Associations of Maternal Pre-Pregnancy Body Mass Index and Gestational Weight Gain with Adult Offspring Cardio-Metabolic Risk Factors: The Jerusalem Perinatal Family Follow-up Study

Running title: Hochner et al.; Maternal attributes and adult offspring health

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Abstract

**Background** - Accumulating evidence demonstrates that both maternal pre-pregnancy body mass index (mppBMI) and gestational weight gain (GWG) are associated with adult offspring adiposity. However, whether these maternal attributes are related to other cardio-metabolic risk factors in adulthood has not been comprehensively studied.

**Methods and Results** - We used a birth cohort of 1400 young adults born in Jerusalem, with extensive archival data as well as clinical information at age 32, to prospectively examine the associations of mppBMI and GWG with adiposity and related cardio-metabolic outcomes. Greater mppBMI, independent of GWG and confounders, was significantly associated with higher offspring BMI, waist circumference (WC), systolic and diastolic BP, insulin and triglycerides and with lower HDL-C. For example, the effect sizes were translated to nearly 5kg/m² higher mean BMI, 8.4cm higher WC, 0.13mmol/L (11.4mg/dL) higher triglycerides and 0.10mmol/L (3.8mg/dL) lower HDL-C among offspring of mothers within the upper mppBMI quartile (BMI>26.4kg/m²) compared to the lower (BMI<21.0kg/m²). GWG, independent of mppBMI, was positively associated with offspring adiposity; differences of 1.6kg/m² in BMI and 2.4cm in waist were observed when offspring of mothers in the upper (GWG>14kg) and lower (GWG<9kg) quartiles of GWG were compared. Further adjustment for offspring adiposity attenuated to null the observed associations.

**Conclusions** - Maternal size both before and during pregnancy are associated with cardio-metabolic risk factors in young adult offspring. The associations appear to be driven mainly by offspring adiposity. Future studies that explore mechanisms underlying the intergenerational cycle of obesity are warranted to identify potentially novel targets for cardio-metabolic risk-reduction interventions.

**Key words:** cohort study, obesity, pregnancy, risk factors
Introduction

Prevalence of overweight and obesity are rising worldwide, affecting all age groups including women of reproductive age. Together with associated cardio-metabolic outcomes, such as hypertension, hypercholesterolemia and diabetes, overweight and obesity have become a major public health concern globally\(^1,2\).

Accumulating evidence suggests that overweight and obesity in adult life and related cardio-metabolic risk factors are influenced by the intrauterine environment. In recent years emerging data from animal and human studies suggests that maternal overnutrition, reflected in part by greater maternal pre-pregnancy BMI (mppBMI) and gestational weight gain (GWG), may impact offspring adiposity later in life\(^1,2\). Population-based studies assessing whether the relationships between these maternal attributes and offspring cardio-metabolic health extend into adulthood are limited, and are restricted mainly to offspring adiposity\(^3-8\), with one study examining young adult offspring blood pressure levels as well\(^9\). We are unaware of previous studies that have examined long-term associations of mppBMI and GWG with other related offspring cardio-metabolic outcomes measured in early adulthood, such as levels of glucose, insulin, lipids and lipoproteins.

Using the Jerusalem Perinatal Family Follow-Up Study birth cohort we extend previous studies by: 1) examining the associations of maternal pre-pregnancy BMI (mppBMI) and gestational weight gain (GWG) with a range of cardio-metabolic risk factors in offspring (BMI, waist circumference (WC), blood pressure (BP) and plasma levels of fasting glucose, insulin, lipids and lipoproteins) 32 years after birth, taking into account characteristics of both early and current environment; and 2) assessing to what extent the possible relationships of mppBMI and GWG with offspring cardio-metabolic outcomes are independent of offspring adiposity.
Methods

The Jerusalem Perinatal Study (JPS) population-based cohort includes a sub-cohort of all 17,003 births to residents of Jerusalem, between years 1974 and 1976. Data consists of demographic and socioeconomic information, medical conditions of the mother during current and previous pregnancies, and offspring birth weight, abstracted either from birth certificates or maternity ward logbooks. Additional information on lifestyle and maternal medical conditions, including gestational age, mother's smoking status, height and pre-pregnancy weight, end of pregnancy weight and gynecological history, was collected by interviews of mothers on the first or second day postpartum. Detailed information on data collection has been previously described. Through data linkage with the Israeli military draft records, information from medical examinations at age 17, including BMI, was obtained for approximately 70% of the JPS cohort.

