**Maternal lipid profile in pregnancy and embryonic growth:**

**a population-based prospective cohort study.**

*Running title: Maternal lipids and embryonic growth*

Dionne V. GOOTJES, MD1,2, Anke G. POSTHUMUS, MD, PhD1,2, Ms. Deveney F. WOLS1,2, Prof. Yolanda B. de RIJKE3,4, Jeanine E. ROETERS VAN LENNEP, MD, PhD4, Prof. Eric A.P. STEEGERS1,2

1Department of Obstetrics and Gynecology, Division of Obstetrics and Fetal Medicine, 2Generation R Study Group, 3Department of Clinical Chemistry, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands, 4Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands, all: Erasmus University Medical Centre, Rotterdam, The Netherlands.

**Correspondence**: D.V. Gootjes, Department of Obstetrics and Gynaecology, Erasmus Medical Centre, room Na 2918, PO Box 2040, 3000CA Rotterdam, the Netherlands. Email [d.gootjes@erasmusmc.nl](mailto:d.gootjes@erasmusmc.nl)

Abstract

Objective To investigate the association between the maternal lipid profile in early pregnancy and embryonic growth.

Design Prospective population-based cohort study.

Setting Rotterdam, the Netherlands.

Population We included 1474 women from the Generation R(otterdam) Study.

Methods The maternal lipid profile was defined as total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), remnant cholesterol, non-high-density (non-HDL-c) lipoprotein cholesterol concentrations and the triglycerides/high-density lipoprotein (TG/HDL-c) ratio. Additionally, maternal glucose concentrations were assessed. Associations were studied with linear regression models, adjusted for confounding factors: maternal age, pre-pregnancy BMI, parity, educational level, ethnicity, smoking and folic acid supplement use

Main Outcome Measures Crown-rump length (CRL).

Results Triglycerides and remnant cholesterol concentrations are positively associated with embryonic growth (fully adjusted models, 0.17 SDS: 95% CI 0.03 ; 0.30, and 0.17 SDS: 95% CI 0.04 ; 0.31, respectively). These associations were not present in women with normal weight (triglycerides and remnant cholesterol: fully adjusted model, 0.44 SDS: 95% CI 0.15 ; 0.72). Associations between maternal lipid concentrations and embryonic growth were not attenuated after adjustment for glucose concentrations. Total cholesterol, HDL-c, LDL-c, non-HDL-c concentrations and the TG/HDL-c ratio were not associated with embryonic growth.

Conclusions Higher triglycerides and remnant cholesterol concentrations in early pregnancy are associated with increased embryonic growth, most notably in overweight women.

Funding The Generation R Study was made possible by financial support from the Erasmus Medical Center, Erasmus University Rotterdam, and The Netherlands Organization for Health Research and Development, The Netherlands Organization for Scientific Research, the Ministry of Health, Welfare, and Sport, and the Ministry of Youth and Families.

Keywords Cholesterol, Low-density lipoprotein (LDL-c), High-density lipoprotein (HDL-c), Triglycerides, Early pregnancy , EPIDEMIOLOGY: GENERAL OBSTETRIC, MATERNAL PHYSIOLOGY

Tweetable abstract The maternal lipid profile in pregnancy is associated with embryonic growth.

# Introduction

In pregnancy, lipids are crucial for the developing fetus and to maintain placental function.(1) Lipids are fatty substances that are either absorbed from food or synthesized by the liver, and comprise of cholesterol, triglycerides and lipoproteins. Cholesterol is crucial to provide structural integrity to the cell membrane.(2, 3)

To facilitate the requirements of the developing fetus, the concentrations of maternal lipids such as triglycerides and total cholesterol rise over the course of pregnancy.(4, 5) Pregnant women with low cholesterol concentrations have a higher risk for fetal growth restriction (FGR), preterm birth, and small-for-gestational age neonates.(6-9) Women affected by the Smith-Lemli-Opitz syndrome, an inherited metabolic disease that results in a decreased cholesterol production, are at a higher risk of giving birth to small-for-gestational age neonates.(2, 7) Low LDL-c has even been proposed as a clinical marker for FGR risk assessment.(10) In contrast, a growing body of evidence from animal and human studies also suggests adverse consequences of increased lipid concentrations in pregnancy. High maternal total cholesterol and triglyceride concentrations are associated with an increased risk of hypertensive disorders of pregnancy, preterm birth and large for gestational age (LGA) neonates.(11-13) Additionally, triglycerides and remnant cholesterol are associated with higher birth weight and infant weight, as well as with the risk of LGA-related complications.(13-15) This is in line with the fetal over-nutrition hypothesis, which suggests that apart from maternal glucose concentrations, other maternal nutrients also contribute to (excess) fetal growth.(16) Additionally, it is proposed that in case of maternal obesity, there is an increased availability of these nutrients and thereby an increased risk for this over-nutrition.(16)

Due to the increase in a sedentary lifestyle and a higher intake of calories, a growing number of women of reproductive age are obese and have abnormally elevated lipid levels.(17, 18) As a consequence of these abnormally elevated lipid levels , more women are at risk for an adverse course and outcome of pregnancy.(11, 12) These adverse outcomes do not only affect health of the offspring in the short term, but also have far reaching effects on the health of the offspring in adulthood.(19-21) Therefore, it is important to identify and mitigate factors that have an adverse effect on embryonic and fetal growth and birth outcomes, both for the long- and short-term health of the offspring.

Until recently, most studies focused on the association between the maternal lipid profile and fetal development in the later phases of pregnancy, and birthweight. However, we hypothesize that an effect of maternal lipids on embryonic growth in early pregnancy may already be present. This is substantiated by the fact that embryonic growth early in pregnancy is strongly associated with fetal growth throughout pregnancy, and birth outcomes.(22, 23) Our aim was therefore to investigate the association between the maternal lipid profile in early pregnancy and embryonic growth.

