternal age; 02 prebmi, maternal pre-pregnancy BMI; 03 gwg, gestational weight gain; 04 glu, maternal fasting glucose level; 05 hdl, maternal plasma high-density lipoprotein cholesterol level; 06 tg, maternal plasma triglyceride level; 07 bwz, birthweight Z-Score; 08 ins, cord blood insulin; 09 sex, neonatal gender; 10 gaw, gestational age at delivery. Same labels also apply to the numbers across the top of the matrix.
DAG definition. Rows are children nodes, columns are parent nodes. 1 represents block from parent node (column) towards child node (row), 0 represents unblock.

Figure S1 ABN block matrix definition

To find the DAG with the best goodness of fit (network score - log marginal likelihood), exact searches were conducted across the parent limits (the limit number of arcs from parent nodes to child node), starting from a minimum of 1 and reaching a maximum of 9. As shown in Figure S2, we found that the goodness of fit (maximum marginal likelihood=-19153.30) does not improve when the number of parent limit is greater than 4.

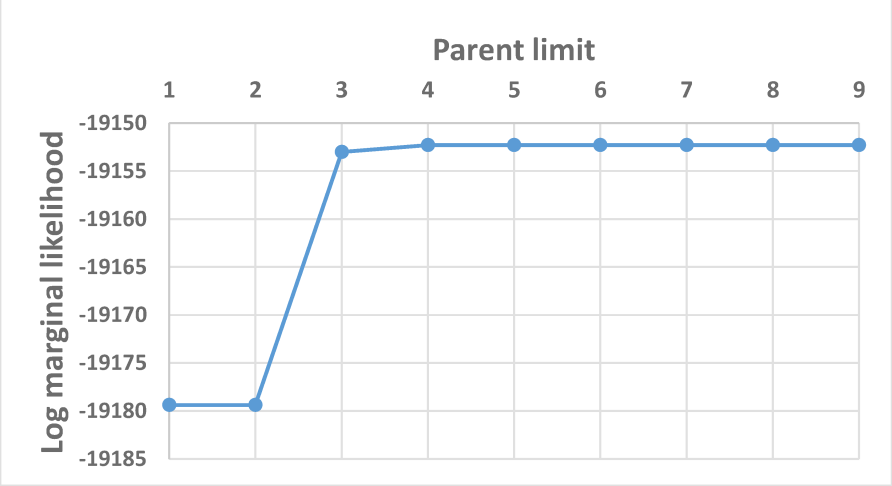
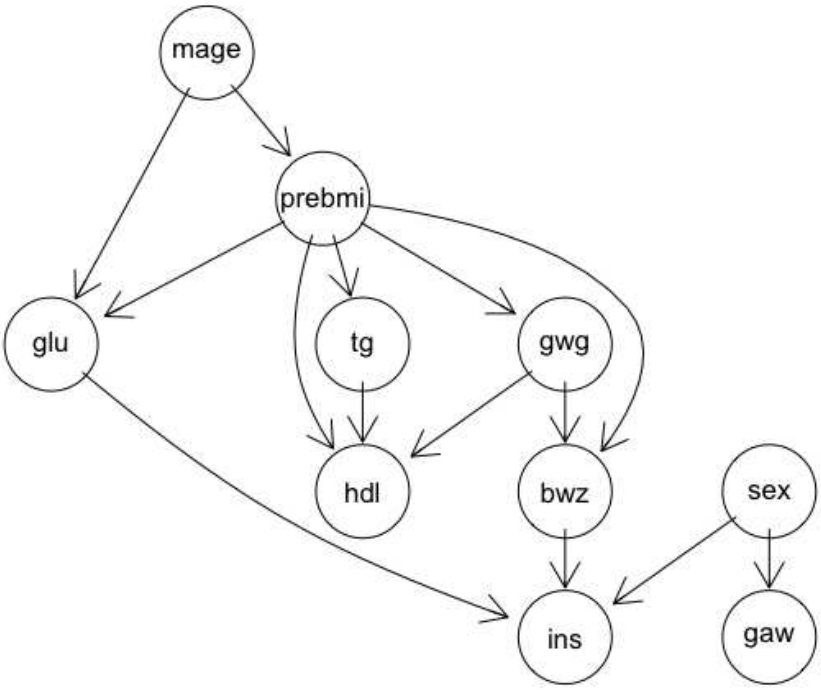