The JPS Family Follow-Up study includes a sample of 1,400 offspring from the original 1974-1976 birth cohort, who were interviewed and examined between 2007 and 2009. Sampling frame included singletons and term (gestational age ≥ 36 weeks) births without congenital malformations. We obtained a stratified sample of eligible individuals, where the strata were defined by mppBMI and birth weight. Both low (≤ 2500 grams) and high (≥ 4000 grams) birth weight as well as over-weight and obese mothers (BMI ≥ 27) were over-sampled. Standard procedures and training protocols were used to measure standing height (without shoes; Seca portable stadiometer), body weight (with indoor light clothes; Seca portable automated scale), waist circumference (at the midpoint between the lower ribs and iliac crest in the midaxillary
line; Seca measurement tape) and blood pressure (measured three consecutive times in the right arm in sitting position following five minute rest; Omron M7 automated sphygmomanometer).

Blood samples at fasting (at least 8 hours of fasting) were taken using standard procedures. Samples were immediately spun and biochemical measurements were assayed in plasma. Insulin levels were determined by radioimmunoassay using Human Insulin-Specific RIA Kit (Millipore) and analyses of glucose, HDL-C and triglycerides were preformed on the VITROS 5,1FS Chemistry System (Ortho Clinical Diagnostics).

Individuals who reported taking BP-lowering medication (n=11), lipid-lowering medication (n=13) or medication to treat diabetes (n=13) were excluded from the corresponding analyses.

Study variables

The following cardio-metabolic outcomes measured at age 32 were examined: body mass index (BMI, calculated by dividing weight (kg) by squared height (m²)); waist circumference (WC, mean of two consecutive measurements (cm)); systolic and diastolic blood pressure (SBP and DBP, mean of three consecutive measures (mmHg)); glucose (mmol/L); insulin (mean of two repeated measures (pmol/L), log-transformed (base 10) due to asymmetrical distribution); low-density lipoprotein cholesterol (LDL-C (mmol/L)); high-density lipoprotein cholesterol (HDL-C (mmol/L)) and triglycerides (TG (mmol/L), log-transformed (base 10) due to asymmetrical distribution). All cardio-metabolic outcomes were treated as continuous variables.

The following explanatory variables were examined: maternal pre-pregnancy body mass index (mppBMI, calculated as weight (kg) divided by squared height (m²), continuous variable) and gestational weight gain (GWG, simple difference between end of pregnancy weight and pre-pregnancy weight (kg), continuous variable).
All models were adjusted for offspring gender and ethnicity. Following an approach suggested by Thomas and Witte, ethnicity of offspring was classified based on country of origin of all four grandparents, using nine major ethnicity strata (Israel, Morocco, Other North Africa, Iran, Iraq, Kurdistan, Yemen, Other Asia and the Balkans and Ashkenazi). Rather than allocating offspring to a single ethnicity, we constructed a covariate for each stratum giving the proportion of grandparents derived from each of the nine ethnic groups (ranging from 0 to 1, reflecting none or all four grandparents originating from the specific ethnic group, respectively) and then included these covariates as adjustment variables in a multiple regression (excluding one strata (Ashkenazi) to eliminate complete multicollinearity).

We addressed potential confounders at two time points in offspring life, at birth and at age 32, reflecting the early environment (i.e. pre- and peri-natal periods) and the environment at young adulthood, respectively. Potential confounders at time of birth were: (1) parity (continuous), (2) mother’s age at child birth (continuous), (3) maternal smoking during the pregnancy (current smoker vs. never smoked or smoked in the past), (4) socioeconomic status (SES) based on father's occupation at time of birth (grouped into three categories: low, medium, high), (5) mother's years of education at time of birth (continuous), (6) maternal medical condition (dichotomous, based on whether the mother had ever suffered from any of the following diseases: diabetes, hypertension, heart disease, toxemia), (7) birth weight (continuous), and gestational week (to adjust for residual confounding within term pregnancies). Potential confounders at age 32 were: (1) smoking status (current smoker vs. never smoked or smoked in the past), (2) physical activity (based on the question: during leisure time are you engaged in moderate or vigorous physical activity that lasts at least 20 minutes, 3 or more times a week) and (3) years of education (continuous).
BMI and WC at age 32 were also assessed as potential mediators in the associations with the other cardio-metabolic outcomes.

**Statistical analyses**

Analyses were carried out using the SPSS version 17.0 statistical package (SPSS, Inc., Chicago, Illinois) and Stata 10.0 (StataCorp, College Station TX).

Linear regression models were used to investigate the associations of mppBMI and GWG, independent of each other, with cardio-metabolic outcomes measured at age 32, after controlling for potential confounders. Two sets of models were constructed: model 1 included both mppBMI and GWG, adjusted for ethnicity and gender, as well as for maternal and offspring characteristics at time of birth (i.e. parity, mother’s age, maternal smoking, SES, mother's years of education, maternal medical condition, birth weight and gestational week) and offspring characteristics at age 32 (i.e. smoking status, physical activity, years of education). In model 2 we assessed whether BMI at age 32 mediates, at least in part, the associations of mppBMI and GWG with all other cardio-metabolic outcomes, by further adjusting model 1 for BMI.

Coefficients presented in the table indicate increment (positive or negative) in cardio-metabolic outcome per one unit increase in mppBMI (kg/m2) or GWG (kg).