# Methods

### Design and study population

This study was embedded in the Generation R Study, a large multi-ethnic population-based prospective cohort study in the city of Rotterdam, the Netherlands.(24, 25) The study protocol has been approved by the Medical Ethics Committee of the Erasmus University Medical Centre (Erasmus MC), Rotterdam (MEC-2007-413). Written informed consent was obtained from all participants. We excluded women with a twin pregnancy, gestational diabetes, diabetes mellitus and women using medication for the regulation of glucose or cholesterol at the moment of study enrolment. The study population comprised 1474 women with a known last menstrual period (LMP), a regular menstrual cycle (28 days, range 24 – 32 days), a live born singleton and of whom information was available on lipid measurements in early pregnancy and ultrasonic assessment of embryonic growth (**Figure 1**).

### Maternal lipid and glucose concentrations in early pregnancy

Non-fasting blood was sampled early in pregnancy (median 12.4 weeks of gestation, 90% range [11.0 - 13.7]) by trained research nurses. Details of the processing procedures have been described earlier.(25) After thawing, the total cholesterol (mmol/L) and HDL-c (mmol/L) concentrations were determined using standard laboratory methods. Concentrations of LDL-c were calculated using the Friedewald equation.(26) This calculation is not valid when the triglyceride level is ≥400 mg/dL. In this study population, there are no women with triglycerides above 400 mg/dL. Remnant cholesterol was calculated as the total cholesterol minus LDL-c and minus HDL-c ([total cholesterol – LDL-c] – HDL-c). Non-HDL-c was calculated by subtracting HDL-c from total cholesterol (total-cholesterol – HDL-c). The TG/HDL-ratio was calculated by TG divided by the HDL-c concentration (TG/ HDL-c) (**Table S1**). Both cholesterol, TG and glucose (mmol/l) were measured with the c702 module on a Cobas 8000 analyzer (Roche, Almere, The Netherlands). Results on maternal lipid levels in this cohort have previously been published.(13)

### Maternal anthropometrics

We collected information about pre-pregnancy weight by questionnaire, and measured height and weight at enrollment. Questionnaire based weight and measured height were then used to calculate BMI (kg/m2). The correlation of pre-pregnancy weight obtained by questionnaire and weight measured at enrollment was high (ρ = 0.97, P < 0.01).(27) Normal weight was defined as a BMI <25.0 kg/m2 and overweight was defined as a BMI ≥ 25.00 kg/m2.

### Embryonic growth and birth weight

Embryonic growth was assessed by ultrasound examinations using an Aloka model SSD-1700 (Tokyo, Japan) or the ATL-Philips Model HDI 5000 (Seattle, WA, USA). Ultrasound examinations for this study were performed by dedicated ultrasonographers at each prenatal visit to the designated research centers.(24) The crown-rump length (CRL) was measured in a true mid-sagittal plane with the genital tubercle and the spine longitudinally in view, according to standard procedures.(22, 28, 29) Intra-class correlation coefficients for intra-observer and inter-observer reproducibility of crown to rump length measurements were 0.998 and 0.995.(28) Gestational age (GA) adjusted standard deviation scores (SDS) were constructed for the CRL measurements. These scores were based on reference growth curves from the whole study population and represent the equivalent of Z-scores.(30) We obtained information on birth weight from midwifery and obstetric medical records. Gestational-age-adjusted SDS for birth weight were constructed using North European growth standards as the reference growth curve and represent the equivalent of z-scores.(30, 31)

### Pregnancy dating

The gestational age is the most important determinant of fetal growth. In clinical practice, pregnancy dating is based on the CRL. However, for the purpose of analyses with CRL as the outcome, gestational age should be based on the LMP.(32) In this study, pregnancy dating was thus based on the last known menstrual period in women with a regular menstrual cycle.(22) The first day of the last menstrual period was derived from the referral letter of the community midwife or hospital.(22) At the ultrasound visit, we checked this date with the mother and obtained additional information on the regularity and duration of the menstrual cycle.

### Covariates

In a consensus meeting (DG, AP, ES, JRvL), we identified confounders for the association between maternal lipid concentrations and embryonic growth. This resulted in a Directed Acyclic Graph (DAG) (**Supplementary Figure 1**).(33) The identified confounders were: maternal age (continuous), pre-pregnancy BMI (continuous), parity (nulliparous, multiparous), educational level (no education finished, lower education, middle education, higher education), ethnicity (Dutch and Western, Turkish and Moroccan, African, Asian), smoking (never smoked during pregnancy, smoked until pregnancy was known, continued smoking in pregnancy), folic acid supplement use (started preconceptionally, started in first 10 weeks of pregnancy, no folic acid supplement intake) and glucose concentrations (continuous). Information on maternal characteristics during pregnancy including maternal age, self-reported pre-pregnancy weight, number of previous pregnancies, ethnicity, educational level and smoking were available from four questionnaires, applied during pregnancy.

### Statistical analysis

First, baseline characteristics and the distribution of the covariates were determined. We examined potential differences in baseline characteristics between women included and excluded from the analysis. Differences in continuous variables with a normal distribution (mean, SD) were analyzed with Students t-test, and variables with a skewed distribution (median, 90% range) with the Mann-Whitney U test. Categorical variables were analyzed with chi-square tests (**Table S2**).

Second, multivariate linear regression analyses were performed to study the association between differences in embryonic growth for the lower and upper tertiles of the maternal lipid concentrations, compared to the middle tertile. We carried out tests for trends based on multiple linear regression models with the maternal lipid concentrations as a continuous variable. To allow mutual comparison of the lipid measures, we constructed Multiple of the Median (MoM) scores of all lipid measures. The crude model was the univariate analysis of maternal lipid concentrations and embryonic growth. In the adjusted model, we additionally corrected for the previously determined confounding factors. We examined whether maternal glucose concentrations mediated the association of maternal lipid concentrations with embryonic growth by adding it to our models (fully adjusted model).

We aimed to investigate the effect of the switch in nutritional source of the embryo, from uterine glands and yolk sac to the placenta, which occurs at around week 12 of gestation. Therefore, sensitivity analyses were performed. Associations between the maternal lipid status and embryonic growth were separately investigated in the period of 10 to 12 weeks GA versus 12 to 14 weeks GA, with gestational-age adjusted MoM’s (**Table S3**). With other sensitivity analyses, we tested the effect of the lowest lipid concentrations by assessing the cases with the lowest 5% of the lipid concentrations (**Table S4**). Results of all linear regression analyses are presented as regression coefficients (β) with a 95% confidence interval (CI).