Figure S2 Comparison of goodness of fits for different parent limits



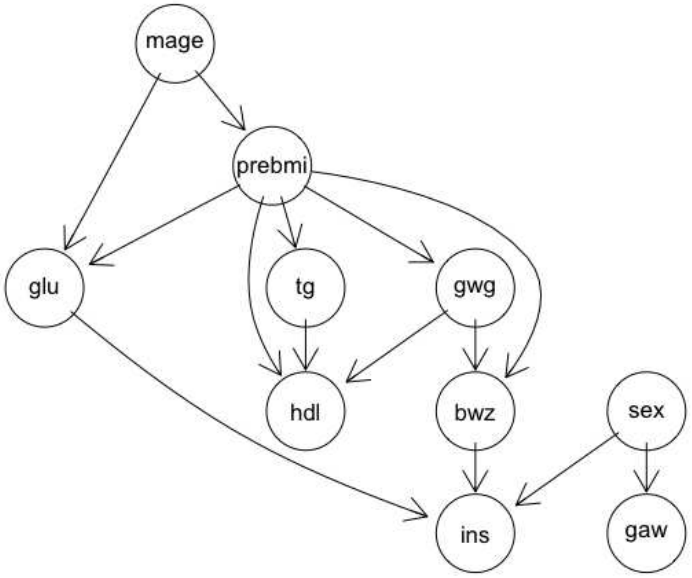
Variables explanation: mage, maternal age; prebmi, maternal pre-pregnancy BMI; gwg, gestational weight gain; glu, maternal fasting glucose level; hdl, maternal plasma high-density lipoprotein cholesterol level; tg, maternal plasma triglyceride level; bwz, birthweight Z-Score; ins, cord blood insulin; sex, neonatal gender; gaw, gestational age at delivery.

Figure S3 The identified optimal DAG from the initial search

Step 2 Adjustment for overfitting: parametric bootstrapping

We have identified the optimal DAG, but there is a risk of overfitting because of the combinatoric nature of Bayesian hypotheses. To address this, 12,800 independent parametric bootstrapping analyses were performed. This involves simulating data sets of the same size as the original dataset, and see how often the different structural features are recovered. Arcs present in less than 50% frequencies of the globally optimal DAGs estimated from the bootstrap data were considered not to be robust and need to be trimmed (removed) from the DAG generated in the first step.

The resulting optimal summary network was inferred from data with a total of 14 high-confidence arcs across 10 variables (Figure S3). The DAGs presented using pruning at 50% was constructed from 12,800 searches with a parent limit of four parents per node. Collating results across these 12,800 searches, all 14 arcs were recovered for at least 12,742 times, as resulting from the frequencies matrix at Figure S4.



Variables explanation: mage, maternal age; prebmi, maternal pre-pregnancy BMI; gwg, gestational weight gain; glu, maternal fasting glucose level; hdl, maternal plasma high-density lipoprotein cholesterol level; tg, maternal plasma triglyceride level; bwz, birthweight Z-Score; ins, cord blood insulin; sex, neonatal gender; gaw, gestational age at delivery.