Gender interactions with mppBMI and GWG on all cardio-metabolic outcomes examined were assessed by introducing both multiplicative terms (i.e. mppBMI*gender and GWG*gender) into the linear regression models. Additionally, to test whether there is evidence for an interaction between mppBMI and GWG on outcomes, an mppBMI*GWG multiplicative term was introduced into the models.

To further illustrate effect sizes and clinical importance, mppBMI and GWG were also examined as categorical variables grouped by quartiles of distribution (mppBMI - Q1:
<21.0kg/m², Q2: 21.0-23.8kg/m², Q3: 23.9-26.4kg/m², Q4: >26.4kg/m²; GWG - Q1: <9kg, Q2: 9-11kg, Q3: 12-14kg, Q4: >14kg). We used estimates for these categorical variables from linear regressions adjusted for confounders described above (in model 1) to determine adjusted means and standard errors (SEs) for offspring cardio-metabolic outcomes for all subjects within the same quartile.

All models used inverse probability weighting to account for the stratified sampling. Due to a limited number of missing values for several potential confounders at time of birth and at age 32, a total of 180 offspring could not be included in analyses. To reduce possible bias we imputed missing data using observed data and assuming that data were missing at random. Linear regression models were repeated with imputed data and yielded similar regression coefficients and standard errors to those obtained by excluding missing values. The following analyses are therefore based on subjects with complete data; 1250 for adiposity and BP and 1130 for blood assays.

This study was approved by the Institutional Review Board of the Hadassah-Hebrew University Medical Center. All participants provided informed consent.

Results

Maternal and offspring characteristics obtained at birth and offspring characteristics and cardio-metabolic outcomes at age 32 are listed in Table 1.

Table 2 presents results of linear regression models examining the associations of mppBMI and GWG with offspring cardio-metabolic outcomes at age 32, in which the coefficient indicates the increment (positive or negative) in cardio-metabolic outcome associated with one unit increase in mppBMI or GWG. mppBMI was positively associated with offspring BMI
(p<0.0001), WC (p<0.0001), SBP (p=0.003), DBP (p=0.017), insulin (p=0.007) and TG (p=0.02) and negatively associated with HDL-C (p=0.03). These associations were independent of GWG and of characteristics at birth and at age 32 (Table 2A, model 1). For example, the coefficients presented in Table 2 can also be interpreted as an increase of 1.8 kg/m² in offspring BMI, 3.5 cm in WC, and 1.7 and 1.1 mmHg in SBP and DBP, respectively, per increase of one standard deviation (SD) in mppBMI. GWG adjusted for mppBMI and all confounders was also positively associated with offspring adiposity traits, including BMI (p=0.0001) and WC (p=0.024), and with TG (p=0.04) (Table 2B, model 1).

The relationships of mppBMI with WC, BP, insulin, HDL-C and TG as well as that of GWG with WC and TG were not independent of concurrent BMI; all significant associations were attenuated to null with further adjustment for BMI at age 32 (Table 2A, B, model 2). Analyses were repeated with further adjustment for WC instead of BMI, yielding similar results (data not shown).

Additional support for a potential mediating role of BMI was provided among a sub-sample of 991 men and women for which in addition to the data at age 32, BMI at age 17 was also available. In this sub-sample, the effect size of mppBMI on SBP at age 32 without adjusting for BMI (B=0.426, p=0.008) was attenuated (B=0.306, p=0.06) after further adjustment for BMI at age 17.

To further illustrate the effects sizes presented in Table 2 we compared adjusted means of selected offspring cardio-metabolic outcomes between quartiles of mppBMI and GWG (Figure 1). This assessment revealed that BMI of offspring whose mothers were in the upper quartile of mppBMI (BMI>26.4 kg/m²) was nearly 5 kg/m² higher compared to offspring of mothers in the lower quartile (BMI<21.0 kg/m²), a difference corresponding to 0.9 standard
deviation (SD) of offspring BMI. WC was 8.4 cm higher among the offspring of mothers in the upper quartile compared to the lower (0.64 SD of WC). The differences in mean BP levels between the two quartiles were 5.2 mmHg for SBP and 3.0 mmHg for DBP (corresponding to approximately 0.4 SD and 0.35 SD, respectively). The effect sizes for the associations of mppBMI with Insulin and TG were translated to 17.4 pmol/L (2.5 U/m) higher insulin, and 0.13 mmol/L (11.4 mg/dL) higher TG among offspring of mothers within the upper mppBMI quartile compared to the lower (Figure 1). Mean HDL-C level among offspring of mothers within the upper quartile of mppBMI was 0.10 mmol/L (3.8 mg/dL) lower compared to offspring of mother in the lower quartile, corresponding to 0.26 SD of HDL-C (data not shown). The differences in BMI and WC among offspring of mothers in the upper (GWG>14 kg) and lower (GWG<9 kg) quartiles of GWG were 1.6 kg/m² in BMI and 2.4 cm in WC (Figure 1).