The following confounders had missing values: pre-pregnancy BMI (14.3%), parity (0.4%), educational level (4.3%), ethnicity (2.1%), smoking (8.8%), folic acid supplement use (19.3%) and glucose (2.7%). To prevent bias associated with missing data, we used multiple imputations for covariates with missing values. We imputed missing data on the basis of the correlation of missing variables with other participant characteristics, according to the Markov Chain Monte Carlo method.(34) Ten datasets were created and analyzed together. A sensitivity analysis was performed to observe differences in observed and expected values of confounders before and after imputation (**Table S5**)**.**

We used IBM Statistical Package of Social Sciences version 25.0 for Windows (SPSS Incl., Chicago, IL, USA) for all statistical analyses. A p-value <0.05 was considered statistically significant.

# Results

Maternal baseline characteristics and first trimester reference ranges(35) for lipid concentrations are presented in **Table 1**. In the study we included 1474 women. Women were on average 30.8 (±4.6) years of age, 1060 (71.9%) women had a Dutch and Western ethnicity and the median pre-pregnancy BMI was 22.6 kg/m2 (90% range 18.9 ; 29.6). **Table S2** shows baseline characteristics of women included and excluded from the analyses. Excluded women were on average younger, less often of Dutch and Western ethnicity, more often lower educated and they more often consumed alcohol in pregnancy.

The associations between maternal lipid concentrations and CRL are shown in **Table 2**. In the crude analyses, a larger CRL was observed in women with higher triglyceride concentrations; a significant linear trend was observed (crude model, 0.16 SDS; 95% CI, 0.05 ; 0.38). In the multivariable analyses, the association remained significant (adjusted model, 0.15 SDS; 95% CI, 0.01 ; 0.28), also after additionally adjusting for glucose concentrations (fully adjusted model, 0.17 SDS; 95% CI, 0.03 ; 0.30). When analyses were performed according to BMI (i.e. normal weight or overweight), the associations only remained in the overweight group (crude model, 0.29 SDS; 95% CI, 0.04 ; 0.53, adjusted model, 0.35 SDS; 95% CI, 0.10 ; 0.61 and fully adjusted model, 0.44 SDS; 95% CI, 0.15 ; 0.72).

The crude analyses between remnant cholesterol and CRLshowed significant positive associations (basic model, 0.17 SDS; 95% CI, 0.05 ; 0.29). After adjustment for confounders in the multivariable analysis, and the fully adjusted analysis, the significant associations remained (adjusted model, 0.15 SDS; 95% CI, 0.02 – 0.29 and fully adjusted model, 0.17 SDS; 95% CI, 0.04 – 0.31, respectively). Again, the associations only remained in the overweight group (crude model, 0.29 SDS; 95% CI, 0.05 ; 0.53, adjusted model, 0.35 SDS; 95% CI, 0.09 ; 0.61 and fully adjusted model, 0.44 SDS; 95% CI, 0.15 ; 0.72) (**Table 2**). Total-cholesterol, HDL-c, LDL-c, non-HDL-c concentrations and the TG/HDL-c ratio in early pregnancy were not associated with CRL. We tested for multicollinearity using the tolerance statistic. As tolerance was >0.20 for all variables in our models, multicollinearity was unlikely.

Sensitivity analysis demonstrated that the associations between triglycerides and remnant cholesterol and embryonic growth attenuated and were no longer significant when the analyses were split for gestational age 10-12 weeks and 12-14 weeks (**Table S3**). Complete case analysis showed similar results to those presented in **Table 2** (data not shown). Also, sensitivity analyses were performed in which we examined the effect of the lowest lipid concentrations within the study population. When investigating the association between the lowest 5% lipid concentrations and embryonic growth, no significant associations were observed (fully adjusted model triglycerides, -0.16 SDS; 95% CI, -0.38 ; 0.13, and fully adjusted model remnant cholesterol, -0.13 SDS; 95% CI, -0.29 ; 0.20, respectively) (**Table S4**).

# Discussion

### Main findings

We showed that both maternal triglycerides and remnant cholesterol in early pregnancy are positively associated with embryonic growth, especially in overweight women and even after adjustment for glucose concentrations.(36)

Lipids such as triglycerides and cholesterol reach the developing embryo or fetus through different mechanisms, which change over the course of pregnancy. In the first 12 weeks of pregnancy, the placenta is developing and not fully functional.(37) In this period, the developing embryo is dependent on the yolk sac and uterine glands for the storage and transport of nutrition.(38, 39) The yolk sac transports maternal lipids into the vitelline vessels that are connected with the circulation of the embryo.(40) Animal studies showed that as the maternal serum lipid concentrations increased, so did the concentrations in the yolk sac, and consequently the secretion by the yolk sac into the embryo.(41) This indicates that the lipid transport to the embryo is dependent on maternal serum lipid concentrations. For triglycerides to pass the yolk sac membrane, they have to be hydrolyzed into free fatty acids by placental lipases.(42) From animal studies it is known that during embryonic growth, approximately 90% of the total energy requirement is derived from yolk lipid fatty acid oxidation.(43) This indicates triglycerides have an important role as energy source in the development of an embryo, supporting our positive association between triglycerides and embryonic growth. Our findings are also in line with the outcomes of a study that demonstrates triglycerides are an important predictor of newborn body fat, even exceeding maternal glucose concentrations.(15) Additionally, since triglycerides and remnant cholesterol only make up a small part of the total cholesterol content, it could explain why we did not find a positive association between the lipid concentrations of LDL-c and HDL-c and total cholesterol concentrations with embryonic growth.

Strikingly, our demonstrated associations between maternal serum lipid concentrations and embryonic growth were most prominent in overweight women. This could be explained by the strong association between both obesity and insulin resistance, and insulin resistance and remnant cholesterol.(44-46) However, we were not able to verify this because in this study, the gold standard for the assessment of insulin resistance, the hyperinsulinemic-euglycemic clamp, was not utilized.(47)

In the performed sensitivity analyses, we did not find associations between the very high versus low lipid concentrations (lowest 5%) and embryonic growth. This could be explained by the fact that there are few cases with lipid concentrations below the 5th percentile (number of participants per type of lipid: range 67 – 73), lowering the statistical power to detect statistically significant differences.

