Gestational diabetes, but not pre-pregnancy overweight predicts cardio-metabolic markers in offspring twenty years later

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Abstract

Context:

Maternal gestational diabetes (GDM) and pre-pregnancy overweight/obesity (body mass index, BMI ≥25kg/m^2) may adversely affect offspring cardio-metabolic health.

Objective:

To assess associations of maternal GDM and pre-pregnancy overweight/obesity with adult offspring cardio-metabolic risk factors.

Design:

Longitudinal cohort study (ESTER and AYLS).

Setting:

Province of Uusimaa and Northern Finland.

Participants:

At mean age 24.1 years (SD 1.3), we classified offspring to offspring of mothers with 1) GDM regardless of pre-pregnancy BMI (OGDM; n=193), 2) normoglycemic mothers with pre-pregnancy overweight/obesity (ONO, n=157) and 3) normoglycemic mothers with pre-pregnancy BMI<25kg/m^2 (controls, n=556).

Main Outcome Measures:

We assessed cardio-metabolic biomarkers from blood and measured resting blood pressure and heart rate.

Results:

Compared with controls, OGDM and ONO had higher fasting glucose [1.6% (95% confidence interval 0.1, 3.1)]; [2.3% (0.5, 4.3), respectively]; and insulin [12.7% (4.4, 21.9)]; [8.7% (0.2, 17.8)]. These differences attenuated to non-significance when adjusted for confounders and/or current offspring characteristics including BMI or body fat percentage. OGDM

showed lower sex hormone binding globulin [SHBG; men: -12.4% (-20.2, -3.9), women: -33.2% (-46.3, -16.8)], high-density lipoprotein [-6.6% (-10.9, -2.2)] and apolipoprotein A1 [-4.5% (-7.5, -1.4), these differences survived the aforementioned adjustments. Heart rate and other biomarkers were similar between groups.

Conclusions:

Adult offspring of mothers with GDM have increased markers of insulin resistance and a more atherogenic lipid profile; these are only partly explained by confounders or current offspring adiposity. Maternal pre-pregnancy overweight/obesity is associated with impaired offspring glucose regulation, which is explained by confounders and/or current adiposity.

99 Précis

100 We measured cardio-metabolic markers in offspring of mothers with GDM, pre-pregnancy

101 overweight and controls. GDM was, unlike maternal overweight, linked with an unhealthier

102 cardio-metabolic profile.

103

Introduction

Metabolic abnormalities, including alterations in lipid and carbohydrate metabolism, are likely among women with gestational diabetes (GDM), overweight (body mass index, BMI ≥ 25kg/m^2) or obesity (BMI ≥ 30kg/m^2) during pregnancy. At a critical period of fetal development, exposure to e.g. hyperglycemia, may induce long-term impacts on the fetus by creating a metabolic memory, previously described as fetal programming (1). Prenatal exposure to a hyperglycemic environment is known to alter growth trajectories and homeostatic regulatory mechanisms, and these changes predispose offspring to epigenetic changes (2, 3). It is likely that both maternal GDM and overweight/obesity provide a prenatal environment making the fetus susceptible to adverse in utero programming. This may cause increased risk of next-generation overweight and obesity, and result in an intergenerational cycle of obesity and insulin resistance.

Offspring of mothers with GDM show markers of insulin resistance and metabolic syndrome, higher BMI and waist circumference by adolescence (4). Further, adult offspring of mothers with GDM represent a risk group for overweight and metabolic syndrome (5). Exposure to maternal obesity during pregnancy also affects offspring health, particularly with increased risk for obesity and metabolic sequelae (6). In a recent review by Nicholas et al. maternal obesity increased offspring risk of both obesity and insulin resistance in childhood, adolescence and adulthood (7). Previous studies have also linked high maternal pre-pregnancy BMI to unfavorable offspring body composition in infancy (8), childhood (9), adolescence (10) and young adulthood (11). Even at a mean age of 62 years a less favorable body composition and higher BMI in the offspring has been reported (12). However, it is not always clear to what extent the associations represent genetic or lifestyle factors, shared by the family, and to what extent they represent causal programming effects. Moreover, it is

uncertain to what extent these offspring consequences are a result of exposure to maternal GDM and to what extent maternal overweight/obesity.

