Preconception insulin resistance and neonatal birth weight in women with obesity

LIFEstyle study group; Wang, Zheng; Nagy, Ruxandra A.; Groen, Henk; Cantineau, Astrid E.P.; van Oers, Anne M.; van Dammen, Lotte; Wekker, Vincent; Roseboom, Tessa J.; Mol, Ben W.J.

Published in:
Reproductive Biomedicine Online

DOI:
10.1016/j.rbmo.2021.08.005

Publication date:
2021

RBMO

ARTICLE

Preconception insulin resistance and neonatal birth weight in women with obesity: role of bile acids

BIOGRAPHY

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KEY MESSAGE

Maternal preconception insulin resistance was positively associated with both circulating levels of bile acids and neonatal birth weight. We could not, however, detect if bile acids influenced the association between insulin resistance and birth weight. This result expands knowledge by illustrating the significance of preconception glycaemic control in improving health.

ABSTRACT

Research question: Does maternal preconception insulin resistance affect neonatal birth weight among women with obesity? Is insulin resistance associated with circulating bile acids? Do bile acids influence the association between maternal preconception insulin resistance and neonatal birth weight?

Design: An exploratory post-hoc analysis of the LIFEstyle randomized controlled trial comparing lifestyle intervention with conventional infertility treatment in women with a BMI of ≥29 kg/m^2. Fasting blood samples were collected at randomization and after 3 and 6 months in 469 women. Insulin resistance was quantified using the homeostasis model assessment of insulin resistance (HOMA-IR). Bile acid sub-species were determined by liquid chromatography with tandem mass spectrometry. Singletons were included (n = 238). Birth weight Z-scores were adjusted for age, offspring gender and parity. Multilevel analysis and linear regressions were used.

Results: A total of 913 pairs of simultaneous preconception HOMA-IR (median [Q25; Q75]: 2.96 [2.07; 4.16]) and total bile acid measurements (1.79 [1.10; 2.94]) μmol/l were taken. Preconception HOMA-IR was positively associated with total bile acids (adjusted B 0.15; 95% CI 0.09 to 0.22; $P < 0.001$) and all bile acid sub-species. At the last measurement before pregnancy, HOMA-IR (2.71 [1.91; 3.74]) was positively related to birth weight Z-score (mean ± SD 0.4 ± 1.1; adjusted B 0.08; 95% CI 0.01 to 0.14; $P = 0.03$). None of the preconception bile acids measured were associated with birth weight.

Conclusion: Maternal preconception insulin resistance is an important determinant of neonatal birth weight in women with obesity, whereas preconception bile acids are not.

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Declaration: A grant was received from ZonMw, the Dutch Organization for Health Research and Development (50-50110-96-518). ZonMw had no role in data collection, analysis, interpretation of data or writing the report. The Department of Obstetrics and Gynecology of the UMCG received an unrestricted educational grant from Ferring pharmaceuticals BV, The Netherlands. BWJM is supported by a NHMRC Investigator grant (GNT1176437). BWJM reports consultancy for Guerbet, has received research funding from Guerbet and Merck and holds Stock options for ObsEva, Geneva.

KEYWORDS

Bile acids, birth weight, obesity, preconception
Insulin resistance

INTRODUCTION

During pregnancy, the development of insulin resistance is a normal physiological adaptation necessary to meet the nutritional requirements of the growing fetus and placenta (*King, 2000*). The consequence of insulin resistance during pregnancy is that maternal energy metabolism shifts from carbohydrate to lipid utilization, thereby saving glucose for the growing fetus (*Sivan et al., 1998*). The biological relevance of this adaptation is illustrated by data showing that, in uncomplicated pregnancies, maternal insulin resistance in mid-pregnancy is positively associated with neonatal birth weight (*Yamashita et al., 2014*). Studies exploring the association between pre-existing maternal insulin resistance before pregnancy and neonatal birth weight, however, are scarce. One study showed an association between preconception maternal blood glucose levels and neonatal birth weight in small for gestational age babies (*Wei et al., 2019*). Although maternal insulin resistance affects fetal growth during pregnancy and hence birth weight, the physiological mechanisms behind the development of insulin resistance before and during pregnancy have been insufficiently characterized (*Das et al., 2010*; *Yamashita et al., 2014*; *Farrar et al., 2016*).

Bile acids, best known for their role in fat absorption in the intestine (*de Aguiar Vallim et al., 2013*), can be divided into primary bile acids synthesized by the liver, such as cholic acid and chenodeoxycholic acid (CDCA), and secondary bile acids that result from modifications by the gut microbiota, such as deoxycholic acid (DCA) and lithocholic acid (LCA) (*Chiang, 2009*). Recent studies have indicated that insulin signalling can substantially affect bile acid metabolism (*Ahmad and Haeusler, 2019*). Insulin resistance has been reported to be positively associated with total plasma bile acids and particularly with primary or 12α-hydroxylated bile acids (*Cariou et al., 2011*; *Haeusler et al., 2013*; *Sun et al., 2016*; *Lee et al., 2019*). Combined, these studies indicate that insulin resistance increases bile acid synthesis and might lead to an altered composition of the bile acid pool. Such changes could conceivably translate into modulation of bile acid signalling via the Farnesoid X receptor or the Takeda G protein-coupled receptor 5, which would conversely result in bile acid-induced changes in glucose metabolism and insulin sensitivity (*Nguyen and Bouscarel, 2008*; *Trauner et al., 2010*).

