History of Maternal Pregnancy Characteristics, Birth Weight, and Subclinical Arterial Disease in Young Adults in the Jerusalem Perinatal Study

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Abstract

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Introduction: There is mounting evidence that the intrauterine environment affects the risk of clinical cardiovascular health in the adult offspring. We studied subclinical disease mechanisms that may account, at least in part, for this association by examining the associations of birth weight (BW), pre-pregnancy BMI (ppBMI), and smoking during pregnancy with offspring measurements of subclinical arterial disease.

Methods: Using the EndoPAT2000 device we measured the Augmentation Index (AI), a measurement of arterial stiffness, and the Reactive Hyperemia Index (RHI), a measurement of endothelial function, in 400 subjects from the Jerusalem Perinatal Family Follow-up Study (JPS-1). The JPS-1 includes data on maternal and pregnancy characteristics collected from an interview taken shortly after birth; weight, height, and blood pressure measurements collected at age 17; and a detailed interview and physical exam conducted at age 32. We repeated the models

adjusting for offspring BMI at age 17 and age 32 and for other maternal and offspring lifestyle, socioeconomic, and demographic characteristics.

Results: We found an inverse linear association between BW and AI (β=-0.439, 95% CI (-0.830,-0.048) for a 100g increase in BW) limited to females, that remained after adjustment for offspring obesity but was attenuated after adjustment for other maternal and offspring characteristics. We did not find an association between BW and RHI. We found an inverse 'U' shape association between ppBMI and RHI that remained after adjustment for maternal and offspring characteristics, including offspring obesity. For example, compared to a ppBMI of 19 kg/m^2, a ppBMI of 24 kg/m^2 was associated with a 0.14 higher mean offspring RHI (95% CI 0.004, 0.28), while compared to a ppBMI of 29 kg/m^2, a ppBMI of 34 kg/m^2 was associated with a 0.18 lower mean offspring RHI (95% CI -0.40, -0.002). We did not find an association between ppBMI and offspring AI. There was no evidence of an association between smoking during pregnancy and offspring arterial characteristics.

Conclusion: Our findings contribute to the evidence that maternal ppBMI and offspring BW may affect offspring clinical cardiovascular health later in life, and suggest that the effect may be partly due to changes in offspring subclinical arterial characteristics.

This work is dedicated to my family, for their unwavering support.

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Birth weight and subclinical arterial characteristics

Introduction

Several studies have suggested that intrauterine growth, as reflected by birth weight, can affect the risk for cardiovascular disease later in life. Birth weight has been shown to be associated with cardiovascular disease in several large cohorts[1-5]. Risk for total mortality in offspring 15 years of age and older was shown to decrease with increase of birth weight in men[3]. Similarly, risk for total cardiovascular disease was shown to decrease with increase of birth weight in adult women[1]. Some have found a J-shape or a U-shape association between birth weight and total mortality later in life, suggesting an increase in risk of disease for those born with high birth weight as well[3,5].

While intrauterine growth also has been associated with cardio-metabolic risk factors, the subclinical disease mechanisms that account the associations of intrauterine growth with cardiovascular morbidity and mortality remains unclear. A possible explanation is presented by the developmental origins of disease hypothesis, which maintains that an unfavorable intra-uterine environment, resulting in excess or lack of nutrients in-utero, may lead to permanent changes to the body's physiology and metabolism[6-8]. The unfavorable intrauterine environment results in changes in intrauterine growth, as reflected by high or low birth weight, and in an increased susceptibility for cardiometabolic disease later in life.

In an attempt to study the subclinical disease mechanisms that may account for the association of intrauterine growth with cardiovascular disease, and to facilitate research in a relatively young offspring population, studies increasingly use measurements of subclinical arterial

characteristics, such as endothelial dysfunction and arterial stiffness. Endothelial dysfunction represents one of the earliest abnormalities in the development of atherosclerosis. It has been shown to be associated with cardiovascular disease risk factors such as hypercholesterolemia, hypertension, diabetes, and smoking, as well as with high sensitivity C-reactive protein (hs_CRP) and lipoporotein-associated phospholipase A2 (Lp-PLA2)[9-11] and has been associated with the development of atherosclerosis and cardiovascular disease[12-15]. Arterial stiffness increases with age[16] and with weight gain[17], and has been associated with traditional cardiovascular disease risk factors such as hypertension and diabetes[18]. In a meta-analysis, arterial stiffness was shown to be associated with cardiovascular events and cardiovascular mortality; an increase in aortic stiffness (as measured by pulse wave velocity) of 1 SD was associated with a relative risk of 1.47 (95% CI 1.31-1.64) for total cardiovascular events and with a relative risk of 1.47 (95% CI 1.29-1.66) for cardiovascular mortality[19].

Several studies have looked at the association between birth weight and subclinical cardiovascular outcomes. Some have found arterial stiffness to be associated with birth weight, while others have not[20-25]. There have also been reports of an inverse association between birth weight and endothelial function[26,27]. In some of the studies the associations were significantly attenuated after adjustment for offspring obesity. However, the majority of the studies were retrospective and were missing data on maternal and pregnancy characteristics as well as subjects' socio-demographic characteristics.

The purpose of our study was to examine whether birth weight is associated with an increase in the risk for subclinical cardiovascular disease in the young adult, as reflected by measures of endothelial function and vascular stiffness (Reactive Hyperemia Index and Augmentation Index,

respectively), while accounting for maternal and offspring socio-demographic and lifestyle characteristics and offspring obesity.

Methods

Study setting and participants

Our study is nested in a cohort from the Jerusalem Perinatal Study (JPS). The JPS is a population based study that collected prenatal, perinatal and postnatal data on 17,003 births to Israeli residents of Jerusalem between 1974 and 1976[28]. Offspring birth weight, demographic and socioeconomic status, and maternal medical conditions in index and previous pregnancies were collected from birth certificates or maternal ward logbooks. Further information was collected through interviews taken on the first or second day post-partum, including gestational age, maternal and paternal smoking status, maternal height, pre-pregnancy weight, end of pregnancy weight, and obstetrical medical history[28]. Offspring body mass index (BMI) from age 17 was obtained through linkage to the Israeli military draft records[29].

The JPS Family Follow-Up Study (JPS1) included a sample of 1500 offspring from the original 1974 to 1976 JPS cohort who were recruited between 2007 and 2009. The protocol included a detailed interview focusing on cardiovascular risk factor history, a physical exam consisting of anthropometric and blood pressure measurements, and a blood draw for genotyping and lipid and glucose analysis. Inclusion criteria for the study included born at term (defined as ≥36 weeks) with no known birth defects, both mother and offspring alive at the 2005 Israeli population registry, being able to communicate in Hebrew, and having a telephone at the time of the exam. A stratified sample was created from all eligible JPS subjects, stratified by maternal BMI (<25, 25-26.9, 27-29.9, 30+ kg/m^2) and by birth weight (≤2500, 2501-3999, 4000+ g). Low birth weight (≤2500g) and high birth weight (≥4000g) were oversampled by a factor of 3.1, and overweight and obese mothers (BMI ≥27 kg/m^2) were oversampled by a factor of 3.3.

A random sample of 400 offspring from those who participated in the JPS1 underwent an EndoPAT measurement exam (see below). None of the participants had exclusion criteria that preclude the completion of the EndoPAT exam and/or the interpretation of the results, such as: 1) Known active Raynaud's disease, 2) Women who have had bilateral or right side mastectomy or lumpectomy with lymph node dissection, 3) Presence of a hemodialysis shunts, and 4) Treatment with alpha blockers, Ca channel blockers or long acting nitroglycerine.

The EndoPAT2000 device is a non-invasive device designed to measure vascular characteristics (Itamar Medical Ltd., Caesarea, Israel). Subjects were instructed to fast and to refrain from smoking for at least 6 hours before the exam. Two finger-mounted probes were placed on the index finger of each hand. After a 10 minute baseline reading, an inflated blood measurement cuff was used to occlude the brachial artery for 5 minutes in the non-dominant hand, while the other hand was used as control[30]. An automated computer program analyzed the data received from the probes. The results for the arterial function are presented as two variables (see supplement for more information):

1. Reactive Hyperemia Index (RHI) – the RHI is based on the ratio of the post-deflation to baseline pulse amplitude in the occluded arm compared to the control arm. Endothelial dysfunction results in diminished post-ischemic vasodilatation and a lower RHI.
2. Augmentation Index (AI) – the AI is based on the ratio of the amplitude difference between the second and first peak of the arterial pulse wave, and the amplitude of the first peak of the arterial pulse wave, corrected for a heart rate of 75. AI uses the shape of the arterial pulse wave to determine the baseline stiffness of the artery wall. A higher AI reflects stiffer arteries.

Statistical analysis

Linear regression models were used to measure the association between birth weight (continuous linear variable) as the exposure and RHI or AI as the outcome (both as continuous linear variables). To better detect a possible J or U shape relations, we repeated the analyses adding a quadratic term to our models. We used inverse probability weighting to account for the stratified sampling.

BMI was calculated by dividing weight (kg) by squared height (m^2). We repeated the models with and without offspring BMI at age 17 and current BMI. Univariate multiple imputation using regression[31,32] was used to account for the missing BMI at age 17 data**,** with gender, birth weight, gestational age, height, current BMI, education, SES, smoking, and ethnic origin as predictors.