Despite previously well-established data on the strong influence of maternal pre-pregnancy overweight/obesity or GDM on offspring's increased risk for obesity and metabolic sequelae during childhood, it is less clear whether the effects extend into adult age.

Taking these observations together, we hypothesized that maternal GDM and pre-pregnancy overweight/obesity may affect cardio-metabolic risk factors in adult offspring. In this study, we investigate the impact of exposure to maternal GDM or pre-pregnancy overweight/obesity on adult offspring cardio-metabolic health.

Materials and Methods

Participants

Participants of the current study come from two prospective birth cohorts (Figure 1) (11): the ESTER Maternal Pregnancy Disorders Study and the Arvo Ylppö Longitudinal Study (AYLS).

The ESTER Study consists of two arms (Figure 1): 1) ESTER Preterm Birth (13) and 2) ESTER Maternal Pregnancy Disorders arms. The present study is based on the latter arm. All ESTER study participants were born in the two northernmost provinces of Finland. Those born in 1985–1986 were recruited from the Northern Finland Birth Cohort 1986 (NFBC 1986) (14) and those born in 1987–1989 through the Finnish Medical Birth Register (FMBR) (13), as previously described (15). We selected all participants of the ESTER Maternal Pregnancy Disorders arm who were confirmed to have maternal GDM (n=157), regardless of the mother's pre-pregnancy BMI. Among ESTER clinical study participants invited as controls (15), participants were stratified into two groups: one group with maternal pre-pregnancy

overweight/obesity included offspring born at term to mothers with pre-pregnancy BMI ≥ 25kg/m^2 and no GDM (n=44), while the control group constituted the remaining controls, all with maternal pre-pregnancy BMI < 25kg/m^2 and no GDM (n=281).

All AYLS participants (Figure 1) were born in the province of Uusimaa, in Southern Finland between 1985 and 1986. This cohort consists of all live-born infants admitted to neonatal wards in obstetric units, or transferred to the neonatal intensive care unit of the Children's Hospital, Helsinki University Central Hospital within 10 days of their birth, with the population ranging from severely ill preterm infants to infants born at term, requiring only brief inpatient observation, and their controls, as previously described (16, 17). Of these AYLS cohort participants, with data available, we selected 1) all who were exposed to maternal GDM, at any maternal BMI (n=37), 2) those who had maternal BMI ≥ 25kg/m^2 and no GDM (n=109) and 3) controls (i.e. originally recruited as controls, maternal BMI < 25kg/m^2 and no GDM; n=266).

For all study participants, perinatal data were collected from healthcare records and questionnaires. Length of gestation, maternal GDM, hypertension (gestational or chronic) and preeclampsia (including superimposed) diagnoses were independently confirmed according to prevailing criteria by reviewing original hospital records (4, 18). Maternal GDM was screened for and diagnosed by oral glucose tolerance test (OGTT). Screening was performed in the maternal welfare clinics between 26 and 28 gestational weeks. Indications for screening were glucosuria, prior GDM, suspected fetal macrosomia, previous macrosomic infant (birth weight >4,500 g), maternal pre-pregnancy BMI ≥ 25 kg/m^2, and maternal age ≥ 40 years. The OGTT was performed after overnight fasting by using a 75-g oral glucose load. At the time of diagnosis in the 1980s, the following cutoff limits for GDM were used for venous blood glucose: >5.5 mmol/l at fasting, >11.0 mmol/l and >8.0 mmol/l,

1 hour and 2 hours after the glucose load, respectively. According to prevailing national guidelines, a diagnosis of GDM required a minimum of one abnormal value in the OGTT (4). For comparison, the International Association of Diabetes and Pregnancy Study Groups (IADPSG) Consensus Panel diagnostic criteria used today are set at fasting plasma glucose ≥5.1mmol/l, and ≥10.0 mmol/l and ≥8.5 mmol/l following a 75g oral glucose load (19). Offspring to mothers with type 1 (n=28) or 2 diabetes (n=1) were excluded from all analyses. We further excluded subjects who were pregnant (n=9) during the clinical examination, reported having cerebral palsy (n=8), mental disability (n=11) or severe physical disability (n=5), as these conditions might affect the measured outcomes. We categorized all ESTER and AYLS cohort participants who underwent biochemical measures into three groups: 1) offspring of mothers with GDM (OGDM) at any level of maternal BMI, 2) offspring of normoglycemic mothers with pre-pregnancy overweight/obesity (ONO) and 3) controls, i.e. offspring of mothers with pre-pregnancy BMI < 25kg/m^2 and no GDM. As a result, 906 subjects were included in the analyses; OGDM n = 193, ONO n= 157 and 556 controls.

