The interleukins IL-6 and IL-1Ra: a mediating role in the associations between BMI and birth weight?

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The biological mechanisms in the association between maternal body mass index (BMI) and birth weight are not well understood, but are likely to involve maternal plasma glucose levels and nutrient transport across the placenta, both important modulators of fetal growth. Adipose tissue contributes to circulating levels of interleukins that may affect glucose metabolism and possibly also placental transport of nutrients. We investigated possible mediating roles of Interleukin 6 (IL-6) and Interleukin 1 Receptor antagonist (IL-1Ra) in 208 pregnant women. Known and hypothesized dependencies between BMI in early pregnancy and fasting glucose, IL-1Ra and IL-6 at gestational weeks 30–32, and birth weight were specified in a path diagram. Standardized regression coefficients, expressing direct, indirect and total effects, were estimated by Bayesian path analysis. Mean (s.d.) BMI was 24.9 kg/m² (4.2) and mean (s.d.) birth weight 3748 g (454). The total effect of BMI on birth weight was 0.24 (95% credibility interval [CrI] [0.12, 0.36]). The direct effect of IL-1Ra on birth weight was not statistically significant, but significant effects of BMI on IL-1Ra (0.61, 95% CrI [0.51, 0.72]), of IL-1Ra on fasting glucose (0.17, 95% CrI [0.01, 0.34]) and of fasting glucose on birth weight (0.14, 95% CrI [0.01, 0.27]) implied an indirect pathway from BMI via IL-1Ra on birth weight. Approximately 20% of the effect of BMI on birth weight was mediated through IL-1Ra. For IL-6, no such effects were found. Our results indicate that IL-1Ra may be a mediator in the association between BMI and birth weight.

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Introduction

Birth weight is a result of a complex interaction between maternal, placental and fetal factors. Of the maternal factors, maternal body mass index (BMI) is a strong, independent and modifiable predictor of birth weight and has been estimated to account for roughly 10–20% of the variance in birth weight. While numerous studies have shown an association between maternal BMI and birth weight, fewer studies have addressed the issue of biological mediators in this association. Considering the increasing prevalence of maternal obesity and the long-term implications of birth weight on later health and disease, an understanding of how excess fat exerts effects on birth weight is important. There are at least two ways in which maternal BMI may affect fetal growth, by modifying nutrient availability or by modifying placental nutrient transport. Traditionally, the link between maternal BMI and birth weight has been attributed in large to maternal hyperglycemia and partly to other metabolic alterations associated with obesity, that is, changes in nutrient availability. However, the fact that BMI remains a significant determinant of birth weight, after correcting for glucose in traditional regression analysis and also in studies of glucose-tolerant women, indicates that other factors associated with maternal obesity are likely to play a role in fetal growth. Studies of non-pregnant populations as well as animal experiments suggest a role of adipose tissue-derived inflammatory factors like interleukins (IL-6 and IL-1Ra), tumor necrosis factor (TNF) and other adipocytokines as molecular links between excess adipose tissue and deranged glucose metabolism, including increased insulin resistance. The few studies concerning inflammatory factors and insulin resistance in pregnancy indicate that at least some of the same mechanisms are present during gestation.

Direct effects of adipose tissue-derived factors on placental properties have also been suggested. There is evidence of maternal obesity affecting placental size and inflammatory properties and preliminary data suggest that placental nutrient transport capacity may be directly affected by interleukins like...
IL-6. Thus, adipokines may have a role as mediators in the associations between maternal fat mass, maternal glucose levels, placental properties and birth weight.

We studied whether the interleukins IL-6 and IL-1Ra had mediating roles in the association between BMI and birth weight. There are conflicting data about the association between TNF and BMI. We therefore chose not to include TNF as a potential mediator. Known and hypothesized dependencies between the chosen variables were depicted in a path diagram. Effect sizes were estimated by path analysis, which is a method where several multiple regression equations are combined to obtain estimates of direct and indirect effects. We used data from healthy pregnant women without infections, sampled from a Norwegian cohort study. To our knowledge, previous studies have not used path analysis in testing for inflammatory factors as mediators in the association between maternal BMI and birth weight.