Bile acids are increasingly recognized for having a role in reproductive health and could potentially affect birth weight in at least two ways. First, we recently identified high levels of bile acids in follicular fluid and delineated the expression of several relevant bile acid transporters in the ovary, suggesting that bile acids could have an underappreciated indirect effect on reproduction at a very early stage (*Nagy et al., 2015*; *2019*). Intriguingly, we also observed in a small-scale study including 60 singleton deliveries that, in normal-weight women undergoing modified natural cycle IVF, preconception serum levels of the primary bile acids, cholic acid and CDCA, were significantly inversely associated with neonatal birth weight, whereas total bile acid concentrations were not (*van Montfoort et al., 2019*). Alternatively, given the role of bile acids as metabolic integrators, bile acids could modulate glucose metabolism and insulin resistance during pregnancy, e.g. through an increase in glucagon-like peptide 1 (GLP-1) secretion from intestinal L-cells mediated through Takeda G protein-coupled receptor 5 (*Kuhre et al., 2018*). This could also potentially result in a lower birth weight through improved glycaemic control.

The aim of the present study was to delineate within the LIFEstyle study a well-characterized randomized controlled trial (RCT) of women with obesity referred for infertility treatment if preconception insulin resistance is associated with neonatal birth weight. Further, given the above referenced association between insulin resistance and bile acid metabolism, an analysis was undertaken of whether maternal preconception insulin resistance was associated with circulating bile acids and if bile acids might influence the association between maternal preconception insulin resistance and neonatal birth weight.

MATERIALS AND METHODS

The present study is an exploratory post-hoc analysis of the multi-centre LIFEstyle RCT. The study was conducted following the principles of the Declaration of Helsinki and approved by the Medical Ethics Committee of the University Medical Centre Groningen (METc code: 2008/284, date of approval: 29 February 2009), as well as by the board of directors of the other participating hospitals (n = 22). All included participants gave written informed consent. The trial was registered in the Netherlands Trial Registry (NTR 1530, date of registration: 16 November 2008).

Participants and study process

The inclusion and exclusion criteria have been described previously (*Mutsaerts et al., 2010*; *2016*). In short, between 2009 and 2012, a total of 577 women with body mass index (BMI) 29 kg/m^2 or above were randomly assigned to a 6-month lifestyle intervention followed by infertility treatment or to prompt infertility treatment. Exclusion criteria included endocrinopathy (such as type 1 diabetes or Cushing's syndrome), severe endometriosis and previous history of pregnancy-induced diseases. The lifestyle intervention consisted of an energy-restricted diet, an increase in physical activity and motivational counselling. The programme included six outpatient visits and four telephone consultations with trained nurses. The main goal was a weight reduction of at least 5% of the original body weight or a reduction in BMI to below 29 kg/m^2 within the intervention period of 6 months. When this weight loss goal was met, participants could stop the lifestyle intervention programme and proceed with infertility treatment in case no natural pregnancy had occurred. Participants could re-enter the intervention in case of a miscarriage. Participants in the control group started infertility treatment promptly after randomization. During the 24 months after randomization, research nurses recorded data in a web-based digital case-record form: Oracle Database (Oracle Corporation, Texas, USA). The accuracy of the database was monitored for inconsistencies and validated by researchers through contacting participating hospitals.

Bile acids and sub-species and homeostasis model assessment of insulin resistance measurements

During hospital visits at randomization, and at 3 and 6 months, body weight (kg) and height (cm) were measured and recorded by research nurses who

were blinded to allocation. The BMI was calculated as body weight (kg)/height (m)^2. Fasting blood samples were collected by venipuncture and were kept at room temperature for half an hour. After centrifuging at 1700 x g for 10 min at 4°C, serum samples were continuously stored at –80°C until analysis.

Bile acid analysis was carried out by Biocrates Life Sciences (Innsbruck, Austria) using their commercially available Bile Acids Kit on a liquid chromatography tandem mass spectrometry platform, as previously reported (*Pham et al., 2016*). In short, 10 µl of internal standards mixture was pipetted onto filter spots suspended in wells of a 96-well filter plate. This filter plate was fixed on top of a deep-well plate serving as a receiving plate for the extract (a combi-plate structure). Subsequently, 10-µl samples were pipetted on the spots, followed by nitrogen drying. Then 100-µl methanol was added to the wells, and the combi-plate was shaken for 20 min. The combi-plate was centrifuged to elute the methanol extract into the lower receiving deep-well plate, which was then detached from the upper filter plate. After adding 60-µl Milli-Q® water to the extracts and shaking briefly, the plate was ready for analysis. For baseline separation, ultra-high pressure liquid chromatography (UHPLC, Shimadzu Nexera X2) was used at a flow rate of 0.5 ml/min and a proprietary reversed-phased UHPLC column (Biocrates Life Sciences, Innsbruck, Austria). Mass spectrometric detection is accomplished with electrospray ionization in negative ion mode (SCIEX QTRAP 5500). Total bile acids were calculated as sum of the individual species. Values below the limit of detection were regarded as missing.

Insulin resistance was quantified using HOMA-IR calculated as fasting insulin concentration multiplied by fasting glucose concentration divided by 22.5 (*Matthews et al., 1985*). Fasting plasma glucose was measured with an enzymatic ultraviolet test (hexokinase method) (*Stein, 1965*). Insulin was measured with the Architect analyzer manufactured by Abbott Diagnostics (Lake Forest, Illinois, USA), using a chemiluminescent micro particle immunoassay. HOMA-IR levels have been published in one of our studies exploring the effect of a lifestyle intervention on cardiometabolic health (*van Dammen et al., 2018*).