Ethics

Our study protocol was in accordance with the Declaration of Helsinki. It was approved by the Ethics Committees of the University of Oulu, the Helsinki City Maternity Hospital, the Helsinki University Central Hospital and Jorvi Hospital, the Ethics Committee of the Northern Ostrobothnia Hospital District and the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District. Written informed consent was obtained from all participants. Because of individual participant consent, these data are not freely available. Researchers requesting data access are asked to contact the corresponding author. Requests may be subject to ethics review and/or participant's re-consent.

Measures and procedures

Anthropometry was measured during clinical examinations conducted in 2009-2011 for ESTER participants and during 2009-2012 for AYLS participants. Height was measured three times without socks and shoes, with a portable stadiometer. Weight was measured during the clinical visit. Most of our participants also underwent bioimpedance measurement and the bioimpedance devise (InBody 3.0, Biospace Co., Ltd., Seoul, Korea) contains a scale. For individuals who did not undergo bioimpedance we used an electronic scale. BMI was calculated using means of the repeated measurements [weight (kg) / height squared (m^2)].

All participants attended a clinical visit in the morning, after an overnight fast. They were examined by a trained study nurse. After a 5-minute rest in a sitting position, blood pressure was measured 3 times from the right upper arm using an automatic oscillometric blood pressure monitor (Omron M10-IT Intellisense, Omron Healthcare Co., Kyoto, Japan). All participants completed questionnaires regarding both participant and parental health status, including medical history and medications. Data on highest parental educational attainment were enquired and categorized into four levels (dummy coded) to serve as an indicator of childhood socioeconomic status.

Laboratory analyses

At the clinical visit venous blood samples were taken in a sitting position with a light stasis into a fluoride-citrate tube (Venosafe, Terumo Europa, Leuven, Belgium) for glucose assays and into a tube containing clot activator (Venosafe) for other assays. Fluoride-citrate plasma and serum were separated by centrifuging, frozen locally immediately after separation, and then transported frozen on dry ice to the biochemistry laboratory of the Genomics and

Biomarker Unit (former the Disease Risk Unit) at National Institute for Health and Welfare (Helsinki, Finland) and the Oulu University Hospital laboratory. All analyses were performed on a clinical chemistry analyzer (Architect ci8200 Abbott Laboratories, Abbott Park, Illinois, USA) at the biochemistry laboratory in the AYLS and ESTER studies, except for fasting plasma glucose, total cholesterol (TC), high- and low-density lipoprotein cholesterol (HDL-C and LDL-C), triglycerides (TGs), alanine aminotransferase (ALT), aspartate transaminase (AST), gamma glutamate (GT) and uric acid regarding ESTER study participants. In the ESTER study these samples were analyzed by using an Advia 2400 automatic chemical analyzer (Siemens Diagnostics, Terrytown, NY, USA) at Oulu University Hospital laboratory, and have been described in detail previously (13) (freely available as web appendix).

For standardizing measurements, the biochemistry laboratory has taken part in Lipid Standardization Program organized by Centers for Disease Control and Prevention (CDC, Atlanta, Georgia, USA) and External Quality Assessment Schemes organized by Labquality (Helsinki, Finland). During the course of the studies, the between-assay coefficient of variation (CV%, mean ±SD), systematic error (Bias%, mean ±SD) and the principle of the methods in the biochemistry laboratory are shown in Supplementary Table 1 (20).