Methods

The present work was performed in a subsample of the STORK study. STORK is a prospective cohort study of healthy women of Scandinavian heritage who registered for obstetric care at Oslo University hospital Rikshospitalet from 2002 to 2008 (n = 1030). Exclusion criteria were multiple pregnancies, known pre-gestational diabetes and severe chronic diseases (lung, cardiac, gastrointestinal or renal). The women were scheduled for four examinations at gestational weeks 14–16, 22–24, 30–32 and 36–38. Maternal height was measured at the first visit and weight at each visit. Fasting glucose was measured at weeks 14–16 and 30–32. Data on age, parity, educational level, smoking status and pregestational BMI were registered. Gestational age was based on ultrasound measures made at weeks 17–19. Data on preeclampsia and hypertension were obtained from hospital records. Birth weight was measured with a calibrated scale.

The present subsample included 240 women from the first part of the STORK cohort (n = 553), enrolled during the period 2002–2005 (Fig. 1). Inflammatory markers were obtained from fasting blood samples at all four visits. A subsample was chosen due to the limited resources for cytokine and other biochemical analyses. Placental insufficiency may be associated with inflammatory changes, and the subsample was therefore restricted to women giving birth to a baby above the 10th birth weight percentile (2962 g) of the cohort. Stratified random sampling based on birth weight below or above 4200 g was used to ensure that women with macrosomic babies were included. Women with possible infections indicated by a C-reactive protein (CRP) value above 10 mg/l, extreme values on IL-1Ra or IL-6 (values beyond 3 S.D. in log scale), or missing data of any variable in the path analysis were excluded from the analyses. The final study sample comprised 208 women (Fig. 1).

The study was approved by the Regional Committees for Medical Research Ethics and all women gave their written informed consent.

Blood sampling and biochemical measurements

The blood samples were drawn in the morning, between 0730 and 0830 after an overnight fast, and were obtained from vein puncture in tubes containing ethylenediaminetetraacetic acid (EDTA). Plasma glucose was measured immediately in EDTA blood by Accu Chek Glucose Test strips (Roche Diagnostics, Switzerland)
Basel, Switzerland). The samples were immediately put on ice and plasma isolated, and stored at −80°C until analyzed. IL-6 (high sensitivity) and IL-1Ra were measured by ELISA using commercially available kits (BIOSOURCE, Invitrogen Corporation). CRP was measured as described by Wu et al.24 All samples were measured in duplicate and serial samples from a given individual were analyzed at the same time to minimize the run-to-run variability. Intra- and inter-assay coefficients of variation were <10% for all assays.

**Statistical methods**

Descriptive statistics are presented as mean and standard deviation (S.D.), frequency and percentage (%) or as median and quartiles. Independent samples t-tests were used to compare the study sample (n = 208) and the remaining eligible women in the STORK cohort (n = 258, Fig. 1). Descriptive analyses and t-tests were performed by SPSS version 15.

Analytical methods in clinical research often rely on multiple regression models with one main outcome variable and explanatory variables treated on equal terms. Path analysis, in contrast, is a multivariable method based on a model with several linked regression equations.20 Within this system of equations, some of the variables can be considered both as outcome variables and as explanatory variables. Path analysis is a form of structural equation modeling that requires that all hypothesized dependencies between the variables are specified in a model and depicted in a path diagram, prior to the analysis. Arrows in a path diagram represent dependencies between variables, and absence of an arrow between two variables indicates that these variables are considered statistically independent in the model. All direct and indirect relations among measured variables can be read off the path diagram.

Based on the literature, we constructed a path diagram for this study, which specified the hypothesized biological pathways between BMI in early pregnancy (weeks 14–16) and fasting glucose, IL-1Ra and IL-6 at weeks 30–32 (Fig. 2). The effect of BMI on birth weight was decomposed into a direct effect and indirect effects. The indirect pathways were hypothesized to be mediated by fasting glucose, the interleukins IL-1Ra or IL-6 or a combination of these. BMI was measured in the first trimester, fasting glucose and interleukins at weeks 30–32 and gestational age at birth. Arrows represent dependencies between variables. Absence of an arrow between two variables indicates that the variables are considered to be statistically independent in the model.

Path analyses were performed using Bayesian estimation procedures,28 with the R2WinBUGS package,29 that runs WinBUGS30 from the statistical software R. Bayesian estimation gives estimates of regression coefficients and corresponding credibility intervals (CrIs), which are comparable with frequentistic confidence intervals. Considerations of statistical significance were based on the coverage of the credibility intervals. Comparison of models was carried out by the deviance information criterion (DIC); lower numbers of DIC are preferable.31 Further details of the Bayesian model specification and model fitting can be found in Appendix A.