Pregnancy outcomes and offspring birth weight measurement

Participants were followed for 24 months after randomization. Data on the course of pregnancy and childbirth was also recorded when a woman conceived within 24 months after randomization, but childbirth occurred after the 24 months of follow-up. Pregnancy complications included gestational diabetes, pregnancy-induced hypertension, preeclampsia and HELLP syndrome. Neonatal outcomes were neonatal birth weight, gestational age, gender and small for gestational age or large for gestational age, defined as birth weight below the 10th or above the 90th percentile according to the Dutch reference curves (*Visser et al., 2009*).

Statistical analysis

For this exploratory post-hoc analysis of the data from the LIFEstyle study, data from all participants, regardless of randomization arm, were pooled. The Kolmogorov–Smirnov test combined with histogram paragraph was used to test normality. Results of descriptive analyses of the participants are expressed as mean ± SD for normally distributed continuous variables and median (Q25; Q75) for non-normally distributed continuous variables and proportions (%) for categorical variables. Data collected in pregnant participants, unknown to be pregnant at time of measurement or blood sampling, were excluded. Exclusion was based on last menstrual date before a clinical pregnancy (in which the gestational sac was visible on ultrasonography). Baseline characteristics were compared between participants who were included in the current study and participants who were not included using Student's t-test for continuous variables and chi-squared test for categorical variables to investigate whether the included group was representative for the total group.

Preconception homeostasis model assessment of insulin resistance and bile acid profiles

Multilevel analysis was carried out using generalized estimating equations (GEE) with an exchangeable correlation matrix to examine the associations between maternal preconception HOMA-IR and bile acid profiles measured at the various time points, i.e. baseline, 3 and 6 months. This allowed all available measurements to be used, which is preferable over complete-case analysis. The association was explored in univariable model and multivariable model adjusting for maternal BMI, as BMI has been shown to be related to bile acid levels (*Prinz et al., 2015*). The changes from preconception HOMA-IR and bile acid levels were also explored over time at 3 and 6 months after randomization by including time points (baseline was used as reference) in the GEE model. A further investigation was made into whether a change in maternal BMI at 3 months was associated with preconception HOMA-IR and bile acid profiles. In this model, a change in BMI (3 months to baseline) and baseline measurements of HOMA-IR or bile acid profiles were included.

Effect of preconception bile acid profiles and homeostasis model assessment of insulin resistance on neonatal birth weights

Baseline measurements along with the last measurement available before pregnancy were used to explore the association between maternal preconception HOMA-IR and neonatal birth weight Z-score and between maternal preconception bile acids and neonatal birth weight Z-score. The birth weight Z-score was calculated adjusting for gestational age, offspring gender and parity (*Land, 2006*). Singletons born between the 28th and 42nd week of gestation were included. Univariable and multivariable linear regression adjusting for potential confounders were used to investigate independent predictors of singleton neonatal birth weight Z-score. To identify potential confounders, baseline characteristics, maternal age at delivery and pregnancy complications, such as gestational diabetes and hypertension, were added to the regression models, one at a time. If the effect estimate changed more than 10% or had already been confirmed to be associated with neonatal birth weight in previously published studies, the variable was included in the final model. Intervention as a confounder was also included as intervention was associated with changes in maternal obesity and some metabolic parameters and might have influenced the neonatal birth weight.

SPSS 25.0 (IBM, Armonk, USA) was used for statistical analyses and GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA) for data visualization. $P < 0.05$ was considered to be statistically significant.