Figure S4 Optimal final DAG (Containing 14 arcs after removal of arcs supported at less than 50% in bootstrapping)

```
> total.dag
        mage prebmi   gwg   glu hdl    tg   bwz ins   sex gaw
mage       0      0     0     0   0     0     0   0     0   0
prebmi 12800      0     0     0   0     0     0   0     0   0
gwg        0  12800     0     0   0     0     0   0     0   0
glu    12800  12800     0     0   0     0     0   0     0   0
hdl        0  12800 12742     0   0 12799     0   0     0   0
tg         0  12800     0     0   0     0     0   0     0   0
bwz        0  12800 12800     0   0     0     0   0     0   0
ins        0      0     0 12800   0     0 12800   0 12800   0
sex        0      0     0     0   0     0     0   0     0   0
gaw        0      0     0     0   0     0     0   0 12800   0
```

Variables explanation: mage, maternal age; prebmi, maternal pre-pregnancy BMI; gwg, gestational weight gain; glu, maternal fasting glucose level; hdl, maternal plasma high-density lipoprotein cholesterol level; tg, maternal plasma triglyceride level; bwz, birthweight Z-Score; ins, cord blood insulin; sex, neonatal gender; gaw, gestational age at delivery.
Rows are children nodes, columns are parent nodes. The number in each cell represents the frequencies at which each arc (from parent node towards child node) was recovered during 12,800 times of bootstrapping.

Figure S5 Frequencies at which each arc in the original DAG was recovered during bootstrapping

Step 3 Estimating marginal from the final DAG

Once the optimal DAG has been identified, we need to examine the strength of the various arcs in our analysis. This process is very similar to when estimating the marginal for the bootstrapping.

Table S1 Association of other maternal metabolic risk factors with birth weight, cord blood insulin level, and the risk of LGA/SGA.

	Pre-pregnancy BMI (Kg/m^2)	Early GWG (Kg)	Glucose (mmol/L)	TC (mmol/L)	LDL-C (mmol/L)
Regression Coefficients(95%CI)					
Birthweight(g)[#]	**29.25 (22.77, 35.73)**	**18.75 (13.06, 24.43)**	**84.32 (42.65, 125.98)**	-0.42 (-19.97, 19.12)	-0.24 (-22.82, 22.34)
Cord blood insulin[##](μU/mL)	0.2 (-0.02, 0.42)	0.08 (-0.11, 0.27)	**2.23 (0.89, 3.57)**	-0.15 (-0.77, 0.48)	-0.15 (-0.88, 0.58)
Odds Ratio (95%CI)					
LGA[§]	**1.24 (1.15, 1.32)**	**1.12 (1.04, 1.20)**	**2.06 (1.31, 3.24)**	1.00 (0.79, 1.25)	1.01 (0.78, 1.31)
SGA[§]	**0.86 (0.78, 0.94)**	0.94 (0.87, 1.00)	0.72 (0.44, 1.18)	0.91 (0.72, 1.15)	0.98 (0.75, 1.27)

BMI, body mass index; GWG, gestational weight gain; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; LGA, large-for-gestational age; SGA, small-for-gestational age.

[#] Adjusted for maternal age, ethnic group, parity, gestational age, neonatal sex, and early pregnancy cigarette exposures. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy. Maternal fasting status was further adjusted for TC and LDL-C.

[##] Adjusted for maternal age, ethnic group, parity, gestational age, neonatal sex, early pregnancy cigarette exposures, delivery mode, and sample storage duration. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy. Maternal fasting status was further adjusted for TC and LDL-C.

[§] Adjusted for maternal age, ethnic group, parity, and early pregnancy cigarette exposures. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy. Maternal fasting status was further adjusted for TC and LDL-C.