We repeated our models adjusting for the following maternal and offspring characteristics: gender, gestational age (continuous linear, weeks), maternal age (continuous linear, years), socio-economic status (SES) based on father's occupation (low, medium, high), mother's ethnic origin (Israeli, Other Middle East, North Africa, and Ashkenazi (Europe/America)), maternal level of education (continuous linear, years), maternal smoking during pregnancy (smoker vs. non-smoker), and offspring smoking (ever vs. never).

The analyses were conducted using Intercooled Stata 9 and 12 (StataCorp LP; College Station, TX) and SPSS (SPSS, Inc. Chicago, IL).

This study was approved by the University of Washington Human Subject Review Committee and by the Institutional Review Board of the Hadassah-Hebrew University Medical Center.

Results

Maternal and offspring characteristics by birth weight are summarized in table 1. Compared to mothers with average birth weight offspring, mothers of low birth weight offspring were more likely to be of Middle Eastern descent and less likely to be of African descent, and mothers of high birth weight offspring were older at birth, had a higher pre-pregnancy BMI, and were less likely to smoke during pregnancy. Compared to average birth weight (2500-3999 g), low birth weight (<2500 g) was associated with a younger gestational age, while high birth weight (4000+ g) was associated with older gestational age, being male, higher BMI, ever smoking, higher SBP, higher waist circumference and higher waist-hip ratio.

Unadjusted models showed an inverse linear association between birth weight and AI (mean difference in AI for a 100g increase in birth weight (BW) -0.439, 95% CI -0.830 to -0.048) (table 2). The association remained after adjustment for BMI at age 17 or current BMI (mean difference in AI for a 100g increase in BW -0.474, 95% CI -0.876 to -0.072 for the model adjusted for BMI at age 17, and mean difference in AI for a 100g increase in BW -0.453, 95% CI -0.845 to -0.061 for the model adjusted for current BMI), but was attenuated when adjusted for other maternal and offspring characteristics, including gender, gestational age, maternal age, SES, mother's ethnic origin, maternal level of education, maternal smoking during pregnancy, and offspring smoking (mean difference in AI for a 100g increase in BW -0.278, 95% CI -0.708 to 0.152 for the adjusted model). The association between birth weight and AI was still evident when the unadjusted analyses were limited to the same sample as the adjusted analyses (Supplement table 1).

There was evidence of gender interaction in the association of birth weight and AI (p for sex×birth weight interaction 0.028). Exploratory analyses stratified by gender showed an association between birth weight and AI in females, where higher birth weight was associated with better arterial characteristics (=lower AI) (mean difference in AI for a 100g increase in BW -0.742, 95% CI -1.337 to -0.147 for the unadjusted model) (table 3, Supplement table 1). The association remained after adjustment for BMI at age 17 or current BMI (mean difference in AI for a 100g increase in BW -0.751, 95% CI -1.35 to -0.153 for the model adjusted for BMI at age 17, and mean difference in AI for a 100g increase in BW -0.737, 95% CI -1.338 to -0.137 for the model adjusted for current BMI), but was attenuated after adjusting for maternal and offspring characteristics (mean difference in AI for a 100g increase in BW -0.603, 95% CI -1.230 to 0.024 for the adjusted model). In contrast, there was no association between birth weight and AI in males (mean difference in AI for a 100g increase in BW -0.057, 95% CI -0.338 to 0.451 for the unadjusted model).

There was no evidence of a linear or a non-linear association between offspring birth weight and RHI (mean difference in RHI for a 100g increase in BW 0.007, 95% CI -0.006 to 0.019 for unadjusted linear model; non-linear analyses not shown). Stratifying by gender did not alter the results (table 4).

Discussion

Our findings show an inverse linear association between birth weight and arterial stiffness, so that higher birth weight is associated with lower AI. The association remained after adjusting for BMI at age 17 and current BMI but was attenuated when adjusted for other maternal and offspring characteristics. There was evidence of gender interaction and the association of birth weight and AI was observed in females but not in males. There was little evidence of an association between birth weight and endothelial function, as measured by RHI.

Several studies examined the association between birth weight and measurements of arterial stiffness with mixed results[20-25,33,34]. Tillling *et al* found a weak association between birth weight and mean intima-media thickness (IMT) in 4635 subjects from the Atherosclerosis Risk in Communities (ARIC) study that was attenuated after adjusting for subjects' current lifestyle characteristics and CVD risk factors[23]. te Velde *et al* did not find an association between birth weight and brachial artery compliance in 281 subjects[24]. In both these studies, birth weight was obtained by recall at time of the exam. Similarly, Montgomery *et al* did not find an association between birth weight and pulse wave velocity in 526 young adults (mean age 25 years)[21]. However, his study does not contain information regarding the subjects' gestational age. Including preterm infants that are appropriate for gestational age among the low birth weight infants might have introduced a bias toward the null. In contrast to these and supporting our findings, the Bogalusa Heart Study reported an inverse association between birth weight and pulse wave velocity in 707 young adults (mean age 35.8 years)[25]. Brachial pulse wave velocity (PWV) decreased by 0.23 m/s (95% CI -0.44 to -0.03 m/s) for each 1 kg increase in birth weight. Similarly, Lurbe *et al* found that augmentation index in 219 subjects was higher in birth weights

of less than 2500g and 2500-2999g compared to those with birth weights of 3000-3500g and over 3500g (11.1±3.5 and 11.7±2.5 vs. 3.4±2.1 and 2.5±1.6, respectively)[34], and Broyd *et al* and found an inverse association between birth weight and stiffness index that persisted after adjusting for current weight, heart rate, blood pressure, and smoking in 220 subjects (mean difference in stiffness index per kg birth weight -1.09±0.23, $p<0.0001$ for men and -0.78±0.16, $p<0.0001$ for women)[20].

There are numerous studies that have examined the effect of postnatal growth on the association between low birth weight and cardiovascular risk factors in adults, with the thought that postnatal life can add to the effects of the intrauterine environment[35-41]. Studies have shown that children and adolescent who had a combination of low birth weight and high growth rate during early childhood were more likely to have high blood pressure and decreased insulin sensitivity[42,43], and adults who were treated for high blood pressure were more likely to be born small and have accelerated postnatal growth compared to those who did not develop hypertension[44]. The combination of small size at birth and accelerated weight gain during childhood is associated with an increased risk for hypertension, type 2 diabetes and coronary heart disease in adulthood[6]. Possible mechanisms for the increased risk for cardiometabolic disease in low birth weight subjects with accelerated postnatal growth include development of a disproportionally high fat mass, overgrowth of a limited cell mass hastening cell death and organ dysfunction, and persistent changes in hormone secretion that were established in-utero[6,35,41].

We do not have measurements from infancy and early childhood, but there was little evidence of interaction of subjects' birth weight and their perception of their body's frame at age 10-12 (described as thin, normal, slightly overweight or overweight) and the association between birth

weight and AI (p for perception×birth weight interaction 0.599, supplement table 2). Similarly, the association between birth weight an AI remained after adjusting for BMI at age 17 and no interaction was observed between BMI at age 17 and the association between birth weight and AI (p for BMI at age 17×birth weight interaction 0.484). When we assessed the risk of having the worst AI quintile by strata created by combining birth weight and BMI at age 17 (birth weight divided to low birth weight (<2500g) or normal birth weight (≥2500g), and BMI at age 17 divided to normal (<25kg/m^2) or overweight or obese (≥25 kg/m^2)), there was some evidence that the risk for worst AI was higher in those with a history of low birth weight and accelerated post natal growth (defined as overweight or obese at age 17) compare to those with normal birth weight and normal BMI at age 17, but the number of subjects with low birth weight was small and the confidence intervals were very wide (supplement table 3). In addition, age 17 is late adolescence and weight or BMI at this age could be influenced by hormonal changes and social pressure and thus may not accurately reflect childhood growth.

In few studies the associations between birth weight and arterial stiffness was attenuated after adjusting for current body size[23,24,33], which might imply that the effect of birth weight on arterial stiffness is due to the association between birth weight and adult BMI. In contrast to these, other studies have found birth weight to be associated with arterial stiffness regardless of subjects' obesity[20,25,34]. We found the association between birth weight and AI to be above and beyond differences in subjects' BMI at age 17 or at age 32.

We found evidence of gender interaction, with an association between birth weight and arterial stiffness in women but not in men. A similar gender interaction was found in a study of 220 young adults, where arterial stiffness was found to be correlated with birth weight in women but

not in men ($p<0.001$ for gender interaction)[20]. The reason for this interaction is not clear. Estrogen has been shown to slow the natural progression of arterial stiffness with age[45]. Studies reported differences in serum dehydroepiandrosterone sulfate (DHEAS) concentrations in small for gestational age children compared to appropriate for gestational age children, suggesting changes in hormonal regulation following intra-uterine growth retardation (IUGR)[46,47]. One possible explanation for the observed gender interaction is that the arterial stiffness protective effects of estrogen are attenuated in low birth weight women, resulting in an increase in arterial stiffness that is not observed in men.