Statistical analyses

All statistical analyses were conducted with IBM SPSS Statistics versions 24 and 25 (SPSS Inc., Chicago, IL, USA). Analyses were performed in a combined dataset of both birth cohorts (ESTER and AYLS). We compared descriptive characteristics between participants with *t*-test (continuous variables) and χ^2-test (categorical variables). The significance level was set to two-tailed $P < 0.05$. As the biochemical measures were not normally distributed, we log-transformed them prior to statistical analyses, to attain normality. We used multiple linear

regression models to compare cardio-metabolic biochemical measures, blood pressure and heart rate between adult offspring of mothers with GDM or pre-pregnancy overweight/obesity with controls. We adjusted for age, sex and birth cohort in model 1. Prenatal and parental confounders were taken into account in model 2, as we additionally adjusted for gestational age, birth weight SD score, maternal hypertension or preeclampsia during pregnancy, maternal smoking during pregnancy, parental educational attainment, parental history of hypertension, diabetes, stroke or myocardial infarction. Participant related factors, including age, sex, birth cohort, BMI, height and daily smoking were adjusted for in model 3. Finally, in the full model 4, all the above mentioned covariates were included.

Results

Perinatal and current characteristics and parental medical history of the OGDM, ONO and control groups are presented in Table 1. For comparison, these data are also shown separately for the two source cohorts in Supplementary Table 2 (20). Mean age of offspring at assessment was 24.1 years (SD 1.3) and 51.3 % were women. Among offspring, 2 OGDM, 2 ONO and 8 controls were born from twin pregnancies, the remainder were all singletons. As a sensitivity analysis, we excluded all twins and reran all analyses. This did not affect our results. Cardio-metabolic biochemical measures, heart rate and blood pressure of the offspring, with corresponding reference or target values are presented in Table 2.

Cardio-metabolic markers in offspring of mothers with gestational diabetes (Table 3)

There were clear associations between maternal GDM and adult offspring cardio-metabolic markers. Fasting glucose and insulin were higher in OGDM compared with controls,

although adjusting for confounders and current offspring characteristics including BMI attenuated these differences to non-significance (Table 3, Supplementary Table 3) (20). Further, OGDM had lower sex hormone binding globulin (SHBG) in both OGDM-men and OGDM-women. Also HDL-C, Lipoprotein (a) (Lp(a)) and Apolipoprotein A1 (ApoA1) were lower; apart from Lp(a), these differences survived adjustment for confounders and current characteristics. No statistically significant differences were seen in heart rate, testosterone, LDL-C, TGs, Apolipoprotein B (ApoB), free fatty acid (FFA), uric acid or liver tests (ALT, AST, GT). In model 1, TC and blood pressure was similar between OGDM and controls, while after adjusting for confounders and current characteristics TC, systolic and diastolic blood pressures were slightly lower in OGDM (Table 3, Supplementary Table 3) (20).

We further reran all analyses, replacing BMI and height with lean body mass and fat percentage in models 3 and 4 (data not shown). The results remained similar for model 3. In model 4, our findings of lower FFA [-9.2% ((-17.6, -0.1)] and hsCRP [-23.2 (-40.3, -1.1)] in OGDM both reached statistical significance.

To further distinguish between effects of maternal GDM and maternal overweight/obesity on adult offspring cardiovascular risk factors, we reran all analyses separately comparing offspring to mothers with 1) GDM and pre-pregnancy BMI < 25kg/m^2 (n=115) 2) GDM and pre-pregnancy BMI ≥ 25kg/m^2 (n=71) with controls (n=556). Most of our results remained similar (Supplementary Table 4) (20). A combination of maternal GDM and overweight/obesity showed a greater effect on fasting glucose and insulin than maternal overweight/obesity alone. Adjusting for confounders attenuated these differences between groups and this was largely due to offspring BMI as a mediator in OGDM with maternal BMI ≥ 25 kg/m^2.

296 *Cardio-metabolic markers in offspring of mothers with overweight/obesity (Table 3)*