### Strengths & limitations

To our knowledge, this study is the first to assess a broad spectrum of the maternal lipid profile in association with growth in early pregnancy. A limitation is that blood samples were obtained in a non-fasting state, which may have led to an underestimation of the observed associations. However, several large-scale, population-based studies have established that plasma lipids change only modestly in response to normal food intake.(48-55) It is therefore stated that only if nonfasting plasma triglycerides are > 5 mmol/L, a fasting blood sample could be considered.(53) In our study, no women had triglycerides that exceeded 5 mmol/L. Moreover, the use of non-fasting samples better reflects the normal physiological state in pregnant women.

A second limitation, is that embryonic growth was measured only once. Therefore, no patterns of embryonic growth could be assessed. Also no information on (changes in) pre-pregnancy lipid concentrations was available. We therefore cannot investigate the effect of preconceptional lipid concentrations on embryonic growth. Next, the use of MoM’s in the analyses makes it harder to clinically interpret the associations. However, these MoM’s enable to compare the different lipid concentrations to each other. The effect sizes for the association between triglycerides and remnant cholesterol and embryonic growth are comparable.

### Moreover, there might be the issue of response bias or self-selection, which is known to happen in cohort studies. Indeed, the median BMI of 22.6 within our study population is within the healthy range and the majority of women did not smoke during pregnancy (73.9%) (Table S1). Indeed, most of the measured maternal lipid concentrations are within the recommended ranges for the first trimester of pregnancy.(35) The selection of a relatively healthy study population did thus not allow to investigate the associations of extreme dyslipidemia. This might imply that effects in the general population with more and severe dyslipidemia may be even larger, and thus has affected the generalizability of our results. Finally, the observational nature of this study does not allow for inference of causality.

### Interpretation

Our findings demonstrate that the previously established associations between higher maternal lipid concentrations and adverse birth outcomes may already be present during the first trimester of pregnancy.(12, 13, 23, 56) It is also in line with the Developmental Origins of Health and Disease (DOHaD) theory, which states that adverse influences in early pregnancy have the potential to affect the change of adverse birth outcomes.(19) The finding that specifically both triglycerides and remnant cholesterol are associated with embryonic growth are not surprising, as plasma triglycerides and remnant cholesterol are highly correlated.(57) Remnant cholesterol is the cholesterol content of triglyceride-rich lipoproteins. In a clinical setting, triglycerides are even proposed as a surrogate marker of remnant cholesterol.(58, 59) Additionally, our results emphasize the potential of triglycerides and remnant cholesterol as markers for first trimester growth.

To unravel the mechanisms of nutrient transport from mother to embryo, and especially lipid transport, more fundamental research is needed. Also changes in nutrient transport due to the switch from yolk sac and uterine glands to the placenta as main nutrient transporter is interesting. Second, due to the small measures of the CRL, the measurement ranges are also small. However, research with repeated CRL measurements would make it possible to investigate embryonic growth patterns.

### Conclusion

The positive association between maternal lipids and early growth in pregnancy, especially in overweight women, emphasizes the importance of healthy maternal nutrition and a healthy weight. We propose maternal serum lipids concentrations, especially triglycerides and remnant cholesterol, may be a marker for early fetal growth. Additionally, they are potentially new targets for an early intervention in overweight pregnant women to prevent excess fetal growth.

**Acknowledgements**

We acknowledge the participation of all participants and the contribution of the general practitioners, hospitals, midwives, and pharmacies in Rotterdam and all those concerned in the Generation R Study. The Generation R Study was conducted by the Erasmus Medical Center, Rotterdam, The Netherlands, in close collaboration with the School of Law and the Faculty of Social Sciences of Erasmus University, Rotterdam, The Netherlands. Furthermore, we gratefully acknowledge Municipal Health Service, Rotterdam area; the Rotterdam Homecare Foundation; the Stichting Trombosedienst and Arts laboratorium Rijnmond, Rotterdam. Lipid measurements were performed by Jeanette Touw. The Generation R Study was made possible by financial support from the Erasmus Medical Center, Erasmus University Rotterdam, and The Netherlands Organization for Health Research and Development, The Netherlands Organization for Scientific Research, the Ministry of Health, Welfare, and Sport, and the Ministry of Youth and Families.

**Disclosure of Interests**

The authors report no conflict of interest.

**Contribution to Authorship**

DG analysed the data and wrote the article. AP and DW contributed to the design of the article and assisted with the analyses and writing of the article. YR contributed to the interpretation of the data and gave input at all stages of the study. JRvL and ES contributed to the design of the paper, writing of the article, interpretation of the data, revisions and gave input at all stages of the study. The authors read and approved the final manuscript.

**Details of Ethics Approval**

The study protocol has been approved by the Medical Ethics Committee of the Erasmus University Medical Centre (Erasmus MC), Rotterdam (MEC-2007-413). Written informed consent was obtained from all participants.

**Funding**

The Generation R Study was made possible by financial support from the Erasmus Medical Center, Erasmus University Rotterdam, and The Netherlands Organization for Health Research and Development, The Netherlands Organization for Scientific Research, the Ministry of Health, Welfare, and Sport, and the Ministry of Youth and Families.

**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Calculation of different lipid measurements.

**Table S2.** Baseline characteristics of women included and excluded in this study.

**Table S3.** Associations of maternal lipid profile in early pregnancy with crown-rump length, split by period 1 (10 to 12 weeks GA) and 2 (12 to 14 weeks GA).

**Table S4.** Associations of low lipid concentrations (<5th percentile) with embryonic growth.

**Table S5.** Observed and expected values for confounders.

**Figure S1.** Directed Acyclic Graph (DAG) representing the pathways between the maternal lipid profile and embryonic growth. Abbreviations: BMI, body mass index; CRL, crown-rump length. These confounders (red) were included in the adjusted regression models.

# References

1. Herrera E, Desoye G. Maternal and fetal lipid metabolism under normal and gestational diabetic conditions. Horm Mol Biol Clin Investig. 2016;26(2):109-27.

2. Baardman ME, Kerstjens-Frederikse WS, Berger RM, Bakker MK, Hofstra RM, Plosch T. The role of maternal-fetal cholesterol transport in early fetal life: current insights. Biol Reprod. 2013;88(1):24.