Our study did not find an association between birth weight and endothelial function. Accounting for maternal and offspring lifestyle and demographics and stratifying by smoking status or gender did not alter the results. Contrary to our results, Leeson *et al* showed that birth weight was associated with flow-mediated dilatation (FMD) in 333 children aged 8-13 years (β=0.027 mm/kg, 95% CI 0.003-0.051)[27]. A similar study of 311 subjects ages 20-28 (mean age 23) also found FMD to have a linear association with birth weight (β=0.18 kg^{-1}, 95% CI 0.004-0.35)[26]. Subjects in both of these studies were younger than ours. One possible explanation for our lack of association could be that birth weight related changes in endothelial function might be masked later in life as the arteries age and more cardiovascular risk factors accumulate.

Possible mechanisms

The developmental origins of disease hypothesis maintains that unfavorable intra-uterine environment may lead to permanent changes to the body's physiology and metabolism[6,7] but the subclinical disease mechanisms behind these changes remain unclear. Researchers have hypothesized that in fetuses whose growth is impaired, arterial wall elastin synthesis is impaired,

resulting in reduced arterial compliance and stiffer arteries[48]. The rate of elastin synthesis is highest in utero and during infancy with a half-life of approximately 40 years[49]. Burkhardt *et al* compared the umbilical arteries from twelve infants with intra-uterine growth retardation (IUGR) to twelve appropriate for gestational age (AGA) and found that, compared to AGA infants, IUGR infants were found to have significantly thinner vessel walls (2.8 vs.3.8 mm2, $P<0.05$) with lower elastin content and significantly lower plasma IGF-1 concentrations (0.32 vs 0.79 ng/ml, $p<0.01$), a regulator of elastin synthesis[50]. In another study, IUGR sheep vessels were thinner, showed altered collagen to elastin ratio and had less organized and more fragmented elastin compared to control[51]. Impaired elastin synthesis would probably have a stronger effect on the arterial stiffness of large arteries such as the aorta than on the arterial stiffness of peripheral muscular arteries.

Strengths and limitations

Our study's strength is our data which includes detailed prenatal and perinatal information along with extensive data on lifestyle, socioeconomic, demographic and physical characteristics of mothers and adult offspring, enabling us to control for these factors. Prenatal and perinatal data were collected at birth. Our study sample is based on the JPS1 which oversampled for low and high birth weight offspring, enabling us to better study these exposures. BMI was available for age 17 and age 32, allowing us to incorporate three points in time (birth, age 17 and age at time of the exam) into our analyses. However, there are several limitations to our study. Our study sample was relatively small. Our measurements at birth include only weight so we were limited to using birth weight as a surrogate measure of intrauterine growth. However, birth weight is a crude measurement of fetal growth and the measurement does not distinguish between a healthy short baby due to parental genetics and a thin tall malnourished one. The inclusion of intrauterine

growth retardation along with healthy small infants in our analysis might have attenuated our findings. Nevertheless, despite its limitations, birth weight is a widely used surrogate for intrauterine growth and has been successfully used in other studies. Last, our study is an observational study and we might have residual confounding factors that are not fully accounted for.

Conclusion

Our goal was to examine the associations between birth weight and subclinical vascular characteristics that could contribute to the understanding of the underlying mechanism of fetal origins of cardiovascular disease. Our study suggests there is an inverse association of birth weight with arterial stiffness that is limited to females. The association remained after adjusting for subjects' obesity but was attenuated once adjusted for lifestyle and maternal characteristics. Birth weight was not similarly associated with measurements of endothelial function. Further study with a larger sample size and validation using other arterial stiffness measurements is recommended.

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Tables

Table 1: Maternal and offspring characteristics by birth weight, a sample from the Jerusalem Perinatal Family Follow-Up Study (JPS1), 2007-2009

	Offspring birth weight								
	<2500			2500-3999			4000+		
Maternal characteristics¶	Raw data	Weighted*	n (39)	Raw data	Weighted*	n (269)	Raw data	Weighted*	n (96)
Age at birth, *yrs*	26.2 (5.8)	26.3 (5.7)	39	27.8 (5.7)	27.2 (5.3)	269	29.4 (5.7)	29.4 (5.5)	96
Pre-pregnancy BMI, *kg/m²*	21.4 (2.7)	21.4 (2.7)	38	25.2 (4.0)	21.9 (2.9)	269	22.7 (3.5)	23.3 (3.8)	92
Smoking during Pregnancy, *% yes*	18.4	18.5	38	13.5	17.8	267	10.5	9.7	95
Ethnicity, *%*			39			269			96
Israel	12.8	10.4		11.9	9.4		13.5	13.68	
Other Middle East	43.6	45.7		29.3	27.8		25	25.88	
North Africa	10.3	10.4		22.0	22.6		29.2	28.74	
Ashkenazi	33.3	33.4		39.3	39.3		32.3	31.69	
Education, *yrs*	12.3 (2.7)	12.3 (2.6)	36	11.5 (3.2)	12.0 (3.2)	259	11.5 (3.8)	11.3 (3.7)	94
SES			39			269			96
low	18.0	18.3		24.2	22.4		18.8	17.0	
medium	41.0	41.9		36.8	34.7		42.7	44.4	
high	41.0	39.9		39.0	42.9		38.5	38.6	
Offspring characteristics¶									
Birth weight, *gr*	2324 (157)	2326 (156)	39	3273 (361)	3213 (331)	269	4185 (206)	4200 (233)	96
Gestational length, *weeks*	38.8 (1.8)	38.9 (1.8)	39	39.7 (1.5)	39.6 (1.5)	269	40.6 (1.5)	40.6 (1.5)	96
Gender, *% male*	43.5	44.1	36	49.8	49.5	269	72.9	73.7	96
BMI age 17, *kg/m²*	20.8 (4.1)	20.6 (4.0)	29	22.3 (3.5)	21.4 (3.1)	221	21.3 (3.12)	21.5 (3.5)	87
Current BMI, *kg/m²*	25.4 (5.9)	25.2 (3.5)	37	27.4 (5.3)	26.1 (3.1)	268	26.4 (5)	28.2 (3.7)	91
Ever smoking, *% yes*	25.6	23.5	39	37.4	36.7	265	51.6	53.7	95
Heart rate, *bpm*	69.8 (10)	69.7 (10)	39	69.6 (9.8)	68.6 (8.6)	267	66.8 (9.7)	66.6 (10.0)	96
Systolic BP, *mmHg*	105.2 (13.5)	105.2 (13.3)	39	107.5 (12.7)	105.0 (12.9)	267	110.5 (13)	110.5 (13)	96
Diastolic BP, *mmHg*	72 (9.9)	71.9 (10.0)	39	72.8 (8.8)	71.2 (8.9)	267	72.5 (8.4)	72.7 (8.2)	96
Glucose, *mg/dL*	84.1 (13)	84.0 (12.9)	38	80.5 (22.6)	79.8 (14.0)	261	81 (12.2)	80.3 (11.2)	95

Triglycerides, *mg/dL*	122.2 (73.2)	121.9 (72.1)	37	113.3 (67.5)	106.7 (67.2)	263	102.5 (63.8)	100.3 (62.5)	95
Cholesterol, *mg/dL*	196.6 (47.1)	196.2 (47.4)	37	188.4 (34.5)	187.1 (32.4)	263	180.2 (33.8)	179.9 (33.2)	95
HDL, *mg/dL*	51.2 (14.8)	50.7 (14.4)	37	50.7 (15.7)	50.7 (14.5)	262	48.1 (14.7)	48.0 (14.5)	95
LDL, *mg/dL*	121.0 (40.8)	121.1 (40.8)	37	115.0 (31.6)	115.5 (29.7)	261	111.6 (29.4)	111.8 (28.8)	95
Waist, *cm*	83.2 (13.3)	83.1 (13.3)	39	89.0 (14.2)	85.9 (13.7)	267	88.9 (13.3)	89.49 (13.6)	96
Waist-hip ratio	0.81 (0.08)	0.81 (0.08)	39	0.84 (0.08)	0.83 (0.07)	267	0.85 (0.08)	0.85 (0.08)	96
RHI[†]	1.86 (0.5)	1.86 (0.5)	39	-1.73 (0.5)	1.70 (0.4)	269	1.76 (0.5)	1.76 (0.4)	95
AI[‡]	-2.29 (11.5)	-2.83 (10.7)	39	-6.62 (12.4)	-7.08 (12.8)	266	-11.53 (11.4)	-11.8 (11.1)	94

* Values were weighted to account for stratified sampling of mother-child pairs based on birth weight and maternal pre-pregnancy BMI

¶ Continuous variables expressed as mean (SD) † RHI – reactive hyperemic index ‡ AI – augmentation index

Table 2: Associations of birth weight and AI*

	beta	95% CI		*p*	n
Unadjusted	-0.439	-0.830	-0.048	0.028	397
Unadjusted + BMI at age 17	-0.474	-0.876	-0.072	0.021	397
Unadjusted + current BMI	-0.453	-0.845	-0.061	0.024	396
Adjusted†	-0.278	-0.708	0.152	0.204	366

* Linear regression models. Coefficient indicates mean difference in AI per birth weight increase of 100 grams. AI – augmentation index
† Adjusted for gestational week, gender, maternal age at birth, maternal education, SES, maternal ethnic origins, offspring ever smoking and maternal smoking during pregnancy