**Results**

Characteristics of the study sample are shown in Table 1. The study sample was not statistically different from those not selected to the present substudy (0.08 < P < 0.95), except from a significantly lower gestational age at visit 3 in the study sample (means 30.8 and 31.0 weeks, respectively, P = 0.05). Gestational age at birth ranged from 37.0 to 42.1 weeks. No significant bivariate correlations between gestational age at birth and BMI, fasting glucose, IL-1Ra or IL-6 were found in our study sample (results not shown).

Path analysis estimates of the model shown in Fig. 2, expressed as standardized regression coefficients and CrIs, are shown in Table 2. Gestational age at birth, maternal BMI in the early second trimester and fasting glucose in late pregnancy all had significant direct effects on birth weight. The strongest of these, with a standardized regression coefficient of 0.40 (95% CrI [0.28, 0.52]), was found for gestational age. This value implies that an increase of 1 S.D. in gestational age (1.2 weeks) gives a mean increase of 0.40 S.D. in birth weight (182 g). An alternative interpretation is that gestational age
Table 1. Sample characteristics

| Sample characteristic                  | Study sample (n = 208) | STORK cohort BW ≥ 2962 g, not selected for present substudy (n = 258) |
|----------------------------------------|------------------------|---------------------------------------------------------------------|
| Pre-gestational                        |                        |                                                                     |
| BMI (kg/m²; self-reported)             | 23.6 (3.7)             | 23.7 (3.9)                                                          |
| Para 0                                 | 98 (47%)               | 136 (53%)                                                          |
| University education                   | 172 (83%)              | 219 (85%)                                                          |
| Visit 1 (weeks 14–16)                  |                        |                                                                     |
| Gestational age (weeks)                | 15.7 (1.4)             | 15.7 (1.4)                                                          |
| Daily smokers                         | 5 (2%)                 | 11 (4%)                                                             |
| Age of mother (years)                  | 31.3 (3.9)             | 31.2 (4.2)                                                          |
| BMI (kg/m²)                            | 24.9 (4.2)             | 25.0 (4.2)                                                          |
| Fasting glucose (mmol/l)               | 4.2 (0.5)              | 4.2 (0.5)                                                           |
| Visit 3 (weeks 30–32)                  |                        |                                                                     |
| Gestational age (weeks)                | 30.8 (1.2)             | 31.0 (1.1)                                                          |
| Fasting glucose (mmol/l)               | 4.4 (0.5)              | 4.5 (0.5)                                                           |
| IL-1Ra (pg/ml; median) [Q1,Q3]         | 165 [136, 212]         | NA                                                                  |
| IL-6 (pg/ml; median) [Q1,Q3]           | 0.18 [0.10, 0.33]      | NA                                                                  |
| Preeclampsia                           | 4 (2%)                 | 10 (4%)                                                             |
| Hypertension                           | 4 (2%)                 | 4 (2%)                                                              |
| Birth                                  |                        |                                                                     |
| Gestational age (weeks)                | 39.8 (1.2)             | 39.9 (1.4)                                                          |
| BW (g)                                 | 3748 (454)             | 3730 (451)                                                          |
| Boys                                   | 114 (55%)              | 138 (53%)                                                           |

BW, birth weight; BMI, body mass index.

Selected characteristics of the study sample (n = 208) and 258 women from the STORK cohort who were not selected for this study. The numbers are mean (s.d.) or frequency (%) unless otherwise stated.

a Complete data on BMI at visit 1, fasting glucose, IL-1Ra and IL-6 at Visit 3 and BW and gestational age at birth. Other numbers may vary due to missing values.

b Complete data on BW and gestational age at birth. Other numbers may vary due to missing values.

c More than 1 cigarette/day.

Table 2. Path analysis

| Outcome variablea | Effect                              | B     | 95% CrI |
|-------------------|-------------------------------------|-------|---------|
| BW                | Direct effect gestational age → BW  | 0.40  | 0.28    | 0.52    |
|                   | Direct effect BMI → BW              | 0.16  | 0.00    | 0.32    |
|                   | Direct effect glucose → BW          | 0.14  | 0.01    | 0.27    |
|                   | Direct effect IL-1Ra → BW           | 0.06  | −0.10   | 0.21    |
|                   | Direct effect IL-6 → BW             | −0.02 | −0.14   | 0.11    |
| Fasting glucose   | Direct effect BMI → fasting glucose | 0.22  | 0.05    | 0.39    |
| IL-1Ra            | Direct effect BMI → IL-1Ra          | 0.61  | 0.51    | 0.72    |
| IL-6              | Direct effect BMI → IL-6            | 0.10  | −0.01   | 0.21    |

BW, birth weight; BMI, body mass index; CrI, credibility intervals.