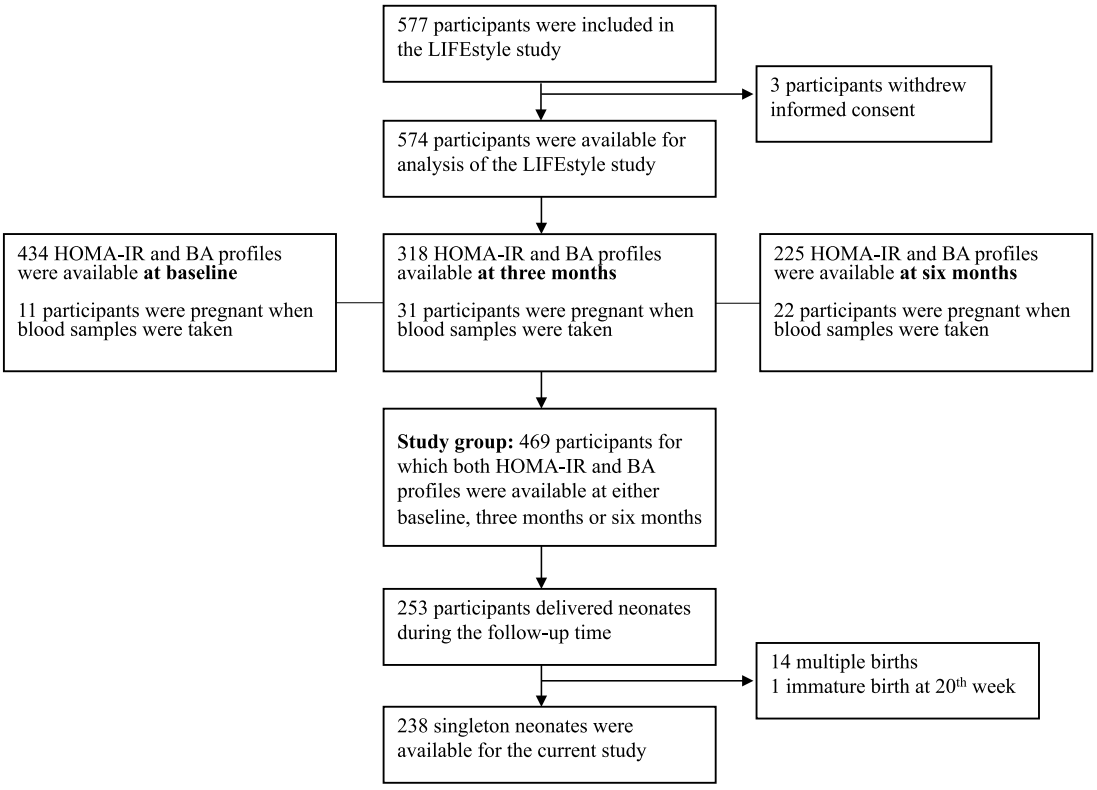

FIGURE 1 Study flow chart. The number of preconception samples used for the measurements of insulin resistance and bile acids decreased over time owing to dropout, failure to attend the hospital visit or pregnancies. BA, bile acids; HOMA-IR, homeostasis model assessment of insulin resistance.

RESULTS

Study participants

The current analysis is presented in FIGURE 1. Among the 577 participants included in the LIFEstyle study, three withdrew informed consent, resulting in 574 participants remaining. Participants with both HOMA-IR and bile acid profiles available at either baseline, 3 months or 6 months after randomization constituted the present study group (n = 469). From 423 participants, HOMA-IR and bile acid profiles were available at baseline, from 287 at 3 months and from 203 at 6 months (160 participants in the present study had measurements at all time points). Baseline characteristics are presented in TABLE 1. The mean age was 29.9 ± 4.5 years, with an average BMI of 35.9 ± 3.3 kg/m^2. In total, 326 (69.5%) participants received intermediate vocational or higher education. A total of 23.6% were current smokers and 77.6% of the participants were nulliparous. No statistically significant differences were found in age, BMI, Western European ethnicity, education, smoking status and nulliparity between participants included in the analysis and those who were not (Supplementary Table 1).

TABLE 1 BASELINE CHARACTERISTICS OF STUDY PARTICIPANTS WITH AT LEAST ONE MEASUREMENT OF HOMA-IR AND BILE ACIDS

	***n* = 469**
Age, years	29.9 ± 4.5
BMI, kg/m^2	35.9 ± 3.3
Western European ethnicity, *n* (%)	412 (87.8)
Education, *n* (%)	
Primary school	22 (4.7)
Secondary education	103 (22.0)
Intermediate vocational education	223 (47.5)
Advanced vocational education and university	103 (22.0)
Unknown	18 (3.8)
Current smoker	110 (23.5)
Nulliparity	364 (77.6)

Participants with HOMA-IR and bile acids available at either baseline, 3 months or 6 months after randomization were included. Data are presented as mean ± SD or proportions (%). BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance.

The association between insulin resistance and bile acids

The median of glucose at baseline was 5.3 (5.0; 5.6) mmol/l; at 3 months, 5.3 (5.0; 5.6) mmol/l; and at 6 months, 5.2 (5.0; 5.5) mmol/l. The median of insulin was 89.6 (65.3; 119.4) pmol/l, 82.6 (59.7; 120.1) pmol/l and 81.2 (57.6; 113.9) pmol/l, respectively. The median of HOMA-IR was 3.07 (2.16; 4.20), 2.96 (2.03; 4.30) and 2.66 (1.90; 3.94), respectively (TABLE 2). In this analysis of pooled data of both groups, no statistically significant differences were found in glucose, insulin or HOMA-IR at 3 and 6 months compared with baseline measurements, although the overall values were

TABLE 2 COMPARISON OF PRECONCEPTION INSULIN RESISTANCE PARAMETERS AND BILE ACID PROFILES AT BASELINE VERSUS 3 OR 6 MONTHS

	Baseline	3 months	Unadjusted B (95% CI)	*P*-value	6 months	Unadjusted B (95% CI)	*P*-value
Glucose, mmol/l	5.3 (5.0; 5.6)	5.3 (5.0; 5.6)	−0.003 (−0.07 to 0.06)	0.93	5.2 (5.0; 5.5)	−0.05 (−0.16 to 0.06)	0.36
Insulin, pmol/l	89.6 (65.3; 119.4)	82.6 (59.7; 120.1)	0.02 (−5.96 to 5.99)	0.996	81.2 (57.6; 113.9)	−4.24 (−10.7 to 2.17)	0.20
HOMA-IR	3.07 (2.16; 4.20)	2.96 (2.03; 4.30)	0.007 (−0.22 to 0.24)	0.96	2.66 (1.90; 3.94)	−0.19 (−0.45 to 0.07)	0.16
Total bile acids	1.84 (1.10; 2.93)	1.79 (1.15; 3.16)	0.01 (−0.21 to 0.24)	0.92	1.62 (1.02; 2.73)	−0.16 (−0.43 to 0.12)	0.26
Cholic acid derivatives[a]	0.24 (0.12; 0.47)	0.24 (0.13; 0.58)	−0.002 (−0.07 to 0.07)	0.96	0.22 (0.13; 0.46)	−0.06 (−0.14 to 0.02)	0.15
DCA derivatives[a]	0.56 (0.32; 0.90)	0.60 (0.33; 0.93)	0.01 (−0.06 to 0.08)	0.78	0.56 (0.31; 0.97)	−0.01 (−0.10 to 0.07)	0.78
CDCA derivatives	0.85 (0.43; 1.47)	0.76 (0.44; 1.40)	−0.02 (−0.14 to 0.10)	0.71	0.71 (0.40; 1.21)	−0.10 (−0.24 to 0.04)	0.16
UDCA derivatives	0.086 (0.037; 0.215)	0.084 (0.035; 0.199)	0.011 (−0.012 to 0.034)	0.33	0.087 (0.037; 0.207)	0.007 (−0.017 to 0.032)	0.56
LCA derivatives	0.024 (0.017; 0.039)	0.022 (0.017; 0.037)	−0.001 (−0.008 to 0.006)	0.75	0.026 (0.017; 0.040)	0.004 (−0.004 to 0.011)	0.33