297 Associations found between maternal pre-pregnancy overweight/obesity and adult
298 offspring cardio-metabolic markers were mostly explained by current offspring
299 characteristics and confounders. Fasting glucose and insulin were both higher in ONO vs.
300 controls, these findings disappeared after adjustments in models 2-4 (Table 3,
301 Supplementary Table 3) (20). In men, serum testosterone was lower in model 1, also this
302 difference attenuated after adjustments in models 2-4. All other cardio-metabolic markers,
303 including heart rate, SHBG, FFA, TC, HDL-C, LDL-C, TGs, LPa, ApoA1, ApoB, liver tests and uric
304 acid were similar between ONO and controls. Only in model 2, hsCRP was higher in the ONO
305 group, adjustment for confounders diluted this finding. However, although systolic blood
306 pressure was not different between groups in model 1, it was somewhat lower in models 2-
307 4 in ONO-participants (Table 3, Supplementary Table 3) (20).
308 As with OGDM, we also reran all analyses comparing ONO vs. controls, replacing BMI and
309 height with lean body mass and fat percentage in models 3 and 4. No changes were seen in
310 the results (data not shown).
311 We further reran all analyses separately comparing offspring to mothers with pre-pregnancy
312 BMI ≥ 30kg/m^2 (n=28) vs. controls. Most results remained similar (data not shown).
313 However, in model 1, hsCRP [66.2% (95% CI. 3.6, 166.3)], TGs [21.5% (95% CI: 2.3, 44.3)] and
314 ApoB [10.8% (95% CI: 0.1, 22.7)] were all higher in in offspring exposed to maternal pre-
315 pregnancy obesity compared with controls. Further, in model 1, heart rate was lower [-5.5%
316 (95%CI: -10.0, -1.0)] and fasting plasma glucose similar [-1.3% (95% CI:-4.3, 1.7)] between
317 obesity exposed and controls. After full adjustment (model 4) all results were similar with
318 ONO vs. controls regarding all cardio-metabolic markers (data not shown).
319

Discussion

We combined data from two longitudinal birth cohorts to study common cardio-metabolic markers in the adult offspring of mothers with GDM or overweight/obesity at start of pregnancy. There are two main findings in this study. First, maternal GDM was associated with increased insulin resistance and risk for an atherogenic lipid profile in adult offspring. Some, but not all, of this association was explained by confounding factors or current offspring characteristics including adiposity. Second, in offspring of mothers with pre-pregnancy overweight/obesity without GDM the consequences were not as clear. They had higher fasting glucose and insulin than controls, in part explained by parental and prenatal confounders or adult BMI or body fat percentage, but similar levels of other cardio-metabolic markers. This pattern differs from that of body composition. In this same cohort exposure to both maternal GDM and overweight/obesity was associated with higher offspring fat percentage and waist circumference, with stronger associations found related to maternal overweight/obesity (11).

Our findings of higher fasting glucose and insulin in OGDM compared with controls, attenuated to non-significance after adjusting for confounders and current characteristics. To further differentiate between the effects of maternal GDM and maternal overweight/obesity on offspring cardio-metabolic health, we divided OGDM participants into two subgroups based on maternal pre-pregnancy weight (BMI < 25 kg/m^2 and BMI ≥ 25 kg/m^2). A combination of maternal GDM and overweight/obesity showed a greater effect on fasting glucose and insulin than maternal overweight/obesity alone. In the fully adjusted model, the adjustments attenuated these differences in fasting glucose and insulin; this attenuation was largely due to offspring BMI as a mediator in OGDM with maternal BMI ≥ 25 kg/m^2.

Further, we report an atherogenic lipid profile in OGDM vs. controls, based on lower HDL-C and ApoA1 in OGDM. ApoA1 is a major component of HDL-C and low levels of ApoA1 are a well established risk factor of atherosclerosis.

In addition to the commonly measured indicators, we found lower SHGB in both OGDM-men and OGDM-women. SHBG is a measure of insulin resistance and hyperandrogenism, and this may reflect increased cardio-metabolic risk later in life.

Traditionally, the global obesity epidemic has been explained by an increase in availability and consumption of energy-dense foods and a simultaneous reduction in physical activity. However, other factors behind this rise in obesity prevalence exist, including genetic factors, an adverse intrauterine milieu and epigenetic changes this milieu may provoke (2). GDM is a common cause of such an adverse milieu and may cause epigenetic changes in offspring. For instance, in a GDM mice model, offspring exposed to GDM showed altered DNA methylation patterns in the pancreas, and this phenotype was characterized by dyslipidemia, insulin resistance and glucose intolerance (3). Our findings suggest that maternal GDM and pre-pregnancy overweight without diagnosed GDM may have distinct effects on offspring health. Previous studies have shown robust associations between higher maternal pre-pregnancy BMI and offspring adiposity as indicated by increased fat mass, fat-free mass and percentage of body fat in both neonates (8), and 6-7 year old children (9); higher BMI and greater waist circumference at adolescence (10), higher BMI, waist circumference, fat mass and fat percentage in young adulthood (11) and higher fat mass and BMI at older age (12). As for pre-pregnancy BMI and offspring cardio-metabolic risk factors, Gaillard et al reported an adverse cardio-metabolic profile (including lipid levels, glucose, insulin, homeostatic model assessment of insulin resistance) in 1392 adolescents at mean age 17 years (21). These associations were independent of maternal GDM and largely mediated by adolescent