We have additionally explored whether there was evidence for gender differences in the associations between mppBMI and GWG with offspring cardio-metabolic outcomes. With the exception of the statistically significant gender interaction with GWG on BP (p_interaction=0.004 and 0.001 for SBP and DBP, respectively), there was little evidence to suggest interactions of gender with either mppBMI or GWG for the other cardio-metabolic outcomes (data not shown).

Finally, we investigated whether the associations of mppBMI with any of the cardio-metabolic outcomes examined were modified by GWG, but found no support for such interactions (data not shown).

Discussion

Summary of findings
This study investigated the associations between maternal pre-pregnancy body size and weight gain during pregnancy with a range of offspring cardio-metabolic risk factors in early adulthood.

We demonstrated that mppBMI was independently and positively associated with offspring BMI and WC at age 32. We extend previous studies by demonstrating that greater mppBMI was also significantly associated with higher offspring SBP, DBP, insulin and TG levels and lower HDL-C. Additionally, we have shown that GWG, independent of mmpBMI, was positively associated with offspring adiposity. The observed associations were independent of characteristics reflecting the pre- peri- and post-natal environment, including current measures of SES and lifestyle. Furthermore, the associations appear to be driven mainly by offspring adiposity.

**Associations with offspring adiposity**

Our findings that mppBMI was positively associated with offspring adiposity in early adulthood are in accordance with other studies. Recently several studies examined the association between GWG and offspring adiposity in adulthood, reflected primarily by offspring BMI, and have generally demonstrated a positive association.

**Associations with offspring BP**

There have been only a few investigations of the association of mppBMI or GWG with offspring BP and due to scarcity of long follow-up data most have examined BP in childhood only. Nevertheless, similar to our findings in adults, positive associations of mppBMI and GWG with offspring BP measured in childhood were demonstrated in several studies. A previous study based on 10,883 subjects from the JPS 1974-1976 birth cohort, examined the association of several prenatal characteristics with offspring BP at adolescence (age 17) and found mppBMI to
be positively associated with offspring BP, while GWG was not related to BP. In an Australian population-based cohort of 2432 individuals aged 21 years, greater GWG was shown to be associated with increased SBP. Although this association was not statistically significant, the authors argue that its magnitude is consistent with the association of GWG with BMI and of BMI with BP. In agreement with the aforementioned studies, we have shown that the association between mppBMI and BP at age 32 was significant, while GWG was not significantly associated with BP. A possible explanation for the lack of association seen for GWG and BP may be the presence of a significant GWG-gender interaction. We do, however, acknowledge that our interaction finding may be due to chance and therefore requires replication in other cohorts.

**Associations with offspring fasting glucose and insulin**

We have shown that mppBMI was associated with offspring fasting insulin levels, while GWG was not associated with either insulin or glucose levels. Data on the associations between mppBMI and GWG with offspring fasting levels of glucose and insulin is scarce. A small case-control study of 52 young adult offspring of obese mothers (BMI≥30 kg/m² before and during pregnancy) and 15 offspring of normal-weight mothers demonstrated that offspring of obese mothers were more likely to be over-weight and obese and more insulin resistant compared to controls.

**Associations with offspring fasting lipids and lipoproteins**

We have shown that mppBMI was significantly associated with HDL-C and TG. No associations of GWG with lipids and lipoproteins were observed (borderline significance with TG - p=0.044). We are unaware of other studies examining these associations with offspring lipids and lipoproteins in young adults. The only previous study that investigated similar associations is a recent study of 9-year-old children from the UK that examined relationships of
mppBMI and GWG with a wide range of offspring cardiovascular risk factors. With some similarity to our findings, the UK study showed that both greater mppBMI and GWG were significantly associated with lower HDL-C, whereas associations with TG levels were positive but confidence intervals were wide. Variation in the findings may be attributed to differences in characteristics of participants (e.g. age) between studies.

Mediating role of adiposity

Our analyses suggest that the significant associations of mppBMI and GWG with BP, insulin and lipids appeared to be largely mediated by offspring concurrent body size, reflected by both BMI and WC.

Similar to our findings in adults, a case-control study comprising of offspring aged 6-13 years, suggested that independent of maternal diabetes status, greater mppBMI was associated with raised BP and this association was mediated by BMI. A recent study of 30,461 mother-child pairs, examining whether intrauterine and/or childhood growth mediate the associations between prenatal factors and SBP at age 7, demonstrated that the association of mppBMI with SBP was independent of intrauterine growth restriction and was attenuated to null after adjustment for offspring BMI trajectory. Additional support for our findings comes from the UK study in children, where the authors reported that the significant relationships demonstrated for mppBMI and GWG were all attenuated to null after adjustment for various measures of adiposity.