All bile acids and derivative concentrations are presented as μmol/l.

Data are presented as median (Q25; Q75).

[a] 12α-hydroxylated bile acids.

CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; HOMA-IR, homeostasis model assessment of insulin resistance; LCA, lithocholic acid; UDCA, ursodeoxycholic acid.

decreasing. Total bile acid levels were 1.84 (1.10; 2.93) μmol/l at baseline, 1.79 (1.15; 3.16) μmol/l at 3 months and 1.62 (1.02; 2.73) μmol/l at 6 months. No statistically significant differences were found in total bile acid levels at 3 and 6 months compared with baseline measurements (unadjusted B 0.01; 95% CI −0.21 to 0.24; $P = 0.92$, and unadjusted B −0.16; 95% CI −0.43 to 0.12; $P = 0.26$, respectively). Detailed bile acid sub-species analyses are presented in TABLE 2. No statistically significant differences were found in cholic acid, DCA, CDCA, ursodeoxycholic acid (UDCA) and LCA derivatives at 3 and 6 months compared with baseline measurements. The change in maternal preconception BMI was significantly and positively associated with HOMA-IR (unadjusted B 0.18; 95% CI 0.12 to 0.24; $P = 0.001$) but not total bile acid levels (unadjusted B 0.05; 95% CI −0.02 to 0.11; $P = 0.14$) and bile acid sub-species (Supplementary Table 2).

The association between preconception HOMA-IR and bile acid profiles is presented in TABLE 3, with detailed number of cases available for GEE analysis and the levels of HOMA-IR and bile acid profiles (all available measurements were included for analysis, regardless of time point). In total, 913 pairs of simultaneous preconception HOMA-IR and total bile acid measurements were taken. The median of HOMA-IR determinations was 2.96 (2.07; 4.16) and the median of total bile acid levels was 1.79 (1.10; 2.94) μmol/l). The scatter plots of HOMA-IR and total bile acids at baseline, at 3 months, at 6 months and the overall group regardless of times points, are separately shown in Supplementary Figure 1. For 869 individual time points, cholic acid derivative measurements were available (0.24 [0.13; 0.50]) μmol/l; for 871 DCA derivatives (0.57 [0.32; 0.93]) μmol/l; for 913 CDCA derivatives (0.79 [0.43; 1.36]) μmol/l; for 864 UDCA derivatives (0.086 [0.036; 0.207]) μmol/l; for 281 LCA derivatives (0.025 [0.017; 0.038]) μmol/l. Preconception HOMA-IR was positively associated with total bile acids (adjusted B 0.15; 95% CI 0.09 to 0.22; $P < 0.001$) and all bile acid sub-species. HOMA-IR was positively associated with 12α-hydroxylated bile acids, cholic acid derivatives and DCA derivatives (adjusted B 0.02; 95% CI 0.01 to 0.04; $P = 0.004$, and adjusted B 0.04, 95% CI 0.02 to 0.07; $P < 0.001$, respectively).

Pregnancy outcomes and neonatal outcomes of singletons

During follow-up of those 469 participants selected in the study group, 253 delivered neonates. Birth weights of 238 singletons were available for analysis after exclusion of multiple births ($n = 14$ participants) and one immature singleton birth at the 20th week of gestation. The mean birth weight of 238 singleton neonates was 3380 ± 601 g corresponding to a birth weight Z-score of 0.4 ± 1.1. The mean gestational age at delivery was 38.8 ± 2.2 weeks and mean maternal age at delivery was 30.8 ± 4.3 years. A total of 18.9% (45/238) of women had gestational diabetes. Other pregnancy and neonatal outcomes are presented in TABLE 4.

The association between preconception insulin resistance and neonatal birth weight, and between bile acids and birth weight

The associations between maternal preconception HOMA-IR and singleton neonatal birth weight, and between maternal preconception bile acid profiles and singleton neonatal birth weight, are presented in TABLE 5. The adjusted model included intervention, smoking status at baseline, maternal age at delivery and preeclampsia. A total of 219 maternal preconception HOMA-IR measurements were taken at baseline, with a median of 2.93 (2.05; 4.02). All 238 maternal preconception HOMA-IR from the last measurement before pregnancy were available (76, 66, and 96 cases from 6 months, 3 months or baseline, respectively), with a median of 2.71 (1.91; 3.74). Maternal preconception HOMA-IR at baseline (adjusted B 0.10; 95% CI 0.03 to 0.17; $P = 0.01$) and at the last measurement before pregnancy (adjusted B 0.08, 95% CI 0.01 to 0.14; $P = 0.03$) were positively and significantly associated with singleton neonatal birth weight. The correlation plots of HOMA-IR and neonatal birth weight Z-score are presented in FIGURE 2. The median of 219 maternal preconception total bile acid levels at baseline was 1.76 (1.04; 2.74) μmol/l, and, in the 238 cases, the median of the last measurement before pregnancy was 1.70 (1.07; 2.73) μmol/l (76, 66 and 96 cases from 6 months, 3 months or baseline, respectively). Maternal preconception total bile acid levels were not significantly associated