Table S2 **Association of other maternal metabolic parameter Z-Scores with birth weight Z-Score and cord blood insulin Z-Score**

	Pre-pregnancy BMI Z-Score	GWG Z-Score	Glucose Z-Score
Birth weight Z-Score			
Model 1	**0.20(0.15, 0.24)**	**0.17(0.12, 0.22)**	**0.08(0.04, 0.12)**
Model 2	**0.20(0.15, 0.24)**	**0.16(0.11, 0.22)**	**0.04(0.00, 0.09)**
Cord blood insulin Z-Score			
Model 3	**0.10(0.05, 0.15)**	0.05(-0.01, 0.12)	**0.13(0.08, 0.18)**
Model 4	**0.08(0.03, 0.14)**	0.05(-0.02, 0.11)	**0.11(0.06, 0.16)**

BMI, body mass index; GWG, gestational weight gain; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides
Model 1: Adjusted for maternal age, ethic group, parity, and early pregnancy cigarette exposures. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.
Model 2: Model 1 + pre-pregnancy BMI Z-Score + GWG Z-Score + Glucose Z-Score + HDL-C Z-Score + TG Z-Score + gestational age of maternal weight measurements during pregnancy.
Model 3: Adjusted for maternal age, ethic group, parity, early pregnancy cigarette exposures, gestational age, neonatal sex, delivery mode, and sample storage duration. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy.
Model 4: Model 3 + pre-pregnancy BMI Z-Score + GWG Z-Score + Glucose Z-Score + HDL-C Z-Score + TG Z-Score + gestational age of maternal weight measurements during pregnancy.

Table S3 Sensitivity analysis of the association between maternal metabolic parameter Z-Score and birth weight Z-score and cord blood insulin Z-Score.

β(95%CI)	HDL-C Z-Score	TG Z-Score	Pre-pregnancy BMI Z-Score	GWG Z-Score	Glucose Z-Score
Birth weight Z-Score [#]					
Non-GDM	-0.04(-0.09, 0.01)	**0.13(0.08, 0.17)**	**0.20(0.15, 0.25)**	**0.18(0.12, 0.24)**	**0.06(0.01, 0.11)**
GDM	-0.07(-0.18, 0.04)	0.04(-0.09, 0.17)	**0.19(0.09, 0.29)**	0.11(-0.03, 0.25)	**0.16(0.07, 0.24)**
P for interaction	0.57	0.20	0.84	0.62	0.06
Lean	-0.02(-0.07, 0.02)	**0.11(0.06, 0.15)**	**0.23(0.16, 0.29)**	**0.15(0.09, 0.21)**	**0.08(0.03, 0.12)**
Overweight	-0.09(-0.22, 0.05)	0.13(-0.01, 0.27)	**0.20(0.03, 0.36)**	**0.13(0.01, 0.25)**	0.05(-0.07, 0.17)
P for interaction	0.36	0.75	0.73	0.79	0.67
Fasting	**-0.05(-0.10, -0.01)**	**0.12(0.08, 0.16)**	-	-	-
Non-fasting	0.01(-0.12, 0.14)	0.07(-0.07, 0.21)	-	-	-
P for interaction	0.39	0.50	-	-	-
Primiparous	-0.05(-0.09, 0.00)	**0.11(0.07, 0.16)**	**0.18(0.13, 0.22)**	**0.15(0.09, 0.21)**	**0.08(0.03, 0.12)**
Multiparous	-0.04(-0.13, 0.05)	**0.14(0.06, 0.22)**	**0.28(0.18, 0.37)**	0.12(-0.01, 0.25)	**0.11(0.01, 0.21)**
P for interaction	0.87	0.57	0.06	0.74	0.52
Before imputation	**-0.04(-0.09, -0.00)**	**0.12(0.08, 0.16)**	**0.20(0.15, 0.24)**	**0.18(0.12, 0.23)**	**0.08(0.04, 0.12)**
After imputation	**-0.05(-0.09, -0.00)**	**0.12(0.08, 0.16)**	**0.20(0.15, 0.24)**	**0.18(0.13, 0.23)**	**0.08(0.04, 0.12)**
Cord blood insulin Z-Score [##]					
Non-GDM	-0.03(-0.08, 0.03)	**0.06(0.01, 0.12)**	**0.03(0.01, 0.05)**	0.05(-0.01, 0.12)	**0.10(0.04, 0.16)**
GDM	-0.13(-0.26, 0.01)	0.03(-0.11, 0.17)	**0.14(0.03, 0.25)**	0.09(-0.07,0,26)	**0.19(0.09, 0.29)**
P for interaction	0.17	0.66	0.38	0.65	0.13
Lean	-0.03(-0.08, 0.03)	**0.07(0.02, 0.12)**	**0.15(0.07, 0.23)**	0.05(-0.01, 0.12)	**0.13(0.08, 0.19)**
Overweight	-0.12(-0.26, 0.03)	-0.03(-0.13, 0.13)	0.07(-0.09, 0.24)	0.10(-0.04, 0.25)	**0.11(0.00, 0.22)**
P for interaction	0.24	0.34	0.41	0.54	0.72
Fasting	-0.04(-0.09, 0.01)	**0.06(0.01, 0.11)**	-	-	-
Non-fasting	-0.01(-0.17, 0.14)	0.11(-0.04, 0.25)	-	-	-
P for interaction	0.73	0.56	-	-	-
Primiparous	-0.03(-0.08, 0.03)	**0.07(0.01, 0.12)**	**0.10(0.04, 0.15)**	0.06(-0.01, 0.13)	**0.14(0.09, 0.19)**
Multiparous	-0.09(-0.19, -0.00)	0.05(-0.05, 0.14)	**0.10(0.00, 0.20)**	-0.00(-0.12, 0.13)	0.09(-0.01,0.19)
P for interaction	0.26	0.68	0.89	0.39	0.42
Before imputation	-0.04(-0.09, 0.01)	**0.07(0.02, 0.12)**	**0.11(0.06, 0.16)**	**0.07(0.00, 0.13)**	**0.14(0.09, 0.19)**
After imputation	-0.04(-0.09, 0.01)	**0.06(0.01, 0.11)**	**0.10(0.05, 0.15)**	0.06(-0.01, 0.12)	**0.13(0.08, 0.18)**