Table 3: Associations of birth weight and AI by gender*

	beta	95% CI		*p*	n
Stratified by gender					
Female					
Unadjusted	-0.742	-1.337	-0.147	0.015	177
Unadjusted + BMI at age 17	-0.751	-1.348	-0.153	0.014	177
Unadjusted + current BMI	-0.737	-1.338	-0.137	0.016	177
Adjusted†	-0.603	-1.230	0.024	0.059	166
Male					
Unadjusted	-0.057	-0.338	0.451	0.778	216
Unadjusted + BMI at age 17	-0.101	-0.483	0.280	0.601	216
Unadjusted + current BMI	-0.015	-0.399	0.369	0.939	215
Adjusted†	-0.052	-0.628	0.525	0.860	201

* Linear regression models. Coefficient indicates mean difference in AI per birth weight increase of 100 grams. AI – augmentation index
† Adjusted for gestational week, gender, maternal age at birth, maternal education, SES, maternal ethnic origins, and maternal smoking during pregnancy

Table 4: Associations of birth weight and RHI*

	beta	95% CI		*p*	n
All subjects					
Unadjusted model	0.007	-0.006	0.019	0.308	394
Unadjusted + BMI at age 17	0.005	-0.007	0.018	0.406	394
Unadjusted + current BMI	0.006	-0.007	0.018	0.367	394
Adjusted†	0.002	-0.016	0.019	0.867	363
Female					
Unadjusted model	0.003	-0.018	0.023	0.809	176
Unadjusted + BMI at age 17	0.002	-0.018	0.023	0.818	176
Unadjusted + current BMI	0.002	-0.018	0.023	0.817	176
Adjusted†	-0.001	-0.022	0.020	0.912	165
Male					
Unadjusted model	0.009	-0.009	0.027	0.339	213
Unadjusted + BMI at age 17	0.006	-0.012	0.023	0.516	213
Unadjusted + current BMI	0.006	-0.012	0.024	0.527	213
Adjusted†	0.007	-0.016	0.031	0.545	200

* Linear regression models. Coefficient indicates mean difference in RHI per BW increase of 100 grams. RHI – reactive hyperemia index

† Adjusted for gestational week, gender, maternal age at birth, maternal education and SES, maternal ethnic origins, offspring ever smoking and maternal smoking during pregnancy

Supplements

Calculating RHI and AI

RHI = (Indexed PAT ratio * Baseline correction factor)

where the Indexed PAT ratio = (RH occluded 90s-120s +RH occluded 120s-150s) / (RH control 90s-120s + RH control 120s-150s)

The RH for each time period above is the mean pulse wave amplitude (PWA) of post occlusion section divided by the mean PWA of baseline region of interest.

Occluded = test arm, the arm on which the cuff is placed; Control = control arm

The Baseline correction factor is based on the observation that subjects with an initial high signal have a smaller potential for dilation even if their endothel is healthy (their artery is already dilated to much of its capacity). The higher the baseline signal is, the more weight a given increase in amplitude receives.

AI = (P2-P1)/P1

where P2 is the pressure at the 2nd peak in systole (the reflected wave from the periphery) and P1 is the pressure at the 1st peak in systole (the systolic contraction of the ventricle). The result is further normalized to heart rate of 75bpm.

Supplement tables

Supplement table 1: The associations of birth weight and AI using the same sample for the unadjusted and adjusted models*.

	beta	95% CI		*p*	n
All subjects					
Unadjusted	-0.439	-0.830	-0.048	0.028	397
Unadjusted limited sample¶	-0.483	-0.898	-0.067	0.023	366
Adjusted†	-0.278	-0.708	0.152	0.204	366
Female					
Unadjusted	-0.742	-1.337	-0.147	0.015	177
Unadjusted limited sample¶	-0.722	-1.345	-0.100	0.023	166
Adjusted‡	-0.603	-1.230	0.024	0.059	166
Male					
Unadjusted	-0.057	-0.338	0.451	0.778	216
Unadjusted limited sample¶	-0.018	-0.446	0.409	0.937	201
Adjusted‡	-0.052	-0.628	0.525	0.860	201

* Linear regression models. Coefficient indicates mean difference in AI per birth weight increase of 100 grams. AI – augmentation index
¶ Sample limited to offspring with no missing values for the adjustment covariates
† Adjusted for gestational week, maternal age at birth, maternal education, SES, maternal ethnic origins, offspring ever smoking, maternal smoking during pregnancy, and gender
‡ Adjusted for gestational week, maternal age at birth, maternal education, SES, maternal ethnic origins, offspring ever smoking, and maternal smoking during pregnancy

Supplement table 2: The association of birth weight and AI, stratified by gender and body perception at age 10-12 years*.

	beta	95% CI		p	n
Female body perception age 10-12					
Thin	-0.381	-0.930	0.168	0.170	72
Normal	-0.475	-0.939	-0.011	0.045	64
Slightly overweight	-0.178	-0.986	0.630	0.657	37
overweight	0.572	-1.660	2.80	0.385	4
Male body perception age 10-12					
thin	-0.254	-0.540	0.031	0.080	100
normal	-0.217	-0.626	0.191	0.293	84
Slightly overweight	-0.011	-0.559	0.537	0.968	25
overweight	-0.677	-1.364	0.010	0.052	7

* Linear regression models. Coefficient indicates mean difference in AI per birth weight increase of 100 grams. AI – augmentation index

Supplement table 3: Joint association of low birth weight† and high BMI at age 17‡ on risk of having the high AI quintile*

BW/BMI at age 17	OR	95% CI		p	n
Normal BW / Normal BMI	ref	ref			251
Normal BW/ High BMI	2.73	0.92	8.09	0.071	57
Low BW/ Normal BMI	1.95	0.73	5.19	0.182	26
Low BW/ High BMI	8.28	0.71	96.49	0.092	3

† Birth weight (BW) divided to low (defined as bellow 2500g) or normal (defined as 2500g and above)

‡ BMI at age 17 divided to normal (defined as bellow 25 kg/m^2) or high (defined as 25 kg/m^2 or above)

* AI – augmentation index. High AI quintile defined as AI > 1.2

Pre-pregnancy BMI and offspring subclinical arterial characteristics

Introduction

Maternal overweight and obesity has been shown to be associated with increased large for gestational age birth weight[1-3], which in turn is associated with an increased risk for obesity and metabolic syndrome in childhood[4,5]. Maternal pre-pregnancy body mass index (BMI) has also been shown to be associated with offspring BMI later in life, regardless of birth weight[6-8]. Studies have shown that, even after adjusting for birth weight, higher pre-pregnancy maternal BMI is associated with an increased risk for higher systolic[6,9] and diastolic[6] blood pressure in the offspring and the association seems to be mainly due to the increase in offspring BMI[10]. Possible mechanisms for the intrauterine effects of maternal obesity on the offspring cardiometabolic risk include permanent changes in offspring ratio of fat to lean body mass, changes in the central nervous system affecting the offspring's appetite and the satiety set point, and increased insulin resistance[11-13].

In an attempt to better understand the subclinical disease mechanisms that may account for the association of the intrauterine environment with cardiovascular disease, and to facilitate research in a relatively young offspring population, studies increasingly use measurements of subclinical arterial characteristics, such as endothelial dysfunction and arterial stiffness. Endothelial dysfunction represents one of the earliest abnormalities in the development of atherosclerosis. It has been shown to be associated with cardiovascular disease risk factors such as hypercholesterolemia, hypertension, diabetes, and smoking, as well as with high sensitivity C-reactive protein (hs_CRP) and lipoporotein-associated phospholipase A2 (Lp-PLA2)[14-16] and has been associated with the development of atherosclerosis and cardiovascular disease[17-20]. Arterial

stiffness increases with age[21] and with weight gain[22], and has been associated with traditional cardiovascular disease risk factors such as hypertension and diabetes[23]. In a meta-analysis, arterial stiffness was shown to be associated with cardiovascular events and cardiovascular mortality; an increase in aortic stiffness (as measured by pulse wave velocity) of 1 SD was associated with a relative risk of 1.47 (95% CI 1.31-1.64) for total cardiovascular events and with a relative risk of 1.47 (95% CI 1.29-1.66) for cardiovascular mortality[24].

Prior human population studies have not examined the associations of pre-pregnancy BMI with offspring subclinical cardiovascular disease. Studies in rats have shown that offspring of rats exposed to high fat diets before or during pregnancy have impaired endothelial function[25-27], and that offspring of rats exposed to a low protein diet during pregnancy have decreased aortic wall thickness and elastin content[28]. If pre-pregnancy BMI in humans was associated with offspring subclinical measurements of arterial disease, such as arterial stiffness or endothelial function, it might point to a novel potential subclinical disease mechanism that accounts, in part, for the fetal origins of cardiovascular disease.

The purpose of our study was to examine whether maternal pre-pregnancy BMI is associated with subclinical cardiovascular disease in the young adult, as reflected by measures of endothelial function and vascular stiffness (Reactive Hyperemia Index and Augmentation Index, respectively), and to examine whether the associations are above and beyond differences in offspring BMI.

Methods

Study setting and participants

Our study is nested in a cohort from the Jerusalem Perinatal Study (JPS). The JPS is a population based study that collected prenatal, perinatal and postnatal data on 17,003 births to Israeli residents of Jerusalem between 1974 and 1976[29]. Offspring birth weight, demographic and socioeconomic status, and maternal medical conditions in current and previous pregnancies were collected from birth certificates or maternal ward logbooks. Further information was collected through interviews taken on the first or second day post-partum, including gestational age, maternal smoking status, maternal height, pre-pregnancy weight, end of pregnancy weight, and gynecological medical history[29]. Offspring BMI from age 17 was obtained through linkage to the Israeli military draft records[30].