3. Nezil FA, Bloom M. Combined influence of cholesterol and synthetic amphiphillic peptides upon bilayer thickness in model membranes. Biophys J. 1992;61(5):1176-83.

4. Grimes SB, Wild R. Effect of Pregnancy on Lipid Metabolism and Lipoprotein Levels. 2000.

5. Lippi G, Albiero A, Montagnana M, Salvagno GL, Scevarolli S, Franchi M, et al. Lipid and lipoprotein profile in physiological pregnancy. Clin Lab. 2007;53(3-4):173-7.

6. Smith DW, Lemli L, Opitz JM. A Newly Recognized Syndrome of Multiple Congenital Anomalies. J Pediatr. 1964;64:210-7.

7. Cooper MK, Wassif CA, Krakowiak PA, Taipale J, Gong R, Kelley RI, et al. A defective response to Hedgehog signaling in disorders of cholesterol biosynthesis. Nat Genet. 2003;33(4):508-13.

8. Edison RJ, Berg K, Remaley A, Kelley R, Rotimi C, Stevenson RE, et al. Adverse birth outcome among mothers with low serum cholesterol. Pediatrics. 2007;120(4):723-33.

9. Pecks U, Brieger M, Schiessl B, Bauerschlag DO, Piroth D, Bruno B, et al. Maternal and fetal cord blood lipids in intrauterine growth restriction. J Perinat Med. 2012;40(3):287-96.

10. Sattar N, Greer IA, Galloway PJ, Packard CJ, Shepherd J, Kelly T, et al. Lipid and lipoprotein concentrations in pregnancies complicated by intrauterine growth restriction. J Clin Endocrinol Metab. 1999;84(1):128-30.

11. Catov JM, Bodnar LM, Kip KE, Hubel C, Ness RB, Harger G, et al. Early pregnancy lipid concentrations and spontaneous preterm birth. Am J Obstet Gynecol. 2007;197(6):610 e1-7.

12. Vrijkotte TG, Krukziener N, Hutten BA, Vollebregt KC, van Eijsden M, Twickler MB. Maternal lipid profile during early pregnancy and pregnancy complications and outcomes: the ABCD study. J Clin Endocrinol Metab. 2012;97(11):3917-25.

13. Adank MC, Benschop L, Kors AW, Peterbroers KR, Smak Gregoor AM, Mulder MT, et al. Maternal lipid profile in early pregnancy is associated with foetal growth and the risk of a child born large-for-gestational age: a population-based prospective cohort study : Maternal lipid profile in early pregnancy and foetal growth. BMC Med. 2020;18(1):276.

14. Harmon KA, Gerard L, Jensen DR, Kealey EH, Hernandez TL, Reece MS, et al. Continuous glucose profiles in obese and normal-weight pregnant women on a controlled diet: metabolic determinants of fetal growth. Diabetes Care. 2011;34(10):2198-204.

15. Barbour LA, Farabi SS, Friedman JE, Hirsch NM, Reece MS, Van Pelt RE, et al. Postprandial Triglycerides Predict Newborn Fat More Strongly than Glucose in Women with Obesity in Early Pregnancy. Obesity (Silver Spring). 2018;26(8):1347-56.

16. Barbour LA. Changing perspectives in pre-existing diabetes and obesity in pregnancy: maternal and infant short- and long-term outcomes. Curr Opin Endocrinol Diabetes Obes. 2014;21(4):257-63.

17. Chooi YC, Ding C, Magkos F. The epidemiology of obesity. Metabolism. 2019;92:6-10.

18. Klop B, Elte JWF, Cabezas MC. Dyslipidemia in obesity: mechanisms and potential targets. Nutrients. 2013;5(4):1218-40.

19. Barker DJ. The origins of the developmental origins theory. J Intern Med. 2007;261(5):412-7.

20. Barker DJ, Osmond C. Diet and coronary heart disease in England and Wales during and after the second world war. J Epidemiol Community Health. 1986;40(1):37-44.

21. Barker DJ, Gluckman PD, Godfrey KM, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. Lancet. 1993;341(8850):938-41.

22. Mook-Kanamori DO, Steegers EA, Eilers PH, Raat H, Hofman A, Jaddoe VW. Risk factors and outcomes associated with first-trimester fetal growth restriction. JAMA. 2010;303(6):527-34.

23. van Uitert EM, Exalto N, Burton GJ, Willemsen SP, Koning AH, Eilers PH, et al. Human embryonic growth trajectories and associations with fetal growth and birthweight. Hum Reprod. 2013;28(7):1753-61.

24. Jaddoe VW, Mackenbach JP, Moll HA, Steegers EA, Tiemeier H, Verhulst FC, et al. The Generation R Study: Design and cohort profile. Eur J Epidemiol. 2006;21(6):475-84.

25. Kruithof CJ, Kooijman MN, van Duijn CM, Franco OH, de Jongste JC, Klaver CC, et al. The Generation R Study: Biobank update 2015. Eur J Epidemiol. 2014;29(12):911-27.

26. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499-502.

27. Gaillard R, Durmus B, Hofman A, Mackenbach JP, Steegers EA, Jaddoe VW. Risk factors and outcomes of maternal obesity and excessive weight gain during pregnancy. Obesity (Silver Spring). 2013;21(5):1046-55.

28. Verburg BO, Mulder PG, Hofman A, Jaddoe VW, Witteman JC, Steegers EA. Intra- and interobserver reproducibility study of early fetal growth parameters. Prenat Diagn. 2008;28(4):323-31.

29. Robinson HP, Fleming JE. A critical evaluation of sonar "crown-rump length" measurements. Br J Obstet Gynaecol. 1975;82(9):702-10.

30. Verburg BO, Steegers EA, De Ridder M, Snijders RJ, Smith E, Hofman A, et al. New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study. Ultrasound Obstet Gynecol. 2008;31(4):388-96.

31. Niklasson A, Ericson A, Fryer JG, Karlberg J, Lawrence C, Karlberg P. An update of the Swedish reference standards for weight, length and head circumference at birth for given gestational age (1977-1981). Acta Paediatr Scand. 1991;80(8-9):756-62.