TABLE 3 ASSOCIATIONS BETWEEN PRECONCEPTION HOMA-IR AND BILE ACID PROFILES

	n	Levels	Unadjusted B (95% CI)	*P*-value	Adjusted[a] B (95% CI)	*P*-value
HOMA-IR	913	2.96 (2.07; 4.16)	NA	NA	NA	NA
Total bile acids	913	1.79 (1.10; 2.94)	0.16 (0.11 to 0.22)	<0.001	0.15 (0.09 to 0.22)	<0.001
Cholic acid derivatives[b]	869	0.24 (0.13; 0.50)	0.03 (0.01 to 0.04)	0.001	0.02 (0.01 to 0.04)	0.004
DCA derivatives[b]	871	0.57 (0.32; 0.93)	0.04 (0.02 to 0.07)	<0.001	0.04 (0.02 to 0.07)	<0.001
CDCA derivatives	913	0.79 (0.43; 1.36)	0.08 (0.05 to 0.11)	<0.001	0.08 (0.05 to 0.11)	<0.001
UDCA derivatives	864	0.086 (0.036; 0.207)	0.009 (0.004 to 0.014)	0.001	0.008 (0.002 to 0.014)	0.01
LCA derivatives	281	0.025 (0.017; 0.038)	0.002 (0.000 to 0.003)	0.01	0.002 (0.000 to 0.003)	0.02

All bile acids and derivatives concentrations are presented as µmol/l.

Data are presented as median (Q25; Q75)

[a] Adjusted for maternal body mass index.

[b] 12α-hydroxylated bile acids.

CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; HOMA-IR, homeostasis model assessment of insulin resistance; LCA, lithocholic acid; UDCA, ursodeoxycholic acid; NA, not applicable.

with neonatal birth weight Z-score at the baseline measurement (adjusted B 0.05; 95% CI −0.05 to 0.15; $P = 0.30$) or at the last measurement before pregnancy (adjusted B 0.04; 95% CI −0.05 to 0.13; $P = 0.34$). The exact number of cases available for analysis and the levels of cholic acid, DCA, CDCA, UDCA and LCA derivatives are presented in TABLE 5. None of the bile acid sub-species measured were significantly associated with neonatal birth weight.

DISCUSSION

This exploratory post-hoc analysis of pooled data of both treatment groups of women with obesity from the LIFEstyle study demonstrates that preconception HOMA-IR levels are positively and significantly associated with neonatal birth weight and bile acid levels (total and sub-species) in women with obesity. Circulating maternal preconception bile acid levels were not significantly associated with neonatal birth weight, precluding further analysis of the effect of the association between insulin resistance and birth weight by bile acids.

The association between maternal glucose or insulin resistance during pregnancy with neonatal birth weight is well established. For example, results from the Hyperglycemia and Adverse Pregnancy Outcome research group demonstrated the significance of maternal glucose levels (less severe in hyperglycaemia than in overt diabetes mellitus) at 24–32 weeks of gestation for neonatal birth weight (*Metzger et al., 2008*). In a Japanese study, maternal insulin resistance determined by HOMA-IR in mid-pregnancy was positively associated with neonatal birth weight in uncomplicated pregnancies (*Yamashita et al., 2014*). Existing data on the association between preconception insulin resistance and neonatal birth weight is, however, rather limited. A large cohort study conducted in China (*Wei et al., 2019*) with over 6.4 million women reported that higher preconception maternal blood glucose levels were associated with a higher risk of adverse neonatal outcomes. Limiting factors for the significance of that study are that insulin was not determined and that birth weight was not explored as a continuous variable. In contrast, we report here that maternal preconception HOMA-IR is positively associated with neonatal birth weight either using baseline or the last measurement before pregnancy. Of note, there were two outliers with high HOMA-IR measurements (14.96 and 16.28). The exclusion of these outliers diminished the respective regression coefficients in the analyses of the baseline measurement and the last measurement before pregnancy (unadjusted B changed from 0.09 to 0.06, and from 0.07 to 0.04, respectively). These results, however, expand current knowledge by illustrating the significance of preconception glycaemic control in improving maternal health as well as neonatal outcomes. More studies with larger sample sizes and uniform standards for preconception measurements are needed to verify our findings.

TABLE 4 PREGNANCY OUTCOMES AND NEONATAL OUTCOMES OF SINGLETONS

	***n* = 238**
Gestational age at delivery, weeks	38.8 ± 2.2
Maternal age at delivery, years	30.8 ± 4.3
Gestational diabetes, *n* (%)	45 (18.9)
Pregnancy-induced hypertension, *n* (%)	42 (17.6)
Preeclampsia, *n* (%)	16 (6.7)
HELLP syndrome, *n* (%)	1 (0.4)
Neonatal birth weight, g	3380 ± 601
Birth weight Z-score[a]	0.4 ± 1.1
LGA, *n* (%)	39 (16.4)
SGA, *n* (%)	10 (4.2)
Neonatal gender	
Male	119 (50)
Female	119 (50)

[a] Adjusted for gestational age, neonatal gender and parity.