[#] Adjusted for maternal age, ethic group, parity, early pregnancy cigarette exposures, and delivery mode. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy. Maternal fasting status was further adjusted for HDL-C and TG (except the analysis between fasting and non-fasting).

[##] Adjusted for maternal age, ethic group, parity, early pregnancy cigarette exposures, gestational age, neonatal gender, delivery mode, and sample storage duration. For gestational weight gain, model was further adjusted for pre-pregnancy BMI and gestational age of maternal weight measurements during pregnancy. Maternal fasting status was further adjusted for HDL-C and TG (except the analysis between fasting and non-fasting).

BMI, body mass index; GWG, gestational weight gain; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; GDM, gestational diabetes mellitus.

Table S4 Effect estimate of additive Bayesian network analysis.

Arcs	Effect estimate (β, 95%CI)	95% CI
Mage → prebmi	0.19	(0.14, 0.24)
Prebmi → gwg	-0.12	(-0.17, -0.06)
mage → glu	0.11	(0.06, 0.16)
Prebmi → glu	0.14	(0.09, 0.19)
Prebmi → hdl	-0.12	(-0.17, -0.07)
Gwg → hdl	0.09	(0.05, 0.14)
Tg → hdl	-0.33	(-0.38, -0.28)
Prebmi → tg	0.23	(0.18, 0.28)
Prebmi → bwz	0.27	(0.22, 0.32)
Gwg → bwz	0.17	(0.12, 0.22)
Glu → ins	0.12	(0.07, 0.17)
Bwz → ins	0.24	(0.19, 0.29)
Sex → ins	0.19	(0.09, 0.29)
Sex → gaw	0.20	(0.10, 0.31)

Variables explanation: mage, maternal age; prebmi, maternal pre-pregnancy BMI; gwg, gestational weight gain; glu, maternal fasting glucose level; hdl, maternal plasma high-density lipoprotein cholesterol level; tg, maternal plasma triglyceride level; bwz, birthweight Z-Score; ins, cord blood insulin; sex, neonatal gender; gaw, gestational age at delivery.

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