Results from the path analysis illustrated in Figure 2. The table shows direct effects with corresponding CrI for the paths depicted in the figure, as well as the total effect of BMI on BW.

a BMI was measured in first trimester, fasting glucose and interleukins at weeks 30–32, and gestational age at birth.
accounts for 40% of the total variation in birth weight. The estimated direct effects for BMI and fasting glucose were 0.16 (95% CrI [0.00, 0.32]) and 0.14 (95% CrI [0.01, 0.27]), respectively.

The estimated direct effect of BMI on fasting glucose was also significant (0.22, 95% CrI [0.05, 0.39]), implying an indirect effect of BMI on birth weight mediated through glucose. An estimate of this indirect effect can be calculated from the direct effects of BMI on fasting glucose and fasting glucose on birth weight: 0.22 · 0.14 = 0.03.

There was no significant direct effect of IL-1Ra on birth weight. The effect of IL-1Ra on fasting glucose, however, was significant (0.17, 95% CrI [0.00, 0.34]), implying an indirect effect of IL-1Ra on birth weight. An estimate of this indirect effect can be calculated from the direct effects involved: 0.17 · 0.14 = 0.02. Hence, the effect of BMI on birth weight mediated through IL-1Ra was split into one path involving IL-1Ra only (estimated effect 0.61 · 0.06 = 0.03) and one path via IL-1Ra and fasting glucose (estimated effect 0.61 · 0.17 · 0.14 = 0.02). In total, the estimated effect of BMI involving IL-1Ra was 0.05 (95% CrI [−0.05, 0.15]).

No significant direct effect of IL-6 on birth weight was found. In addition, the effect of IL-6 on IL-1Ra was not significant, indicating neither direct nor indirect effect of IL-6 on birth weight. The direct effect of BMI on IL-6 was significant (0.17, 95% CrI [0.03, 0.31]), but the indirect effect of BMI via IL-6 on birth weight was negligible in comparison to the other effects estimated in the model (<0.01, calculations not shown).

The total effect of maternal BMI on birth weight was estimated to be 0.24 (95% CrI [0.12, 0.36]). The total effect is the sum of the direct effect (0.16) and indirect effects via glucose only, via IL-1Ra only, via IL-1Ra and glucose and via IL-6 (Fig. 2), calculated above to be 0.03, 0.03, 0.02 and <0.01, respectively. The decomposition of the total effect of BMI on birth weight is emphasized in Fig. 3. The figure shows the relative percentages of the total BMI effect, through the different pathways in the model. Approximately 20% (0.05/0.24) of the total BMI effect worked through paths involving IL-1Ra, whereas a negligible percentage (<1%) involved IL-6. Approximately 13% (0.03/0.24) of the effect worked through glucose without involving IL-1Ra. The remaining 67% (0.16/0.24) of the BMI effect represent effects not explained by variables or structures in our model.

Considering the above results, a reduced model without IL-6 was formulated and the corresponding effects estimated (results not shown). DIC decreased substantially (from 2193 to 1603). The large reduction was mostly attributable to the weak association between BMI and IL-6, as the predictive capabilities of the model as a whole improves when leaving IL-6 out of the model. The effect of IL-1Ra on fasting glucose was not affected by the changes in the model. The direct effect of IL-1Ra on birth weight was still not significant, and the proportion of the total effect of BMI mediated through IL-1Ra was still approximately 20%.

**Discussion**

There are numerous reports on the effect of maternal BMI on birth weight, but the biological mechanisms behind this association still remain to be elucidated. We have studied the possible mediating role of interleukins (IL-6 and IL-1Ra) in the association between BMI and birth weight. Path analysis indicated a mediating role of IL-1Ra, but less impact of IL-6.

The use of path diagrams and analysis of structural models is expanding in the field of epidemiology, including studies of pregnancy outcome, and there is a need for biological understanding. The crucial task in path analysis is to formulate a plausible path diagram based on existing evidence and current biological concepts. Maternal BMI may modify both nutrient availability and nutrient transport. Maternal BMI is a strong determinant of glucose plasma levels, and might thus indirectly affect birth weight through increasing nutrient availability for fetal growth. We chose to include fasting glucose in the path diagram as some studies, including ours, indicate fasting glucose to correlate more strongly with both BMI and birth weight than the 2 h glucose value. Late gestation glucose levels were included in the diagram, that is, when fetal growth is at its maximum.