LGA, large for gestational age; SGA, small for gestational age.

TABLE 5 ASSOCIATIONS BETWEEN SINGLETON NEONATAL BIRTHWEIGHT Z-SCORE AND MATERNAL PRECONCEPTION HOMA-IR AS WELL AS BILE ACID PROFILES

	Overall *n*	*n* at T0	*n* at T1	*n* at T2	Levels	Unadjusted B (95% CI)	*P*-value	Adjusted[a] B (95% CI)	*P*-value
Baseline measurement									
HOMA-IR	219	219	NA	NA	2.93 (2.05; 4.02)	0.09 (0.02 to 0.16)	0.01	0.10 (0.03 to 0.17)	0.01
Total bile acids	219	219	NA	NA	1.76 (1.04; 2.74)	0.04 (–0.06 to 0.13)	0.42	0.05 (–0.05 to 0.15)	0.30
Cholic acid derivatives[b]	206	206	NA	NA	0.22 (0.11; 0.46)	0.06 (–0.27 to 0.38)	0.72	0.11 (–0.22 to 0.44)	0.53
DCA derivatives[b]	211	211	NA	NA	0.48 (0.28; 0.74)	0.17 (–0.10 to 0.45)	0.21	0.18 (–0.09 – 0.46)	0.19
CDCA derivatives	219	219	NA	NA	0.80 (0.39; 1.32)	0.02 (–0.17 to 0.21)	0.81	0.04 (–0.16 to 0.23)	0.69
UDCA derivatives	207	207	NA	NA	0.059 (0.034; 0.192)	0.17 (–0.57 to 0.90)	0.65	0.44 (–0.34 to 1.22)	0.26
LCA derivatives	63	63	NA	NA	0.023 (0.017; 0.035)	16.3 (–6.89 to 39.5)	0.17	13.1 (–11.7 to 37.8)	0.29
Last measurement[c]									
HOMA-IR	238	96	66	76	2.71 (1.91; 3.74)	0.07 (0.00 to 0.13)	0.05	0.08 (0.01 to 0.14)	0.03
Total bile acids	238	96	66	76	1.70 (1.07; 2.73)	0.03 (–0.05 to 0.12)	0.45	0.04 (–0.05 to 0.13)	0.34
Cholic acid derivatives[b]	229	92	65	72	0.23 (0.13; 0.45)	0.08 (–0.24 to 0.40)	0.62	0.10 (–0.23 to 0.42)	0.56
DCA derivatives[b]	234	94	68	72	0.53 (0.30; 0.83)	0.10 (–0.16 to 0.37)	0.45	0.12 (–0.15 to 0.39)	0.40
CDCA derivatives	238	96	66	76	0.74 (0.42; 1.26)	0.03 (–0.12 to 0.18)	0.67	0.04 (–0.11 to 0.20)	0.58
UDCA derivatives	230	90	70	70	0.082 (0.033; 0.210)	0.26 (–0.51 to 1.02)	0.51	0.57 (–0.25 to 1.39)	0.17
LCA derivatives	90	45	27	18	0.021 (0.016; 0.031)	10.2 (-0.33 – 20.8)	0.06	10.1 (–0.92 to 21.2)	0.07

All bile acid and derivatives concentrations are presented as µmol/l.

Data are presented as median (Q25; Q75)

[a] Baseline measurement was adjusted for smoking status at baseline, maternal age at delivery, and preeclampsia. Last measurement was adjusted for intervention, smoking status at baseline, maternal age at delivery and preeclampsia.

[b] 2α-hydroxylated bile acid.

[c] If the measurement was available at 6 months (T2), we used the measurement at 6 months (T2); if not, we used the measurement at 3 months (T1), otherwise, we used baseline measurement (T0).

CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; HOMA-IR, homeostasis model assessment of insulin resistance; LCA, lithocholic acid; UDCA, ursodeoxycholic acid; NA, not applicable.

Bile acid metabolism and insulin resistance are closely associated (*Cariou et al., 2011*; *Haeusler et al., 2013*; *Sun et al., 2016*; *Legry et al., 2017*; *Lee et al., 2019*). Cross-sectional work (*Sun et al., 2016*) described that, in both sexes, circulating bile acid levels are elevated in an insulin resistant population independent of glucose levels. Specifically, insulin resistance has been associated with a disproportionate increase in 12α-hydroxylated bile acids (cholic acid, DCA and their conjugated forms) over non-12α-hydroxylated bile acid sub-species. In overt type-2 diabetes, however, such a relationship is no longer apparent (*Haeusler et al., 2013*). Our results in non-diabetic participants with obesity confirmed the positive association between maternal preconception insulin resistance and bile acid profiles, although these associations were not specific for the 12α-hydroxylated bile acid. Our data, however, do not allow conclusions to be drawn about causality between insulin resistance and bile acids, i.e. whether high bile acid levels contribute to the rise of insulin resistance or *vice versa*. Of note, the association between bile acids and HOMA-IR remained significant if HOMA-IR was used as an outcome and bile acid levels as predictors (data not shown) in the GEE model.