The JPS Family Follow-Up Study (JPS1) included a sample of 1500 offspring from the original 1974 to 1976 JPS cohort who were recruited between 2007 and 2009. The protocol included a detailed interview focusing on cardiovascular risk factor history, a physical exam consisting of anthropometric and blood pressure measurements, and a blood draw for genotyping and lipid and glucose analysis. Inclusion criteria for the study include born at term (defined as ≥36 weeks) with no known birth defects, both mother and offspring alive at the 2005 Israeli population registry, being able to communicate in Hebrew, and having a telephone at the time of the exam. A stratified sample was created from all eligible JPS subjects, by maternal BMI (<25, 25-26.9, 27-29.9, 30+ kg/m^2) and by birth weight (≤2500, 2501-3999, 4000+ g). Low birth weight (≤2500g) and high birth weight (≥4000g) were oversampled by a factor of 3.1, and overweight and obese mothers (BMI ≥27 kg/m^2) were oversampled by a factor of 3.3.

A random sample of 400 offspring from those who participate in the JPS1 study underwent an EndoPAT measurement exam (see below). None of the participant had exclusion criteria that preclude the completion of the EndoPAT exam and/or the interpretation of the results, such as: 1) Known active Raynaud's disease, 2) Women who have had bilateral or right side mastectomy or lumpectomy with lymph node dissection, 3) Presence of a hemodialysis shunts, and 4) Treatment with alpha blockers, Ca channel blockers or long acting nitroglycerine.

The EndoPAT2000 is a non-invasive device designed to measure vascular characteristics (Itamar Medical Ltd., Caesarea, Israel). Subjects were instructed to fast and to refrain from smoking for at least 6 hours before the exam. Two finger-mounted probes were placed on the index finger of each hand. After a 10 minute baseline reading, an inflated blood measurement cuff was used to occlude the brachial artery for 5 minutes in the non-dominant hand, while the other hand was used as control[31]. An automated computer program analyzed the data received from the probes. The results for the arterial function are presented as two variables (see supplement for more information):

1. Reactive Hyperemia Index (RHI) – the RHI is based on the ratio of the post-deflation to baseline pulse amplitude in the occluded arm compared to the control arm. It is a dynamic characteristic of the arteries and measures the change in pulse wave amplitude in response to physiological stressors (such as ischemia induced by transient artery occlusion). The change in amplitude reflects endothelium-dependent vasodilation through endothelial derived nitric oxide (NO). Endothelial dysfunction results in diminished post-ischemic vasodilatation and a lower RHI.

2. Augmentation Index (AI) – the AI is based on the ratio of the amplitude difference between the second and first peak, and the amplitude of the first peak of the arterial pulse wave, standardized for a heart rate of 75. AI measures arterial stiffness, which is a static or structural characteristic of the arteries. It uses the shape of the arterial pulse wave to determine the baseline stiffness of the artery wall and is influenced by the properties of the medial layer of the artery composed of smooth muscle cells and an extracellular matrix of elastin and collagen. A higher AI reflects stiffer arteries.

Statistical analysis

BMI was calculated by dividing weight (kg) by squared height (m^2). Linear regression models were used to measure the association between pre-pregnancy BMI as the exposure (continuous linear variable) and RHI or AI as the outcome (both as continuous linear variables). To better detect a possible J or U shape relations, we repeated the analyses adding a quadratic term to our models. We used inverse probability weighting to account for the stratified sampling.

We added the following maternal and offspring characteristics to our models: gender, gestational age (continuous linear, weeks), maternal age (continuous linear, years), socio-economic status (SES) based on father's occupation (low, medium, high), mother's ethnic origin (Israeli, Asia (Middle East), North Africa, and Ashkenazi (Europe/America)), maternal level of education (continuous linear, years), maternal smoking during pregnancy (smoker vs. non-smoker), and offspring smoking (ever vs. never). We repeated the models with and without offspring birth weight, BMI at age 17, and current BMI (all continuous linear). We also repeated the model adjusting for gestational weight gain (continuous linear), calculated as the difference between the weight at the end of the pregnancy and the pre-pregnancy weight.

The analyses were conducted using Intercooled Stata 9 and 12 (StataCorp LP; College Station, TX) and SPSS (SPSS, Inc. Chicago, IL).

This study was approved by the University of Washington Human Subject Review Committee and by the Institutional Review Board of the Hadassah-Hebrew University Medical Center.

Results

Maternal and offspring characteristics by maternal pre-pregnancy BMI are summarized in table 1. Mothers with a pre-pregnancy BMI of 25 kg/m^2 or higher were older at birth, less likely to smoke during pregnancy, and less educated compared to those with a pre-pregnancy BMI of less than 25 kg/m^2. Compared to offspring of mothers with a pre-pregnancy BMI of less than 25 kg/m^2, offspring of mothers with pre-pregnancy BMI of 25 kg/m^2 and above were more likely to have higher birth weight, higher BMI at age 17, higher current BMI, higher systolic and diastolic blood pressure, higher triglycerides, higher waist circumference and a higher waist-hip ratio.

We found a non-linear association between pre-pregnancy BMI (ppBMI) and endothelial function as measured by RHI (figure 1, table 2). For example, based on the adjusted model, an additional 5 kg/m^2 BMI, comparing a maternal pre-pregnancy BMI of 24 kg/m^2 to a maternal pre-pregnancy BMI of 19 kg/m^2, is associated with a 0.14 higher mean offspring RHI (95% CI 0.004-0.28), while an additional 5 kg/m^2 BMI, comparing a maternal pre-pregnancy BMI of 34 kg/m^2 to a maternal pre-pregnancy BMI of 29 kg/m^2, is associated with a 0.18 lower mean offspring RHI (95% CI -0.40 to -0.002) (table 3). The association remained after adjustment for maternal and offspring characteristics and after adjustment for birth weight, BMI at age 17, and current offspring BMI. The association was also seen after adjustment for pregnancy weight gain (supplement table 1) and was also evident when the unadjusted analysis was limited to the same sample as the adjusted analysis (supplement table 2).

There was no evidence of an association between maternal pre-pregnancy BMI and arterial stiffness as measured by AI using a linear model or a non-linear one (mean difference in AI for a

change of 1 kg/m^2 in ppBMI 0.066, 95% CI -0.587 to 0.718, for the adjusted linear model) (table 4).

Discussion

Our findings show an association between maternal pre-pregnancy BMI and offspring endothelial function as measured by RHI at an average age of 32 years. The association seems to have an inverted U shape, so that both low pre-pregnancy BMI and high pre-pregnancy BMI are associated with decreased endothelial function. The association remained after adjusting for maternal and offspring characteristics and pregnancy weigh gain, and after adjusting for offspring birth weight, BMI at age 17 or current BMI. We did not find an association between maternal pre-pregnancy BMI and arterial stiffness, as measured by AI.

Comparison with other studies

Prior studies in human populations have not examined the association of maternal pre-pregnancy BMI and offspring subclinical arterial characteristics later in life. While addressing a somewhat different question, animal experimental studies examined the effect of nutrition throughout the pregnancy on the offspring arterial characteristics (these studies did not assess the effect of maternal obesity during conception and early pregnancy). Studies in rats have shown that offspring exposed to high fat diets during pregnancy or caloric restriction during pregnancy have impaired endothelial function compared those exposed to regular diet[25-27,25]. However, the effect of prenatal caloric restriction on endothelial function depended on the diet the offspring received immediately after birth[28,32]. Since rats are born at a much earlier stage of development than human infants and continue to develop after birth, it is possible that in rats the effect of postnatal nutrition on endothelial function is stronger than the effect of prenatal nutrition. These differences in fetal development raise questions regarding the relevance of these animal-experimental findings to human health.

Kumaran *et al* examined the association of maternal weight during pregnancy and arterial stiffness (measured using pulse wave velocity) in 425 subjects age 40-61 years[33]. Arterial compliance was reduced in subjects born to mothers whose pregnancy weight was at the lower tertile. However, pre-pregnancy BMI was not available in this study. The authors collected pregnancy weight from maternal antenatal visit records, and since the timing of the visit was different for each mother, calculated the approximate gestational age of the visit by calculating how many days before delivery the mother attended the clinic and used gestational age-adjusted pregnancy weight for their analyses. The pregnancy weight in this case reflects both the mothers' pre-pregnancy BMI as well as weight gain during pregnancy.

We did not find an association between pre-pregnancy weight and arterial stiffness. In contrast, our study did show an inverse "U" shaped association between maternal pre-pregnancy weight and endothelial function that remained after adjustment for maternal and offspring characteristics. To the best of our knowledge this is the first study to examine the association of pre-pregnancy BMI and offspring endothelial function. Pre-pregnancy BMI has been previously shown to be positively associated with birth weight[1-3] and with offspring BMI later in life[6-8]. However, the association between pre-pregnancy BMI and RHI in our study did not seem to be driven by offspring adiposity and remained after adjustment for birth weight and offspring BMI.