In recent years, a growing body of literature has established a relation between BMI and low-grade systemic inflammation. There is increasing evidence that the same relation is present during pregnancy. Adipose tissue-derived inflammatory factors, including interleukins, have received considerable attention as potential mediators in the link between excess fat and the dysregulation of glucose metabolism including increased insulin resistance in obesity. Our selection of potential inflammatory mediators was based on several considerations. In general, data from the non-pregnant population indicate that IL-6 and IL-1Ra are both central interleukins and interact with each other. They are upstream markers of inflammation, which are both elevated in obesity and have been implicated in glucose regulation in

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**Fig. 3.** The figure visualizes the decomposition of the total effect of maternal BMI on birth weight. The total effect is the sum of all arrows, that is, the direct and indirect effects. The arrow widths represent the relative proportions of the total effect through a specific pathway.
epidemiological and experimental studies. Furthermore, IL-6 has been found to be elevated in obese pregnant women. Data are lacking for IL-1Ra and maternal obesity, but IL-1Ra is one of the most consistent markers of obesity in the non-pregnant population.

Fetal growth is also dependent on nutrient transport across the placenta. Maternal obesity has been found to affect placental size, structure and function. Inflammatory molecules do not easily pass the placenta and maternal inflammation does not seem to be associated with umbilical cord inflammation. Thus, an effect of adipokines on birth weight will expectedly work through altered placental transport capacity or function. Indeed, preliminary data show an effect on transport proteins in the placenta after exposure to IL-6. IL-6 may also act on the placenta and regulate fetal growth through upregulating leptin, which in turn regulates placental growth, nutrient transfer and fetal fat accretion. Finally, experimental studies have demonstrated increased litter fat mass after prenatal exposure to IL-6 and one study has linked maternal IL-6 levels directly to neonatal fat mass. Thus, the literature suggests a biological role for interleukins in the association between maternal BMI and birth weight.

The hypothesized associations were partly confirmed in our data. About 20% of the effect of BMI on birth weight was mediated through paths involving IL-1Ra. However, IL-1Ra is a dual marker; it is an anti-inflammatory cytokine, binding to IL-1 receptor without inducing an effect, but at the same time reflects an activation of the IL-1 system and is also a marker of inflammation in general. Based on this, we cannot rule out that the measured effect of IL-1Ra reflects the action of IL-1β. This emphasizes the need for experimental studies to assess molecular mechanisms and also emphasizes the importance of interpreting results from observational studies with caution concerning causality. However, this result indicates a substantial role for the interleukin 1-system in the deranged glucose metabolism associated with higher maternal BMI during pregnancy and consequently an important role for interleukins as mediators between maternal fat mass, glucose and birth weight. We did not find significant direct effects of the interleukins on birth weight. There might be several explanations for this finding. It may be that interleukins like IL-6 and IL-1Ra do not play an important role in regulating fetal growth through changing placental properties. The result may however also be due to the fact that cytokines display pleiotropic effects and show considerable biological variation. We chose two markers as representative of the inflammatory status in obese women, being aware that other markers may be important as mediators in the association between BMI and birth weight. In addition, effects of cytokines on birth weight are probably not an effect of a single mediator, but rather the result of the interactions of several and in combinations. Therefore, we cannot rule out that cytokines in combinations may have a direct effect on placental properties and birth weight even if we were not able to find such an effect.

We recognize that the hypothesized path diagram (Fig. 1), in which maternal BMI leads to increased inflammation with secondary downstream effects on glucose regulation and fetal growth, has limitations. Integrative physiology is much more complex than reflected in this simplified model. For example, fetal growth relies primarily on glucose as an energy substrate; however lipids and amino acids are also nutrient substrates for fetal growth. An expansion of the model to include lipids would be interesting.

Nevertheless, the analyses based on our simplified path diagram support the notion that inflammatory mediators are involved in the association between maternal BMI and birth weight.

BMI and birth weight are both surrogate markers of fat mass. We used BMI at early gestation in the path diagram, as maternal BMI and fat mass have been shown to correlate more strongly in early than in late pregnancy. Glucose and inflammatory markers were analyzed at gestational weeks 30–32 but there is evidence that the inflammatory and metabolic derangements associated with pregravid maternal obesity are sustained throughout pregnancy. The use of birth weight as a marker of fetal growth might explain why our results were not in accordance with the previously reported association between maternal IL-6 and prenatal growth reflected by neonatal fat mass.