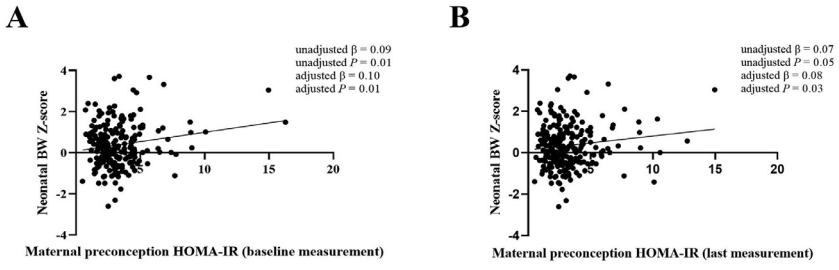

FIGURE 2 Scatter plot of maternal preconception homeostasis model assessment of insulin resistance (HOMA-IR) with neonatal birth weight Z-score. (A) Scatter plot of preconception HOMA-IR at baseline measurement with neonatal birth weight; (B) scatter plot of preconception HOMA-IR at the last measurement before pregnancy with neonatal birth weight. Baseline measurement was adjusted for smoking status at baseline, maternal age at delivery and preeclampsia. Last measurement was adjusted for intervention, smoking status at baseline, maternal age at delivery and preeclampsia. BW, birth weight.

Intriguingly, previous results from our group showed that, in a setting close to normal reproductive physiology, preconception serum levels of primary bile acids were significantly inversely associated with neonatal birth weight in 60 singleton deliveries of normal-weight women (*van Montfoort et al., 2019*). In the present analysis, we could not replicate this relationship. Plausible

explanations might be either power or, in our view more likely, the substantial differences in maternal BMI between the two study groups (in the former study 23.1 ± 3.3 kg/m^2; and in the present study: 35.9 ± 3.3 kg/m^2). In this setting, metabolic (dys)regulation during pregnancy, such as insulin resistance, could become more important than pre-conceptional parameters in determining neonatal outcome birth weight. In regulating bile acids, it is worth noting that cholestasis of pregnancy can also occur (*Tolunay et al., 2021*), which might be associated with lower neonatal birth weight (*Li et al., 2018*). Lack of these parameters somewhat limits the validity of our model. Another potential explanation is the time gap between the bile acid measurement and the date of conception and birth (13.4 ± 5.1 months between the date of neonatal birth and the date of the last measurement of maternal preconception insulin resistance and bile acids). In our former study, serum bile acids were determined in fasted serum on the day of ovum retrieval, 3 days before embryo transfer and, therefore, close to conception, which minimizes the effect of time.

A strength of our work is the longitudinal design allowing total bile acids and subspecies and HOMA-IR changes over time within individuals to be investigated. Further, we made use of a well-characterized RCT, in which participants were closely followed and relevant clinical parameters were well documented. Certain limitations of the study, however, are also worth pointing out. First, this is an exploratory post-hoc analysis of an RCT. Unfortunately, glucose concentrations, measures of insulin resistance and bile acid levels were not determined during pregnancy owing to the focus of the original study design. Therefore, the association between preconception bile acids and gestational bile acids, and between gestational bile acids and neonatal birth weight, could not be explored in the present study. In general, the association between preconceptional and gestational bile acid homeostasis is unclear and represents a topic worth addressing in future studies. It can be envisioned that bile acid levels or turnover during pregnancy have more relevance for the regulation of birth weight than in the preconception phase, particularly in an obese population. Similarly, a more elaborate testing of glucose tolerance as well as insulin resistance before and during pregnancy would potentially provide an improved mechanistic understanding. This was not, however, feasible in the present study. Further, because of dropout, failure to attend the hospital visit or pregnancies, the number of preconception samples used for the measurements of insulin resistance and bile acids decreased over time, so results should be interpreted with caution. We compared the baseline characteristics and no statistically significant differences were found between participants included in the present study and participants that were not included. Therefore, we concluded that the included group is representative of the total group. In addition, a time gap occurred between the measurement of insulin resistance and bile acids, and conception. To accommodate this limitation, we explored the association between maternal HOMA-IR and neonatal birth weight and between maternal bile acids and neonatal birth weight by either using the baseline measurement or the last measurement within the intervention period to include as many cases as possible and to be as close to conception as feasible. Lastly, despite a clear positive association between preconception HOMA-IR and bile acids and their subspecies, the nature of the study does not allow conclusions to be drawn on the association between insulin resistance and bile acids.

In conclusion, maternal preconception insulin resistance was positively and significantly associated with both neonatal birth weight in singletons and circulating levels of total bile acids as well as all bile acid subspecies. The present data, however, suggest that preconception bile acids are not an important determinant of neonatal birth weight in women with obesity.

ACKNOWLEDGEMENTS

We thank all the women who participated in this study. We thank all participating hospitals and their staff for their contribution to this study, and the lifestyle coaches, research nurses, research midwives and office members of the Dutch Consortium (www.studies-obsgyn.nl) for their hard work and dedication. We thank Aafke van Montfoort for performing the BW Z-score calculation. The following individuals are members of the LIFEstyle study group: Anne M van Oers, Meike AQ Mutsaerts, Henk Groen, Jolande A Land, Walter K Kuchenbecker, Ron van Golde, Gerrit J E Oosterhuis, Niels EA Vogel, Ben WJ Mol, Annemieke Hoek, Denise AM Perquin, Carolien AM Koks, Eugenie M Kaaijk, Frank J Broekmans, Cornelis B Lambalk, Nicole F Klijn, Patricia EAM Mercelina, Yvonne M van Kasteren, Annemiek W Nap, Egbert A Brinkhuis, Ed TCM Gondrie, Jan P de Bruin.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rbmo.2021.08.005.

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Received 9 April 2021; received in revised form 29 July 2021; accepted 2 August 2021.