Mechanisms underlying the observed associations

There are several pathways that may underlie the associations of mppBMI and GWG with offspring adiposity. First, mppBMI and GWG are correlated with birth weight and therefore their association with offspring body size may simply reflect tracking of body size throughout
life. However, consistent with other studies\textsuperscript{7, 9, 15, 16}, adjustment for birth weight did not alter the observed associations. Second, shared genetic and environmental characteristics between mother and offspring that are related to both adiposity and weight gain may account for these relationships. This study did not assess the contribution of directly measured genetic factors to the observed associations. Yet we have attempted to account for various shared environmental characteristics by adjusting for characteristics reflecting the early environment, such as SES and maternal smoking, as well as for characteristics of the environment in early adulthood, including offspring level of education, smoking and physical activity. Our models demonstrated significant associations, independent of the various environmental characteristics. Third, intrauterine mechanisms may account for the long-term associations with offspring adiposity. This was examined in a recent study of approximately 150,000 Swedish male conscripts (aged 18) using a unique study design to compare within-siblings and between non-siblings associations\textsuperscript{15}. The authors reported that among overweight and obese mothers not only shared genetic and environmental factors explain the association between weight gain during pregnancy and later offspring obesity but there is also evidence for the contribution of intrauterine mechanisms to this association.

Intrauterine mechanisms explaining long-term associations with offspring obesity and related cardio-metabolic outcomes have been proposed by the ‘developmental overnutrition hypothesis’. Greater fat during pregnancy (due to higher mppBMI or greater GWG) result in greater delivery of glucose, amino acids and free fatty acids from mother to fetus and thus may lead to permanent changes in appetite control, neuroendocrine functioning and energy metabolism in the developing fetus with long-term consequences on risk of adiposity and related cardio-metabolic disease\textsuperscript{1}. Relevant here is the concept of ‘developmental origins of health and
disease’, in which ample evidence exists linking birth weight, a surrogate measure of intrauterine growth and development, with cardio-metabolic outcomes in adult life, such as obesity, hypertension and diabetes. As mentioned above, taking birth weight into account did not attenuate the observed associations of maternal attributes with offspring cardio-metabolic outcomes. This finding can imply that birth weight does not measure the intrauterine environment well enough, although in our data birth weight appears to be associated to some extent with several of the cardio-metabolic outcomes examined (e.g. adiposity, BP, insulin, TG), as well as with clinical outcomes, such as mortality, demonstrated in previous studies in this cohort. Alternatively, it could suggest that the associations of maternal adiposity (both mppBMI and GWG) and birth weight with offspring cardio-metabolic outcomes reflect different pathways linking early life events with adult health.

Lastly, there is a rising recognition that epigenetic processes, linking environmental and genetic factors, are important components in the transmission of acquired information from the uterus to the offspring in later life. It has been suggested that the obesogenic environment experienced in utero may induce epigenetic modifications causing changes in gene expression, tissue structure and organ development resulting in subsequent cardio-metabolic health consequences in the adult offspring. In support of epigenetic mechanisms, it should be noted that although adiposity and related risk factors show significant heritability (≥50%), common genetic variation identified thus far appear to have modest impact on these traits. Furthermore, in our cohort mothers were noticeably leaner than their offspring when they were at childbearing age. Clearly, the genetic makeup did not change during these years, but the environment has changed dramatically; Jerusalem during the last three decades has gradually become a more affluent society. It may therefore be the differences in the environment
experienced by the offspring in utero and the one experienced later in life that have interacted with genes to exert the observed intergenerational changes. On a more specific note, in a recent study of 9-year-old children from the UK it was suggested that long-term associations of GWG and offspring cardio-metabolic outcomes may vary between outcomes depending on the timing during gestation, possibly pointing to differences in underlying mechanisms between traits. In the UK study, GWG at all stages of gestation was found to be associated with offspring adiposity, whereas other cardio-metabolic outcomes, such as lipids and inflammatory profiles, were related to GWG only in mid to late gestation. The influence of timing of intrauterine exposures has also been demonstrated in the Dutch famine cohort, where people exposed to famine in late or mid gestation showed a different cardio-metabolic risk pattern in adulthood than those exposed in early gestation, and these differences may involve persistent changes in DNA methylation that depend on gestational timing. Thus one possible explanation for the fact that in the present study GWG was found to be associated with adiposity traits and not with other cardio-metabolic outcomes is that it may reflect mechanistic differences, where obesity is more robustly affected by weight gain at any time during the pregnancy, while other outcomes are sensitive to weight gain in specific time windows in gestation. We were unable to examine this possibility, as repeated measures of GWG were unavailable.

**Strengths and limitations**

The major strength of our study is the combination of high-quality detailed records of pre- and peri-natal maternal and offspring characteristics with comprehensive long-term follow-up data 32 years after birth. Availability of information collected in early life, including both pregnancy-related factors and lifestyle and socio-demographic characteristics, together with
characteristics of offspring at early adulthood, improved the characterization of the environment during pregnancy and birth as well as in adulthood, permitting control for these important factors.