Gestational weight gain has been shown to be positively associated with offspring BMI[6,34-36] and a few studies have suggested that it might also be associated with other offspring cardiovascular risk factors[6,34,37]. Adjustment for pregnancy weight gain did not alter the association between maternal pre-pregnancy BMI and RHI (supplement table 1). Maternal weight gain, assessed using maternal weight at the end of the pregnancy, includes the weight of the fetus, amniotic

fluid, and placenta. Our findings were similar when the analysis was repeated deducting the birth weight from the maternal weight gain (supplement table 1).

Possible mechanisms

The association of low pre-pregnancy BMI and decreased endothelial function may be explained by the developmental origins of disease hypothesis, which maintains that an unfavorable intra-uterine environment resulting in lack of nutrients in-utero may lead to permanent changes to the body's physiology and metabolism[38,39]. Similarly, the developmental overnutrition hypothesis proposes that the increased nutrient supply to the fetus in mothers with high BMI results in permanent changes in the offspring metabolism and appetite regulation that can result in obesity and cardiometabolic risk later in life[40,41]. Pregnant obese mothers develop earlier insulin resistance and higher hyperlipidemia compared to non-obese mothers, and obese pregnant women have a general increase in inflammatory markers and an increase in placental inflammatory response, potentially influencing the fetus[42].

Environmental characteristics shared by the mothers and offspring might explain some of the association we found, since characteristics that affect maternal pre-pregnancy weight may also affect endothelial function in the offspring. For instance, mothers who smoke tend to be thinner[43] and smoking is associated with decreased endothelial function[44]. Similarly, shared dietary habits can explain obesity in both mothers and offspring, which in turn can lead to decreased endothelial function[45]. However, our findings were similar after adjusting for several maternal and offspring environmental and lifestyle characteristics including maternal smoking during pregnancy and offspring obesity.

Strength and limitations

Our study's strength is the availability of detailed prenatal and perinatal information along with extensive data on lifestyle, socioeconomic, demographic and physical characteristics of mothers and adult offspring at age 32 years, enabling us to control for some of the factors that could potentially act as possible confounders or effect modifiers. Additionally, the inclusion of these characteristics in our models might improve the precision of our estimates of the association of pre-pregnancy BMI with our outcomes. There are several limitations to our study. Our sample size is relatively small. However, by oversampling for maternal pre-pregnancy weight we enriched our population with overweight and obese mothers, better enabling us to study this pregnancy characteristic. Pre-pregnancy weight was collected retrospectively from the mothers by interview a few days after delivery and not from clinical records. Studies have shown that there is a high correlation between maternal recollection of pre-pregnancy weight and clinical records and that maternal recall is reproducible[46,47]. Having the interview mere days after the delivery probably increased the likelihood for accurate recollection. Last, our study is an observational study and we might still have residual confounding factors of the shared maternal and offspring environment that we have not accounted for.

Conclusion

To the best of our knowledge, this is the first study to examine the associations of maternal pre-pregnancy weight and subclinical vascular characteristics. Our study suggests there is an inverse U shaped association between pre-pregnancy BMI and endothelial function, so that offspring of mothers with low pre-pregnancy BMI and those with high pre-pregnancy BMI are more likely to have impaired endothelial function and are potentially at a higher risk for future cardiovascular disease. The association was observed after taking into account differences in offspring birth

weight or obesity. Further study with a larger sample size and validation using other endothelial function measurements is recommended.

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Tables and figures

Table 1: Maternal and offspring characteristics by pre-pregnancy BMI, a sample from the Jerusalem Perinatal Family Follow-Up Study (JPS1), 2007-2009

	Pre-pregnancy BMI (kg/m^2)					
	<25			25+		
Maternal characteristics¶	Raw data	Weighted*	n (210)	Raw data	Weighted*	n (189)
Age at birth, *yrs*	27.6 (5.6)	27.2 (5.3)	210	28.5 (5.6)	28.4 (5.7)	189
Pre-pregnancy BMI, *kg/m^2*	21.1 (1.9)	21.0 (1.9)	210	27.8 (2.6)	27.5 (2.6)	189
Smoking during pregnancy, *% yes*	17.3	18.6	208	9.0	8.8	188
Ethnicity, *%*			210			189
Israel	11.0	9.1		13.3	13.4	
Other Middle East	29.5	29.7		28.0	28.8	
North Africa	22.8	21.2		28.0	27.8	
Ashkenazi	36.7	40.0		30.7	30.0	
Education, *yrs*	12.0 (3.2)	12.1 (3.1)	206	11.1 (3.3)	11.1 (3.3)	180
SES			210			189
low	20.5	21.7		24.3	23.4	
medium	39.1	34.9		38.6	39.7	
high	40.5	43.4		37.0	36.9	
Offspring characteristics¶						
Birth weight, *gr*	3371 (726)	3222 (421)	210	3420 (432)	3461 (461)	189
Gestational length, *weeks*	39.7 (1.6)	39.6 (1.5)	206	39.9 (1.5)	40.0 (1.5)	188
Gender, *% male*	56.2	50.5	210	52.4	54.1	189
BMI age 17, *kg/m^2*	20.9 (3.0)	21.1 (2.9)	185	23.1 (3.7)	23.0 (3.8)	149
Current BMI, *kg/m^2*	25.6 (2.6)	25.4 (2.4)	208	31.7 (2.8)	31.5 (2.7)	187
Ever smoking, *% yes*	39.1	36.8	207	40.1	41.9	187
Heart rate, *bpm*	68.2 (9.2)	68.4 (8.4)	209	69.9 (10.5)	69.5 (10.4)	188
Systolic BP, *mmHg*	107.0 (13.2)	104.8 (13.0)	209	109.2 (12.6)	109.1 (12.6)	188
Diastolic BP, *mmHg*	71.5 (8.8)	70.9 (8.9)	209	74.0 (8.6)	73.9 (8.7)	188
Glucose, *mg/dL*	80.4 (11.2)	79.8 (10.3)	205	81.2 (26.1)	81.2 (26.3)	184

Triglycerides, *mg/dL*	103.7 (65.4)	104.8 (66.9)	206	120.1 (68.7)	118.3 (67.2)	184
Cholesterol, *mg/dL*	185.2 (35.8)	186.4 (32.5)	206	189.5 (36.0)	189.3 (35.8)	184
HDL, *mg/dL*	50.5 (14.6)	50.6 (14.2)	206	49.9 (16.3)	49.8 (16.3)	183
LDL, *mg/dL*	114.2 (31.6)	115.3 (29.6)	205	115.3 (32.8)	115.7 (32.5)	183
Waist, *cm*	85.3 (13.06)	85.2 (13.5)	210	91.7 (14.2)	91.6 (14.2)	187
Waist-hip ratio	0.83 (0.07)	0.82 (0.07)	210	0.85 (0.09)	0.85 (0.09)	187
RHI†	1.76 (0.46)	1.70 (0.44)	207	1.74 (0.49)	1.75 (0.50)	185
AI‡	-7.67 (12.83)	-7.26 (12.85)	209	-6.98 (11.62)	-7.42 (11.67)	188

* Values were weighted to account for stratified sampling of mother-child pairs based on offspring birth weight and maternal pre-pregnancy BMI

¶ Continuous variables expressed as mean (SD) † RHI – reactive hyperemic index ‡ AI – augmentation index

Figure 1: The association between pre-pregnancy BMI and RHI using the unadjusted quadratic regression model

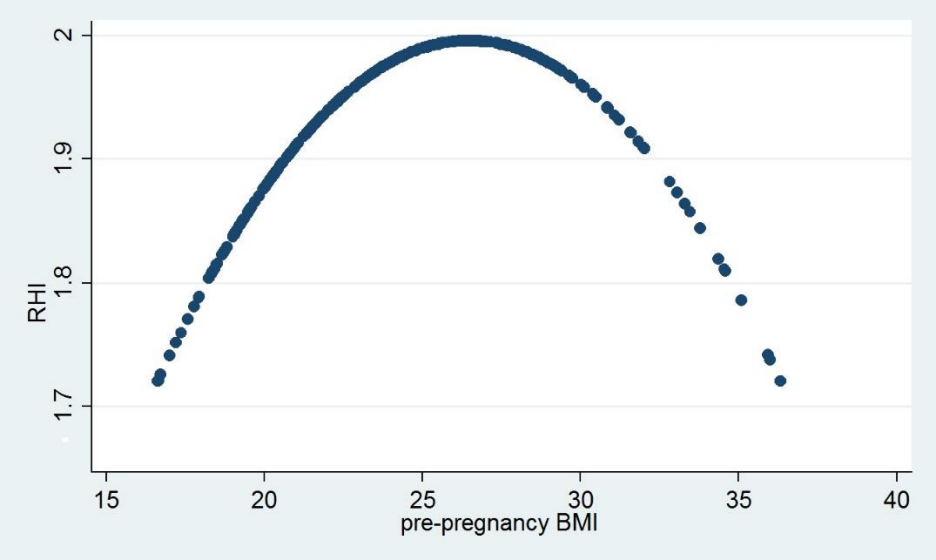

Table 2: Association of pre-pregnancy BMI and RHI*

	beta	95% CI		p	n
Linear regression model					
Unadjusted	-0.015	-0.001	0.030	0.059	393
Adjusted†	0.012	-0.007	0.031	0.215	366
Quadratic regression model					
Unadjusted					
ppbmi‡	0.151	0.008	0.293	0.038	393
ppbmi^2	-0.003	-0.006	-0.00004		
Adjusted†					
ppbmi‡	0.166	0.012	0.319	0.035	366
ppbmi^2	-0.003	-0.006	-0.0002		
Adjusted† + birth weight					
ppbmi‡	0.165	0.012	0.318	0.034	366
ppbmi^2	-0.003	-0.006	-0.0002		
Adjusted† + BMI at age 17					
ppbmi‡	0.170	0.015	0.325	0.031	366
ppbmi^2	-0.003	-0.006	-0.0003		
Adjusted† + current BMI					
ppbmi‡	0.174	0.016	0.332	0.031	366
ppbmi^2	-0.003	-0.006	-0.0003		

† Adjusted for gestational week, maternal age at birth, maternal education and SES, maternal ethnic origins, maternal smoking during pregnancy and offspring ever smoking

‡ ppbmi - pre-pregnancy BMI * RHI – reactive hyperemia index

Table 3: Mean differences in estimated RHI* associated with 5 kg/m^2 additional pre-pregnancy BMI, based on the adjusted quadratic regression model†

Reference pre-pregnancy BMI (kg/m^2)	Mean difference in RHI	95% CI	
19	0.14	0.004	0.28
24	-0.02	-0.12	0.07
29	-0.18	-0.40	-0.002

* RHI – reactive hyperemia index

† Adjusted for gestational week, maternal age at birth, maternal education and SES, maternal ethnic origins, maternal smoking during pregnancy and offspring ever smoking.