Bayesian methods have been used to a limited extent in clinical research, but the WinBUGS software has made Bayesian methods available. Traditional frequentistic analyses are based on normality assumptions and central limit theory, whereas the WinBUGS analyses are based on prior assumptions and simulation techniques. For both approaches, the linearity of the regression equations should be explored. Frequentistic path analysis is sensitive to violations of normality assumptions in small samples and non-linearity or combinations of different types of variables can be difficult to handle. In Bayesian models, in contrast, non-normality and non-linearity are more easily dealt with. Such methods are also flexible with respect to several types of variables. In studies of complex biological mechanisms, the samples will typically be small due to the costs and restraints in collecting the data. In addition, inflammatory biological markers tend to be skewed, and sometimes display non-linear relations. Therefore, Bayesian methods represent a valuable tool in such studies.

The representativity of the STORK cohort, considering voluntarily participation and a closer follow-up than in usual obstetric care in Norway, has been described earlier. As our study focused on general physiological mechanisms, presumably similar in all healthy pregnant women, neither close follow-up nor self-selection effects would be likely to affect our results substantially.

We wanted to avoid confounding from other biological processes than those studied. Women with low infant birth weight were not included in this study because fetal growth restriction may be associated with placental inflammatory changes. No formal definition of fetal growth restriction exists, and the use of the 10th birth weight percentile as an exclusion criterion was in
acCORDANCE WITH A PRAGMATIC TRADITION. WE USED A CRP VALUE ABOVE 10 mg/l TO EXCLUDE WOMEN WITH POSSIBLE INFECTIONS,24–26 THEREBY ADJUSTING FOR CONFOUNDOING CAUSED BY INFECTIONS. UNMEASURED LIFESTYLE FACTORS (LIKE DIET OR PHYSICAL ACTIVITY), GENETIC FACTORS OR BIOLOGICAL FACTORS MIGHT CONFOUND WITH THE RELATIONS WE STUDIED, AND THEREBY EITHER ATTENUATE OR INCREASE EFFECTS. HOWEVER, IT IS HARD TO TELL IN WHAT DIRECTION THE EFFECT SIZES WOULD BE AFFECTED.64 GESTATIONAL AGE AT BIRTH WAS MODELED AS A POTENTIAL CONFOUNDER OF THE DIRECT EFFECTS ON BIRTH WEIGHT, BUT NOT ON THE CAusal PATHWAY TO BIRTH WEIGHT. OUR ESTIMATES WOULD BE STRONGLY AFFECTED IF THIS WAS THE CASE.65,66 HOWEVER, NO SIGNIFICANT BIVARIATE CORRELATIONS BETWEEN GESTATIONAL AGE AND THE OTHER VARIABLES WERE FOUND IN OUR STUDY SAMPLE.

This study is based on a relatively large sample with measurements of inflammatory markers, which is a strength due to substantial biological variance of such markers.57 Furthermore, moderate effect estimates were anticipated. As a basis for comparison, maternal BMI, one of the major determinants of weight, accounts for approximately 10–20% of the variation in birth weight.1–4 Our result was similar, but only borderline significant, possibly due to the homogeneity of our study sample and a lack of power to detect small effects. Based on these considerations, we reported our model with all the original arrows present, although not all the direct effects were significant. Correspondingly, indirect effects were estimated with significant and non-significant direct effects included.

We conclude that the results of our study, combining current biological concepts and empirical data, suggest that adipose tissue-derived inflammatory factors may be mediators in the association between BMI and birth weight. Mechanisms like metabolic pathways are complex, yet simplified models like the current one may still be useful.

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Statement of Interest
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Appendix A

All path estimates presented in this paper are based on MCMC samples from the joint posterior distribution of the parameters given in the data. We used three parallel MCMC chains in our calculations, each based on 30,000 iterations from which the first 10,000 were discarded as a ‘burn-in’ to achieve convergence, and a thinning factor of five to avoid autocorrelation in the samples. Inference was based on the remaining 12,000 iterations. Convergence of the MCMC series was confirmed using several plots and diagnostics available in the coda-package, including density plots, trace plots, autocorrelation plots, the Gelman-Rubin diagnostic and the Raftery-Lewis diagnostic. Vague prior probability distributions were used for all parameters. Different parameter specifications of the priors were tried to check for the influence of choice of priors. Computing code used to implement the models is available as supplementary material at the journal website (S1).