There are several limitations to our study. First, it includes only a sample of offspring from the original 1974-1976 JPS cohort who were invited to participate in the follow-up study. However, using a stratified sampling approach and over-sampling in the ends of the distribution ensured that offspring with a full range of mppBMI and birth weight were included in our study. Second, both mppBMI and GWG were reported by mothers in interviews conducted by nurses while hospitalized after delivery. Verification from clinical records was not available. Nevertheless, the associations demonstrated in the present study between reported maternal attributes and measured cardio-metabolic traits more than 30 years later, as well as with long-term clinical outcomes in mothers described previously in this cohort\textsuperscript{29}, together with the agreement with findings from studies in other populations (e.g. \textsuperscript{5, 9, 16}), lend support to the validity of the data. Additionally, studies have shown that maternal recollection of pre-pregnancy weight and height is reproducible and valid\textsuperscript{39-41}. High correlation was also reported between documented and maternal self-reported GWG when recall was within 9 months of delivery\textsuperscript{42}. Importantly, evaluation of the impact of misclassification in GWG on associations with various pregnancy outcomes has demonstrated that associations were attenuated when GWG was based on recall rather than on measurement, indicating a bias towards the null\textsuperscript{43, 44}. In our study, it seems reasonable to assume that given the timing of the interview, i.e. within several days of delivery, the majority of mothers could provide valid information on GWG, yet even if reporting error was present it most likely resulted in an underestimation in our findings. Additionally, we were not able to examine the associations of GWG at various stages of pregnancy with offspring...
cardio-metabolic outcomes, as repeated measures of GWG were not available. Lastly, this study did not assess the impact of direct measurements of genetic and epigenetic factors on the associations examined. Further studies that examine whether genetic and/or epigenetic variation may account for the observed associations are warranted.

Implications

Our study adds to and extends accumulating evidence of long-term relationships of maternal excess weight and weight gain in pregnancy with offspring cardio-metabolic health in adulthood. It should be emphasized that at the time this birth cohort was established obesity was no way near its current magnitude. Therefore the distribution of mppBMI in our study reflects a much leaner population than nowadays and this is likely true for GWG, given its rising trends. Nevertheless, significant associations with mppBMI and GWG were demonstrated for a range of cardio-metabolic risk factors in offspring, with clinically significant effect sizes on offspring adiposity in particular. Furthermore, the associations appear to be driven mainly by offspring adiposity, emphasizing the potential impact maternal adiposity may have through offspring adiposity on various predictors of sub-clinical and clinical disease, including diabetes, myocardial infarction and stroke. Whether this predicts even worse outcomes for the next generation is unknown, yet the possibility is nonetheless concerning.

Future studies exploring mechanisms underlying the relationships between these maternal pregnancy-related characteristics and cardio-metabolic outcomes in offspring. Achieving a better understanding of the mechanisms underlying the intergenerational cycle of obesity may help facilitate the identification of novel targets for cardio-metabolic risk-reduction interventions.
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**Conflict of Interest Disclosures:** None

**References:**


Table 1: Study characteristics at birth and at age 32, by gender

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<thead>
<tr>
<th>Characteristics obtained at birth*</th>
<th>Women (N=634)</th>
<th>Men (N=622)</th>
<th>Total (N=1256)</th>
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<tr>
<td>Maternal pre-pregnancy BMI, kg/m²</td>
<td>24.27 (3.87)</td>
<td>23.63 (3.64)</td>
<td>23.96 (3.77)</td>
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<tr>
<td>Gestational weight gain, kg</td>
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<td>11.38 (4.55)</td>
<td>11.05 (4.62)</td>
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<tr>
<td>Smoking mothers %</td>
<td>17.98</td>
<td>18.97</td>
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</tr>
<tr>
<td>Maternal ethnic origin %</td>
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<tr>
<td>Israel</td>
<td>12.30</td>
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<td>Middle East</td>
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<td>Parity</td>
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<td>28.19 (5.21)</td>
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<td>Socioeconomic status %</td>
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<td>High</td>
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<td>Birth weight, kg</td>
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<td>3.404 (0.614)</td>
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<td>Gestational week</td>
<td>39.98 (1.56)</td>
<td>39.98 (1.52)</td>
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<td>Mothers with any background disease† %</td>
<td>8.99</td>
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<thead>
<tr>
<th>Characteristics obtained at age 32*</th>
<th>Women (N=634)</th>
<th>Men (N=622)</th>
<th>Total (N=1256)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Education years</td>
<td>15.02 (2.59)</td>
<td>15.38 (3.74)</td>
<td>15.20 (3.21)</td>
</tr>
<tr>
<td>Smokers %</td>
<td>18.61</td>
<td>35.85</td>
<td>27.15</td>
</tr>
<tr>
<td>Physically active %</td>
<td>48.58</td>
<td>54.02</td>
<td>51.27</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cardio-metabolic outcomes at age 32‡</th>
<th>Women</th>
<th>Men</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>25.86 (5.51)</td>
<td>26.74 (4.63)</td>
<td>26.30 (5.11)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>81.40 (12.48)</td>
<td>91.19 (12.06)</td>
<td>86.25 (13.21)</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>98.77 (9.41)</td>
<td>113.83 (10.26)</td>
<td>106.24 (12.39)</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>68.51 (8.06)</td>
<td>74.75 (7.99)</td>
<td>71.61 (8.61)</td>
</tr>
<tr>
<td>Glucose§ mmol/L</td>
<td>4.26 (0.56)</td>
<td>4.51 (0.62)</td>
<td>4.38 (0.61)</td>
</tr>
<tr>
<td>Insulin§ pmol/L</td>
<td>80.14 (50.16)</td>
<td>91.55 (62.55)</td>
<td>85.90 (57.01)</td>
</tr>
<tr>
<td>LDL-C§ mmol/L</td>
<td>2.79 (0.75)</td>
<td>3.03 (0.74)</td>
<td>2.91 (0.76)</td>
</tr>
<tr>
<td>HDL-C§ mmol/L</td>
<td>1.46 (0.38)</td>
<td>1.12 (0.29)</td>
<td>1.29 (0.76)</td>
</tr>
<tr>
<td>Triglycerides§ mmol/L</td>
<td>1.02 (0.57)</td>
<td>1.37 (0.97)</td>
<td>1.20 (0.82)</td>
</tr>
</tbody>
</table>