Table 4: Associations of pre-pregnancy BMI and AI*

	beta	95% CI		p	n
Linear regression model					
Unadjusted model	0.167	-0.461	0.794	0.601	398
Adjusted model†	0.194	-0.437	0.826	0.545	371
Quadratic regression model					
Unadjusted					
ppbmi‡	1.502	-4.408	7.412	0.869	398
ppbmi^2	-0.028	-0.141	0.085		
Adjusted†					
ppbmi‡	1.841	-4.290	7.973	0.817	371
ppbmi^2	-0.034	-0.153	0.084		

† Adjusted for gestational week, maternal age at birth, maternal education and SES, maternal ethnic origins, offspring ever smoking and maternal smoking during pregnancy

‡ ppbmi - pre-pregnancy BMI * AI – augmentation index

Supplements

Calculating RHI and AI

RHI = (Indexed PAT ratio * Baseline correction factor)

where the Indexed PAT ratio = (RH occluded 90s-120s +RH occluded 120s-150s) / (RH control 90s-120s + RH control 120s-150s)

The RH for each time period above is the mean pulse wave amplitude (PWA) of post occlusion section divided by the mean PWA of baseline region of interest.

Occluded = test arm, the arm on which the cuff is placed; Control = control arm

The Baseline correction factor is based on the observation that subjects with an initial high signal have a smaller potential for dilation even if their endothel is healthy (their artery is already dilated to much of its capacity). The higher the baseline signal is, the more weight a given increase in amplitude receives.

AI = (P2-P1)/P1

where P2 is the pressure at the 2nd peak in systole (the reflected wave from the periphery) and P1 is the pressure at the 1st peak in systole (the systolic contraction of the ventricle). The result is further normalized to heart rate of 75bpm.

Supplement tables

Supplement table 1: Association between pre-pregnancy BMI and RHI[¶], adjusted for gestational weight gain

	beta	95% CI		p	n
Quadratic regression model					
Unadjusted					
ppbmi[‡]	0.151	0.008	0.293	0.038	393
ppbmi^2	-0.003	-0.006	-0.00004		
Adjusted[†]					
ppbmi[‡]	0.166	0.012	0.319	0.034	366
ppbmi^2	-0.003	-0.006	-0.0002		
Adjusted[†] plus pregnancy weight gain[*]					
ppbmi[‡]	0.175	0.020	0.329	0.027	366
ppbmi^2	-0.003	-0.006	-0.0004		
Adjusted[†] plus pregnancy weight gain w/o birth weight[**]					
ppbmi[‡]	0.175	0.020	0.329	0.028	366
ppbmi^2	-0.003	-0.006	-0.0004		

¶ RHI – reactive hyperemia index ‡ ppbmi - pre-pregnancy BMI

† Adjusted for gestational week, maternal age at birth, maternal education and SES, maternal ethnic origins, offspring ever smoking and maternal smoking during pregnancy

* Calculated as end of pregnancy weight minus pre-pregnancy weight

** Calculated as end of pregnancy weight minus pre-pregnancy weight minus birth weight

Supplement table 2: Association of pre-pregnancy BMI and RHI[*] using the same sample for the unadjusted and adjusted models

	beta	95% CI		p	n
Quadratic regression model					
Unadjusted					
ppbmi[‡]	0.151	0.008	0.293	0.038	393
ppbmi^2	-0.003	-0.006	-0.00004		
Unadjusted limited sample[¶]					
ppbmi[‡]	0.172	0.018	0.325	0.028	366
ppbmi^2	-0.003	-0.006	-0.0003		
Adjusted[†]					
ppbmi[‡]	0.166	0.012	0.319	0.035	366
ppbmi^2	-0.003	-0.006	-0.0002		

*RHI – reactive hyperemia index ‡ ppbmi - pre-pregnancy BMI

¶ Sample limited to offspring with no missing values for the adjustment covariates

† Adjusted for gestational week, maternal age at birth, maternal education and SES, maternal ethnic origins, maternal smoking during pregnancy and offspring ever smoking

Maternal smoking during pregnancy and offspring subclinical arterial characteristics

Introduction

According to the 2008 Pregnancy Risk Assessment and Monitoring System (PRAMS) data from 29 states, approximately 12.8% of women reported smoking during the last three months of pregnancy[1]. Maternal smoking during pregnancy has been known to adversely affect fetal growth, both through direct fetal toxicity of the cigarette smoke and due to effects of smoking on the placenta vasculature[2]. Smoking during pregnancy is associated with poor pregnancy outcomes including placenta previa, abruptio placentae, preterm birth, low birth weight, and sudden infant death syndrome (SIDS)[3-5].

Maternal smoking during pregnancy has also been shown to be associated with offspring cardiovascular risk factors[6-13]. Although infants of mothers who smoke tend to be smaller at birth, several studies have shown that offspring of mothers who smoke during pregnancy are at increased risk for obesity later in life[6]. In addition, studies have shown that smoking in pregnancy is associated with increased diastolic[7] and systolic[7-9,14] blood pressure in the offspring. In several of the studies offspring obesity appeared to account for some[9] or all[14] of this association. Studies have found an association between maternal smoking during pregnancy and offspring cholesterol and triglyceride levels[11,12]. An association between maternal smoking during pregnancy and offspring changes in glucose metabolism was also reported and attributed to offspring adiposity[13].

Studying the effects of maternal smoking during pregnancy on offspring subclinical measurements of arterial disease, such as arterial stiffness or endothelial function, could

contribute to the understanding of the underlying mechanism of fetal origins of cardiovascular disease. There is limited data regarding the effect of smoking during pregnancy on these measurements in the offspring. Children of mothers who smoked during pregnancy had significantly lower arterial distensibility (a measurement of arterial stiffness) compared to offspring of mothers who did not smoke during pregnancy[15]. An increase in intima-media thickness (IMT) was found in newborn infants, children, and young adults who were exposed to smoking during pregnancy[15-17]. In newborn, smoking during pregnancy has been associated with decreased endothelial function[18]. However, the effect of maternal pregnancy smoking on offspring endothelial function beyond infancy has not been studied.

The purpose of our study was to examine whether maternal smoking during pregnancy is associated with subclinical cardiovascular disease in the young adult offspring, as reflected by measures of endothelial function and vascular stiffness (Reactive Hyperemia Index and Augmentation Index, respectively).

Methods

Study setting and participants

Our study is nested in a cohort from the Jerusalem Perinatal Study (JPS). The JPS is a population based study that collected prenatal, perinatal and postnatal data on 17,003 births to Israeli residents of Jerusalem between 1974 and 1976[19]. Offspring birth weight, demographic and socioeconomic status, and maternal medical conditions in current and previous pregnancies were collected from birth certificates or maternal ward logbooks. Further information was collected through interviews taken on the first or second day post-partum, including gestational age, maternal and paternal smoking status, maternal height, pre-pregnancy weight, end of pregnancy weight, and obstetrical medical history[19]. Offspring BMI at age 17 was obtained through linkage to the Israeli military draft records[20].

The JPS Family Follow-Up Study (JPS1) included a sample of 1500 offspring from the original 1974 to 1976 JPS cohort who were recruited between 2007 and 2009. The protocol included a detailed interview focusing on cardiovascular risk factor history, a physical exam consisting of anthropometric and blood pressure measurements, and a blood draw for genotyping and lipid and glucose analysis. Inclusion criteria for the study include born at term (defined as ≥36 weeks) with no known birth defects, both mother and offspring alive at the 2005 Israeli population registry, being able to communicate in Hebrew, and having a telephone at the time of the exam. A stratified sample was created from all eligible JPS subjects, by maternal BMI (<25, 25-26.9, 27-29.9, 30+ kg/m^2) and by birth weight (≤2500, 2501-3999, 4000+ g). Low birth weight (≤2500g) and high birth weight (≥4000g) were oversampled by a factor of 3.1, and overweight and obese mothers (BMI ≥27 kg/m^2) were oversampled by a factor of 3.3.