32. Slama R, Khoshnood B, Kaminski M. How to control for gestational age in studies involving environmental effects on fetal growth. Environ Health Perspect. 2008;116(7):A284; author reply A-A5.

33. Textor J, van der Zander B, Gilthorpe MS, Liskiewicz M, Ellison GT. Robust causal inference using directed acyclic graphs: the R package 'dagitty'. Int J Epidemiol. 2016;45(6):1887-94.

34. Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ. 2009;338:b2393.

35. Abbassi-Ghanavati M, Greer LG, Cunningham FG. Pregnancy and laboratory studies: a reference table for clinicians. Obstet Gynecol. 2009;114(6):1326-31.

36. Wahab RJ, Scholing JM, Gaillard R. Maternal early pregnancy dietary glycemic index and load, fetal growth, and the risk of adverse birth outcomes. Eur J Nutr. 2020.

37. Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. Philos Trans R Soc Lond B Biol Sci. 2015;370(1663):20140066.

38. Burton GJ, Jauniaux E, Charnock-Jones DS. Human early placental development: potential roles of the endometrial glands. Placenta. 2007;28 Suppl A:S64-9.

39. Burton GJ, Watson AL, Hempstock J, Skepper JN, Jauniaux E. Uterine glands provide histiotrophic nutrition for the human fetus during the first trimester of pregnancy. J Clin Endocrinol Metab. 2002;87(6):2954-9.

40. Lanford RE, Bronson DL, Estlack LE, Wians FH, Jr. Plasma protein and apolipoprotein synthesis by human yolk sac carcinoma cells in vitro. In Vitro Cell Dev Biol. 1991;27A(3 Pt 1):205-10.

41. McConihay JA, Horn PS, Woollett LA. Effect of maternal hypercholesterolemia on fetal sterol metabolism in the Golden Syrian hamster. J Lipid Res. 2001;42(7):1111-9.

42. Powell KA, Deans EA, Speake BK. Fatty acid esterification in the yolk sac membrane of the avian embryo. J Comp Physiol B. 2004;174(2):163-8.

43. Noble RC, Cocchi M. Lipid metabolism and the neonatal chicken. Prog Lipid Res. 1990;29(2):107-40.

44. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature. 2001;414(6865):782-7.

45. Ohnishi H, Saitoh S, Takagi S, Ohata J, Isobe T, Kikuchi Y, et al. Relationship between insulin-resistance and remnant-like particle cholesterol. Atherosclerosis. 2002;164(1):167-70.

46. Schaefer EJ, McNamara JR, Shah PK, Nakajima K, Cupples LA, Ordovas JM, et al. Elevated remnant-like particle cholesterol and triglyceride levels in diabetic men and women in the Framingham Offspring Study. Diabetes Care. 2002;25(6):989-94.

47. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol. 1979;237(3):E214-23.

48. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. Circulation. 2008;118(20):2047-56.

49. Langsted A, Nordestgaard BG. Nonfasting lipids, lipoproteins, and apolipoproteins in individuals with and without diabetes: 58 434 individuals from the Copenhagen General Population Study. Clin Chem. 2011;57(3):482-9.

50. Mora S, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. Circulation. 2008;118(10):993-1001.

51. Sidhu D, Naugler C. Fasting time and lipid levels in a community-based population: a cross-sectional study. Arch Intern Med. 2012;172(22):1707-10.

52. Steiner MJ, Skinner AC, Perrin EM. Fasting might not be necessary before lipid screening: a nationally representative cross-sectional study. Pediatrics. 2011;128(3):463-70.

53. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points-a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. Eur Heart J. 2016;37(25):1944-58.

54. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points-a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. Eur Heart J. 2016;37(25):1944-58.

55. Langsted A, Nordestgaard BG. Nonfasting versus fasting lipid profile for cardiovascular risk prediction. Pathology. 2019;51(2):131-41.

56. Gaillard R, Steegers EA, de Jongste JC, Hofman A, Jaddoe VW. Tracking of fetal growth characteristics during different trimesters and the risks of adverse birth outcomes. Int J Epidemiol. 2014;43(4):1140-53.

57. Varbo A, Benn M, Tybjærg-Hansen A, Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG. Remnant cholesterol as a causal risk factor for ischemic heart disease. J Am Coll Cardiol. 2013;61(4):427-36.

58. Varbo A, Nordestgaard BG. Remnant lipoproteins. Current Opinion in Lipidology. 2017;28(4):300-7.

59. Nordestgaard BG, Varbo A. Triglycerides and cardiovascular disease. Lancet. 2014;384(9943):626-35.

**Table 1.** Baseline characteristics of the study population.

|  |  |  |
| --- | --- | --- |
| **Maternal characteristics**  **N=1474** |  | **Reference ranges**  **lipid concentrations(35)** |
| Age at intake, years | 30.8 (4.6) |  |
| Pre-pregnancy BMI, kg/m2 | 22.6 (18.9 ; 29.9) |  |
| Overweight women, n (%) | 347 (25.4) |  |
| Parity (nulliparous) | 877 (59.5) |  |
| Educational level (high) | 785 (53.3) |  |
| Ethnicity (Dutch and Western) | 1060 (71.9) |  |
| Smoking (continued smoking in pregnancy) | 232 (15.7) |  |
| Alcohol (continued alcohol consumption in pregnancy) | 650 (44.1) |  |
| Folic acid supplement use (start preconceptional) | 756 (51.3) |  |
| Embryonic sex (male) | 723 (49.1) |  |
| Glucose, mmol/L | 4.41 (0.83) |  |
| Total cholesterol, mmol/L | 4.69 (0.81) | 3.65 – 5.44 |
| Triglycerides, mmol/L | 1.19 (0.70 ; 2.24) | 0.50 – 1.80 |
| HDL-c, mmol/L | 1.77 (0.34) | 1.04 – 2.02 |
| LDL-c, mmol/L | 2.34 (0.67) | 1.55 – 3.96 |
| Remnant cholesterol, mmol/L | 0.54 (0.32 ; 1.01) | - |
| Non-HDL-c, mmol/L | 2.93 (0.77) | - |
| TG/HDL-c ratio | 0.67 (0.34 ; 1.63) | - |

Abbreviations: HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TG, triglycerides; BMI, body mass index. Values are means (SD) for continuous variables with a normal distribution, or medians (90% range) for continuous variables with a skewed distribution. Confounders were imputed. Non-imputed values represent valid percentages.