* Values are expressed as mean (SD) or percent.
† Diabetes, hypertension, heart disease or toxemia.
‡ N=1248 for adiposity and BP (629 women, 619 men) and N=1134 for blood assays (563 women, 571 men).
§ Based on blood plasma assays at fasting (at least 8 hours).
‖ Conversions: glucose, mg/dL = (mmol/L)/0.0555; insulin, μU/mL = (pmol/L)/6.945; triglycerides, mg/dL = (mmol/L)/0.0113; HDL and LDL cholesterol, mg/dL = (mmol/L)/0.0259.
Table 2. Associations* of maternal pre-pregnancy BMI and gestational weight gain with offspring cardio-metabolic outcomes at age 32

A. Exposure: MATERNAL PRE-PREGNANCY BMI

<table>
<thead>
<tr>
<th>Offspring cardio-metabolic outcomes</th>
<th>Coefficient (95% CI)</th>
<th>P</th>
<th>Coefficient (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m2</td>
<td>0.481 (0.377, 0.585)</td>
<td>&lt;0.0001</td>
<td>-0.094 (-0.263, 0.074)</td>
<td>0.272</td>
</tr>
<tr>
<td>WAIST CIRCUMFERENCE, cm</td>
<td>0.927 (0.668, 1.185)</td>
<td>&lt;0.0001</td>
<td>-0.094 (-0.263, 0.074)</td>
<td>0.272</td>
</tr>
<tr>
<td>SYSTOLIC BP‡, mmHg</td>
<td>0.441 (0.149, 0.732)</td>
<td>0.003</td>
<td>-0.004 (-0.010, 0.002)</td>
<td>0.184</td>
</tr>
<tr>
<td>DIASTOLIC BP‡, mmHg</td>
<td>0.287 (0.051, 0.523)</td>
<td>0.017</td>
<td>-0.004 (-0.010, 0.002)</td>
<td>0.184</td>
</tr>
<tr>
<td>GLUCOSE§**, mmol/L</td>
<td>-0.001 (-0.019, 0.016)</td>
<td>0.875</td>
<td>-0.004 (-0.010, 0.002)</td>
<td>0.184</td>
</tr>
<tr>
<td>INSULIN§#**, pmol/L</td>
<td>0.008 (0.002, 0.014)</td>
<td>0.007</td>
<td>-0.004 (-0.010, 0.002)</td>
<td>0.184</td>
</tr>
<tr>
<td>LDL-C‖**, mmol/L</td>
<td>0.012 (-0.008, 0.031)</td>
<td>0.240</td>
<td>-0.002 (-0.024, 0.019)</td>
<td>0.828</td>
</tr>
<tr>
<td>HDL-C‖**, mmol/L</td>
<td>-0.010 (-0.019, -0.0007)</td>
<td>0.033</td>
<td>0.003 (-0.006, 0.012)</td>
<td>0.502</td>
</tr>
<tr>
<td>TRIGLYCERIDES‖**, mmol/L</td>
<td>0.007 (0.001, 0.012)</td>
<td>0.020</td>
<td>-0.002 (-0.008, 0.003)</td>
<td>0.417</td>
</tr>
</tbody>
</table>