BMI. In another study, including 1400 participants at 32 years of age, higher maternal pre-pregnancy BMI was associated with higher offspring blood pressure, insulin and triglycerides and lower HDL-C (22), again independent of maternal GDM and fully explained by current BMI of the offspring. This is consistent with our finding of higher fasting glucose and insulin among ONO who, however, had similar serum lipids and other biomarkers as controls.

As to offspring of mothers with GDM, a previous study in 16-year-olds, in one of our source cohorts, showed that they have a higher BMI and waist circumference at adolescence than offspring of mothers without GDM (4). Further, in that study fasting insulin was higher and homeostatic model assessment-insulin sensitivity was lower in offspring to mothers with GDM, while blood lipids and glucose where similar to controls. To some extent maternal GDM is also associated with adult offspring body composition. In the same cohort participants, in which we now report on cardio-metabolic markers in the current study, higher fat percentages were seen in offspring exposed to GDM (11). As for maternal diabetes and offspring cardio-metabolic risk factors at adult age; recently a large Canadian cohort study including 467 850 mother-infant dyads, described an association between both maternal type 2 diabetes (T2D) during pregnancy and GDM with T2D in offspring by age 30 (23). In this study exposure to maternal T2D during pregnancy conferred a greater risk to offspring compared with GDM exposure (3.19 vs. 0.80 cases of T2D per 1000 person-years) (23). In line with the Canadian study, we also showed increased markers of insulin resistance, i.e. higher fasting insulin and lower SHBG, HDL-C and ApoA1, in offspring with prenatal exposure to maternal GDM as compared to controls.

Recently, Bellatorre et al reported an increase in liver fat, independent of offspring adiposity, in both childhood (mean age 10.4 years) and adolescence (mean age 16.4 years) in offspring of mothers with pre-pregnancy obesity (BMI ≥ 30 kg/m^2), while no such effect

was found in offspring of mothers with GDM (24). In our study we did not find any associations between GDM or maternal pre-pregnancy overweight/obesity and biochemical markers of increased liver fat; levels of offspring liver enzymes were similar between groups.

Strengths of our study include a rather large sample size and long follow-up time. One of the indications for screening GDM was having a pre-pregnancy BMI ≥ 25 kg/m^2, thus mothers with GDM could be reliably separated from our group of normoglycemic mothers with overweight/obesity. To further distinguish between the effects of maternal GDM and maternal overweight/obesity on offspring cardio-metabolic health, we reran all analyses with OGDM participants further divided into two subgroups based on maternal pre-pregnancy weight (BMI < 25 kg/m^2 and BMI ≥ 25 kg/m^2 vs. controls). In these subgroup analyses with diminished numbers of participants, our results remained similar. Our participants come from an ethnically homogenous Finnish population, combined from two longitudinal study cohorts. The homogeneity of our study population may decrease the generalizability of our findings. Further, in the analyses we adjusted for important confounders, including perinatal and pregnancy related factors, parental hypertension, diabetes, stroke and myocardial infarction, and current participant related factors. However, residual confounding remains possible. Both treatment and GDM screening guidelines have changed during the previous 25 years. This may have introduced bias, depending on the adequacy of the screening. Thus, the GDM offspring in the current study may represent a more severe end of the GDM spectrum in today's pregnant women. Unfortunately, we do not have data on maternal glucose levels throughout pregnancy.

In sum, we found that maternal GDM is associated with increased levels of insulin resistance and a more atherogenic lipid profile in young adult offspring, as compared with controls. These findings suggest increased risk of cardio-metabolic diseases later in life. Maternal pre-pregnancy overweight or obesity alone was associated with offspring insulin resistance, but the association was weaker and explained by current adiposity.

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