**Table 2.** Associations of maternal lipid profile in early pregnancy with embryonic growth, by normal weight versus overweight.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Study population (n=1474)** | **Crude model** | | | **Adjusted model** | | | **Fully adjusted model** | | |
|  | **Whole group** | **Normal weight** | **Overweight** | **Whole group** | **Normal weight** | **Overweight** | **Whole group** | **Normal weight** | **Overweight** |
|  | **β (95% CI)** | **β (95% CI)** | **β (95% CI)** | **β (95% CI)** | **β (95% CI)** | **β (95% CI)** | **β (95% CI)** | **β (95% CI)** | **β (95% CI)** |
| **Total cholesterol, mmol/L** |  |  |  |  |  |  |  |  |  |
| Lowest tertile MoM (<0.94) | -0.03 (-0.15 ; 0.09) | -0.08 (-0.23 ; 0.07) | 0.01 (-0.26 ; 0.28) | -0.07 (-0.20 ; 0.07) | -0.08 (-0.24 ; 0.09) | -0.13 (-0.44 ; 0.17) | -0.06 (-0.20 ; 0.07) | -0.07 (-0.24 ; 0.10) | -0.14 (-0.46 ; 0.17) |
| Second tertile MoM (0.94 – 1.08) | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* |
| Highest tertile MoM (>1.08) | 0.02 (-0.10 ; 0.14) | -0.001 (-0.15 ; 0.15) | 0.12 (-0.13 ; 0.37) | -0.02 (-0.15 ; 0.11) | -0.03 (-0.20 ; 0.13) | -0.02 (-0.30 ; 0.26) | -0.02 (-0.15 ; 0.11) | -0.05 (-0.22 ; 0.12) | -0.04 (-0.32 ; 0.25) |
| Trend analyses MoM | 0.09 (-0.19 ; 0.37) | 0.21 (-0.15 ; 0.56) | 0.15 (-0.47 ; 0.77) | 0.13 (-0.19 ; 0.44) | 0.13 (-0.26 ; 0.51) | 0.13 (-0.56 ; 0.82) | 0.11 (-0.21 ; 0.43) | -0.40 (-1.38 ; 0.57) | 0.07 (-0.32 ; 0.47) |
| **Triglycerides, mmol/L** |  |  |  |  |  |  |  |  |  |
| Lowest tertile MoM (<0.87) | -0.08 (-0.20 ; 0.04) | -0.12 (-0.26 ; 0.03) | 0.06 (-0.24 ; 0.36) | -0.08 (-0.21 ; 0.06) | -0.09 (-0.25 ; 0.07) | 0.03 (-0.30 ; 0.36) | -0.08 (-0.21 ; 0.06) | -0.09 (-0.25 ; 0.07) | 0.05 (-0.28 ; 0.39) |
| Second tertile MoM (0.87 – 1.18) | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* |
| Highest tertile MoM (>1.18) | 0.04 (-0.08 ; 0.16) | -0.01 (-0.16 ; 0.15) | 0.18 (-0.07 ; 0.43) | 0.04 (-0.10 ; 0.18) | -0.06 (-0.23 ; 0.11) | 0.23 (-0.05 ; 0.50) | 0.06 (-0.07 ; 0.20) | -0.06 (-0.23 ; 0.12) | 0.28 (-0.01 ; 0.56) |
| Trend analyses MoM | **0.16 (0.05 ; 0.38)** | 0.12 (-0.03 ; 0.28) | **0.29 (0.04 ; 0.53)** | **0.15 (0.01 ; 0.28)** | 0.02 (-0.15 ; 0.18) | **0.35 (0.10 ; 0.61)** | **0.17 (0.03 ; 0.30)** | 0.03 (-0.15 ; 0.20) | **0.44 (0.15 ; 0.72)** |
| **HDL-c, mmol/L** |  |  |  |  |  |  |  |  |  |
| Lowest tertile MoM (<0.92) | 0.04 (-0.08 ; 0.16) | 0.01 (-0.14 ; 0.17) | 0.18 (-0.08 ; 0.43) | 0.08 (-0.06 ; 0.22) | 0.06 (-0.11 ; 0.24) | 0.13 (-0.16 ; 0.43) | 0.10 (-0.04 ; 0.23) | 0.09 (-0.09 ; 0.26) | 0.15 (-0.15 ; 0.45) |
| Second tertile MoM (0.92 – 1.08) | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* |
| Highest tertile MoM (>1.08) | 0.04 (-0.08 ; 0.16) | 0.07 (-0.08 ; 0.22) | -0.03 (-0.31 ; 0.25) | 0.05 (-0.08 ; 0.19) | 0.06 (-0.10 ; 0.22) | -0.03 (-0.34 ; 0.28) | 0.05 (-0.08 ; 0.18) | 0.07 (-0.09 ; 0.23) | -0.05 (-0.37 ; 0.27) |
| Trend analyses MoM | -0.01 (-0.26 ; 0.25) | 0.17 (-0.16 ; 0.50) | -0.51 (-1.07 ; 0.06) | 0.03 (-0.28 ; 0.32) | 0.18 (-0.20 ; 0.55) | -0.39 (-1.03 ; 0.25) | -0.01 (-0.32 ; 0.29) | 0.15 (-0.23 ; 0.54) | -0.48 (-1.16 ; 0.20) |
| **LDL-c, mmol/L** |  |  |  |  |  |  |  |  |  |
| Lowest tertile MoM (<0.88) | 0.04 (-0.08 ; 0.17) | 0.01 (-0.14 ; 0.16) | -0.09 (-0.37 ; 0.19) | -0.01 (-0.15 ; 0.13) | -0.06 (-0.22 ; 0.11) | -0.05 (-0.37 ; 0.27) | -0.01 (-0.15 ; 0.13) | -0.04 (-0.21 ; 0.13) | -0.05 (-0.38 ; 0.28) |
| Second tertile MoM (0.88 – 1.13) | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* |