B. Exposure: GESTATIONAL WEIGHT GAIN

<table>
<thead>
<tr>
<th>Offspring cardio-metabolic outcomes</th>
<th>Coefficient (95% CI)</th>
<th>P</th>
<th>Coefficient (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m2</td>
<td>0.178 (0.088, 0.267)</td>
<td>0.0001</td>
<td>-0.100 (-0.235, 0.035)</td>
<td>0.146</td>
</tr>
<tr>
<td>WAIST CIRCUMFERENCE, cm</td>
<td>0.277 (0.036, 0.518)</td>
<td>0.024</td>
<td>-0.100 (-0.235, 0.035)</td>
<td>0.146</td>
</tr>
<tr>
<td>SYSTOLIC BP‡, mmHg</td>
<td>0.206 (0.003, 0.408)</td>
<td>0.047</td>
<td>0.063 (-0.135, 0.260)</td>
<td>0.534</td>
</tr>
<tr>
<td>DIASTOLIC BP‡, mmHg</td>
<td>0.174 (-0.004, 0.353)</td>
<td>0.055</td>
<td>0.061 (-0.112, 0.234)</td>
<td>0.490</td>
</tr>
<tr>
<td>GLUCOSE§**, mmol/L</td>
<td>0.005 (-0.008, 0.019)</td>
<td>0.480</td>
<td>0.003 (-0.010, 0.017)</td>
<td>0.639</td>
</tr>
<tr>
<td>INSULIN§#**, pmol/L</td>
<td>0.002 (-0.003, 0.008)</td>
<td>0.339</td>
<td>-0.003 (-0.007, 0.002)</td>
<td>0.275</td>
</tr>
<tr>
<td>LDL-C‖**, mmol/L</td>
<td>0.007 (-0.010, 0.023)</td>
<td>0.429</td>
<td>0.001 (-0.016, 0.018)</td>
<td>0.902</td>
</tr>
<tr>
<td>HDL-C‖**, mmol/L</td>
<td>-0.006 (-0.014, 0.002)</td>
<td>0.164</td>
<td>-0.0004 (-0.008, 0.007)</td>
<td>0.909</td>
</tr>
<tr>
<td>TRIGLYCERIDES‖**, mmol/L</td>
<td>0.005 (0.0001, 0.009)</td>
<td>0.044</td>
<td>0.001 (-0.003, 0.005)</td>
<td>0.692</td>
</tr>
</tbody>
</table>

* Linear regression models; coefficient indicates increment (positive or negative) in cardio-metabolic outcome per one unit increase in maternal pre-pregnancy BMI (kg/m2) or gestational weight gain (kg).
† Model 1: includes both maternal pre-pregnancy BMI and gestational weight gain, adjusted for ethnicity and gender, characteristics at time of birth (i.e. parity, mother's age, maternal smoking, SES, mother's years of education, maternal medical condition, birth weight and gestational week) and offspring characteristics at age 32 (i.e. smoking status, physical activity, years of education). Model 2: as model 1 plus additional adjustment for offspring BMI at age 32.
‡ Individuals who reported taking antihypertensive medication (N=11) were excluded from analysis
§ Individuals who reported taking diabetes medication (N=13) were excluded from analysis.
‖ Individuals who reported taking lipid-lowering medication (N=13) were excluded from analysis.
** Conversions: glucose, mg/dL = (mmol/L)/0.0555; insulin, μU/mL = (pmol/L)/6.945; triglycerides, mg/dL = (mmol/L)/0.0113; HDL and LDL cholesterol, mg/dL = (mmol/L)/0.0259.
**Figure Legend:**

**Figure 1.** Adjusted means of offspring selected cardio-metabolic outcomes at age 32 by quartiles of maternal pre-pregnancy BMI and gestational weight gain. Maternal pre-pregnancy BMI (mppBMI) and gestational weight gain (GWG) were grouped by quartiles of distribution: mppBMI - Q1: <21.0kg/m², Q2: 21.0-23.8kg/m², Q3: 23.9-26.4kg/m², Q4: >26.4kg/m²; GWG - Q1: <9kg, Q2: 9-11kg, Q3: 12-14kg, Q4: >14kg. Estimates for the categorical variables from linear regression models adjusted for ethnicity, gender, characteristics at time of birth (i.e. parity, mother’s age, maternal smoking, SES, mother's years of education, maternal medical condition, birth weight and gestational week) and offspring characteristics at age 32 (i.e. smoking status, physical activity, years of education) were used to determine adjusted means and standard errors (SEs) for offspring cardio-metabolic outcomes for all subjects within the same quartile. Error bars represent SEs. Difference between each value displayed on the Y axis corresponds to approximately two standard errors of the respective cardio-metabolic outcome. Conversions: insulin, μU/mL=(pmol/L)/6.945; triglycerides, mg/dL=(mmol/L)/0.0113.
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Hagit Hochner, Yechiel Friedlander, Ronit Calderon-Margalit, Vardiella Meiner, Yael Sagy, Meytal Avgil-Tsadok, Ayala Burger, Bella Savitsky, David S. Siscovick and Orly Manor

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