| Highest tertile MoM (>1.13) | 0.03 (-0.09 ; 0.15) | 0.03 (-0.12 ; 0.19) | 0.03 (-0.22 ; 0.27) | -0.01 (-0.14 ; 0.12) | -0.04 (-0.21 ; 0.13) | 0.02 (-0.25 ; 0.30) | -0.01 (-0.15 ; 0.12) | -0.05 (-0.22 ; 0.12) | 0.01 (-0.27 ; 0.29) |
| Trend analyses MoM | -0.02 (-0.18 ; 0.15) | 0.03 (-0.18; 0.25) | 0.10 (-0.26 ; 0.47) | 0.01 (-0.18 ; 0.20) | 0.03 (-0.20 ; 0.26) | 0.02 (-0.39 ; 0.43) | 0.004 (-0.19 ; 0.19) | -0.01 (-0.24 ; 0.23) | 0.001 (-0.42 ; 0.42) |
| **Remnant cholesterol, mmol/L** |  |  |  |  |  |  |  |  |  |
| Lowest tertile MoM (<0.87) | -0.08 (-0.20 ; 0.04) | -0.13 (-0.28 ; 0.02) | 0.09 (-0.21 ; 0.38) | -0.08 (-0.21 ; 0.06) | -0.10 (-0.25 ; 0.06) | 0.06 (-0.27 ; 0.39) | -0.08 (-0.21 ; 0.06) | -0.10 (-0.26 ; 0.06) | 0.09 (-0.25 ; 0.42) |
| Second tertile MoM (0.87 – 1.17) | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* |
| Highest tertile MoM (>1.17) | 0.05 (-0.08 ; 0.17) | -0.04 (-0.20 ; 0.12) | 0.24 (-0.01 ; 0.49) | 0.04 (-0.10 ; 0.18) | -0.08 (-0.25 ; 0.09) | 0.27 (-0.003 ; 0.55) | 0.06 (-0.08 ; 0.20) | -0.08 (-0.25 ; 0.10) | **0.32 (0.04 ; 0.61)** |
| Trend analyses MoM | **0.17 (0.05 ; 0.29)** | 0.13 (-0.02 ; 0.29) | **0.29 (0.05 ; 0.53)** | **0.15 (0.02 ; 0.29)** | 0.02 (-0.15 ; 0.19) | **0.35 (0.09 ; 0.61)** | **0.17 (0.04 ; 0.31)** | 0.03 (-0.15 ; 0.20) | **0.44 (0.15 ; 0.72)** |
| **Non-HDL-c, mmol/L** |  |  |  |  |  |  |  |  |  |
| Lowest tertile MoM (<0.89) | 0.03 (-0.09 ; 0.15) | 0.02 (-0.13 ; 0.17) | -0.13 (-0.41 ; 0.15) | -0.01 (-0.14 ; 0.13) | -0.003 (-0.17 ; 0.16) | -0.22 (-0.54 ; 0.09) | -0.01 (-0.15 ; 0.13) | 0.01 (-0.16 ; 0.18) | -0.23 (-0.55 ; 0.10) |
| Second tertile MoM (0.89 – 1.11) | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* |
| Highest tertile MoM (>1.11) | 0.07 (-0.05 ; 0.19) | 0.09 (-0.06 ; 0.25) | 0.03 (-0.22 ; 0.27) | 0.05 (-0.09 ; 0.18) | 0.03 (-0.14 ; 0.20) | -0.03 (-0.30 ; 0.24) | 0.05 (-0.09 ; 0.18) | 0.02 (-0.16 ; 0.19) | -0.04 (-0.32 ; 0.24) |
| Trend analyses MoM | 0.06 (-0.12 ; 0.24) | 0.09 (-0.14 ; 0.32) | 0.25 (-0.15 ; 0.66) | 0.08 (-0.13 ; 0.28) | 0.03 (-0.21 ; 0.28) | 0.21 (-0.24 ; 0.67) | 0.08 (-0.13 ; 0.28) | 0.01 (-0.25 ; 0.26) | 0.21 (-0.26 ; 0.68) |
| **TG/HDL-c ratio** |  |  |  |  |  |  |  |  |  |
| Lowest tertile MoM (<0.83) | -0.07 (-0.19 ; 0.05) | -0.01 (-0.16 ; 0.13) | -0.15 (-0.45 ; 0.15) | -0.04 (-0.17 ; 0.09) | 0.003 (-0.15 ; 0.16) | -0.11 (-0.44 ; 0.22) | -0.04 (-0.17 ; 0.09) | 0.01 (-0.15 ; 0.17) | -0.15 (-0.49 ; 0.18) |
| Second tertile MoM (0.83 – 1.23) | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* | *Reference* |
| Highest tertile MoM (>1.23) | 0.01 (-0.11 ; 0.13) | 0.02 (-0.14 ; 0.18) | 0.10 (-0.16 ; 0.35) | 0.05 (-0.09 ; 0.19) | -0.01 (-0.19 ; 0.16) | 0.26 (-0.03 ; 0.54) | 0.07 (-0.07 ; 0.21) | 0.002 (-0.18 ; 0.18) | 0.28 (-0.01 ; 0.57) |
| Trend analyses MoM | 0.06 (-0.01 ; 0.13) | 0.03 (-0.06 ; 0.12) | **0.15 (0.01 ; 0.29)** | 0.04 (-0.03 ; 0.12) | -0.02 (-0.12 ; 0.07) | **0.16 (0.01 ; 0.31)** | 0.06 (-0.02 ; 0.13) | -0.01 (-0.11 ; 0.09) | **0.26 (0.06 ; 0.45)** |

Abbreviations: CI: confidence interval, HDL-c: high-density lipoprotein cholesterol, LDL-c: low-density lipoprotein cholesterol, MoM: Multiple of the median, n.a.: not applicable. Values are regression coefficients with the 95% CI and are based on linear regression models. Crude model: univariate regression analysis. Adjusted model: basic model additionally adjusted for maternal age, parity, educational level, ethnicity, smoking and folic acid supplement use. Fully adjusted model: adjusted model additionally adjusted for maternal glucose concentrations. Estimates of MoM trend analyses represent the unit increase in the outcome per 1 multiple of the median increase in lipid, compared to the reference category.

# 