A random sample of 400 offspring from those who participated in the JPS1 underwent an EndoPAT measurement exam (see below). None of the participants had exclusion criteria that precluded the completion of the EndoPAT exam and/or the interpretation of the results: 1) Known active Raynaud's disease, 2) Women who have had bilateral or right side mastectomy or lumpectomy with lymph node dissection, 3) Presence of a hemodialysis shunts, and 4) Treatment with alpha blockers, Ca channel blockers or long acting nitroglycerine.

The EndoPAT2000 is a non-invasive device designed to measure vascular characteristics (Itamar Medical Ltd., Caesarea, Israel). Subjects were instructed to fast and to refrain from smoking for at least 6 hours before the exam. Two finger-mounted probes were placed on the index finger of each hand. After a 10 minute baseline reading, an inflated blood measurement cuff was used to occlude the brachial artery for 5 minutes in the non-dominant hand, while the other hand was used as control[21]. An automated computer program analyzes the data received from the probes. The results for the arterial function are presented as two variables (see supplement for more information):

1. Reactive Hyperemia Index (RHI) – the RHI is based on the ratio of the post-deflation to baseline pulse amplitude in the occluded arm compared to the control arm. It is a dynamic characteristic of the arteries and measures the change in pulse wave amplitude in response to physiological stressors (such as ischemia induced by transient artery occlusion). The change in amplitude reflects endothelium-dependent vasodilation through endothelial derived nitric oxide (NO). Endothelial dysfunction results in diminished post-ischemic vasodilatation and a lower RHI.

2. Augmentation Index (AI) – the AI is based on the ratio of the amplitude difference between the second and first peak, and the amplitude of the first peak of the arterial pulse wave, corrected for a heart rate of 75. AI measures arterial stiffness, which represents structural characteristics of the arteries. It uses the shape of the arterial pulse wave to determine the baseline stiffness of the artery wall and is influenced by the properties of the medial layer of the artery composed of smooth muscle cells and an extracellular matrix of elastin and collagen. A higher AI reflects stiffer arteries.

Statistical analysis

Linear regression models were used to examine the association between maternal smoking during pregnancy (yes/no) as the exposure and RHI or AI as the outcome (both as continuous linear variables). Mothers who reported in the post-partum interview as having smoked throughout the pregnancy (n=51) or having smoked through part of the pregnancy (n=5) were grouped as pregnancy smokers, while mothers who reported never smoking (n=347) or that they had stopped smoking before the pregnancy (n=20) were grouped as not having smoked during pregnancy. Fathers' smoking during pregnancy was defined in a similar fashion. Three mothers who defined themselves as smokers in the post-partum questionnaire but as "never smoked" in the follow-up interview were excluded from the study. To account for secondhand smoking during the pregnancy by the fathers, we repeated our analyses limiting to offspring of fathers who did not smoke during pregnancy. In sensitivity analyses, we added paternal smoking, by creating variables where both parents smoked or either parent smoked during pregnancy as the exposure, and compared them to offspring who were not exposed to smoking during pregnancy by either parent. We used inverse probability weighting to account for the stratified sampling.

We repeated our models adjusting for the following maternal and offspring characteristics: gender, gestational age (continuous linear, weeks), maternal age (continuous linear, years), socio-economic status (SES) based on father's occupation (low, medium, high), mother's ethnic origin (Israeli, other Middle East, North Africa, and Ashkenazi (Europe/America)), maternal level of education (continuous linear, years), and offspring smoking (ever vs. never).

The analyses were conducted using Intercooled Stata 9 and 12 (StataCorp LP; College Station, TX) and SPSS (SPSS, Inc. Chicago, IL).

This study was approved by the University of Washington Human Subject Review Committee and by the Institutional Review Board of the Hadassah-Hebrew University Medical Center.

Results

Maternal and offspring characteristics by maternal smoking status during the pregnancy are summarized in table 1. Compared to mothers who did not smoke during pregnancy, those who did were of lower SES. Compared to offspring of mothers who didn't smoke during pregnancy, offspring of those who did smoke had lower birth weight. Fathers of offspring of mothers who smoked during pregnancy were more likely to smoke themselves compared to fathers of offspring of mothers who did not smoke during pregnancy.

There was no evidence of an association between maternal smoking during pregnancy and offspring endothelial function as measured by RHI (mean difference in RHI -0.071, 95% CI -0.274 to 0.132, and mean difference in RHI -0.021, 95% CI -0.278 to 0.237 for the unadjusted and adjusted models, respectively) (table 2). Similarly, there was little evidence to support an association between maternal smoking during pregnancy and AI (mean difference in AI 0.138, 95% CI -5.716 to 5.992, and mean difference in AI -0.469, 95% CI -6.294 to 5.355 for the unadjusted and adjusted models, respectively) (table 2). There was little evidence to support an interaction with sex (p=0.082 for RHI, and p=0.655 for AI) or an interaction with offspring smoking (p=0.736 for RHI and p=0.671 for AI). The results were similar when the analysis was limited to offspring of fathers who did not smoke during pregnancy (mean difference in RHI 0.014 95% CI -0.246 to 0.275 and mean difference in AI 3.472, 95% CI -3.953 to 10.897 for the adjusted models) (table 2).

We then examined possible additional effect of paternal smoking during pregnancy on offspring arterial characteristics (table 3). We found little evidence of an association between any parental smoking during pregnancy (either mother or father) and offspring RHI or AI (mean difference in

RHI -0.042, 95% CI -0.194 to 0.111 and mean difference in AI -2.931, 95% CI -6.997 to 1.134 for the adjusted models). Similarly, we found little evidence of an association between parental (both parents) smoking during pregnancy and offspring RHI or AI (mean difference for RHI -0.068 95% CI -0.418 to 0.283 and mean difference for AI -2.119, 95% CI -9.851 to 5.613 for the adjusted models).

D**iscussion**

We did not find evidence supporting an association between maternal smoking during pregnancy and offspring arterial characteristics, as measured by AI and RHI. The results were similar after accounting for paternal smoking.

Comparison with other studies

Endothelial function:

To the best of our knowledge, only one study examined the association of maternal smoking during pregnancy and endothelial function in humans. Chirico *et al* examined post-ischemic transcutaneous oxygen levels (PO_2) in the forearm in response to a transient arterial occlusion in 40 infants shortly after birth. Infants who were exposed to smoking during pregnancy, either directly due to maternal smoking during pregnancy or indirectly due to paternal smoking during pregnancy, showed a significant reduction in PO_2 levels post-occlusion (18.5±5.5mmHg in non-smokers vs 8.4±3.0mmHg in active smokers and 13.5±3.4mmHg in passive smoking, $p<0.0001$ and $p<0.025$, respectively), which suggests that any exposure to smoking during pregnancy impairs offspring endothelial-induced dilatation[16]. However, the study did not adjust for maternal characteristics that might confound the observed association, such as age, ethnicity, SES and education. Our study did not find an association between maternal smoking during pregnancy and offspring endothelial function, as measured by RHI. Similarly, we did not find an association between any parental, or both maternal and paternal smoking during pregnancy and offspring RHI.

Arterial stiffness:

Few studies have examined the effect of smoking during pregnancy and arterial stiffness. Gunes *et al* showed that newborn exposed to smoking during pregnancy had increased weight-adjusted aortic intima-media thickness (IMT) compared to newborn of mothers who did not smoke (0.151±0.005 mm/kg vs. 0.118±0.014 mm/kg, $p<0.0001$)[18]. Similarly, Geerts *et al* found carotid IMT to be increased by 18.8 µm (95% CI 1.1-36.5) and a 15% lower distensibility (95% CI 2-30) in 5 year-old offspring of mothers who smoked during pregnancy compared to offspring of non-smoking mothers[15]. The association was not found in offspring of mothers who did not smoke during the pregnancy but smoked thereafter, implying the differences were the result of intrauterine exposure to smoking and less so due to environmental exposure to smoking after birth. In another study, Greets *et al* examined carotid IMT in 732 young adults (mean age 28.4) and found that offspring of mothers who smoked during pregnancy had a 13.4 µm thicker carotid IMT (95% CI 5.5-21.3) compared to offspring of mothers who did not smoke during pregnancy[17]. The differences were slightly attenuated after adjusting for offspring IMT risk factors (including age, gender, BMI, pulse pressure, and LDL cholesterol) and were similar after accounting for offspring and current parental smoking status. The association of smoking during pregnancy and carotid IMT was strongest when both parents smoked[15,17].

We found little evidence supporting an association between maternal smoking during pregnancy and offspring AI or an association between any parental, or both maternal and paternal smoking during pregnancy and offspring AI. Most previous studies focused on intima-media thickness, a measurement of structural changes in the arterial wall, while our study used the AI measurement, which is a functional measurement of arterial stiffness. Kampus *et al* tried to compare the two measurements and showed that, though these are both indicators of arterial stiffness, they relate

differently to cardiovascular risk factors in asymptomatic individuals[22]. In his study, both measurements were correlated with age, weight, and heart rate, but carotid IMT was also shown to correlate with white blood cell count and LDL, while AI was correlated with gender, CRP, mean arterial pressure, and height. A study by Gomez-Marcos *et al* demonstrated an association between AI and carotid IMT in subjects with diabetes, but in non-diabetic subjects the association was lost after adjustment for age, sex, and heart rate[23]. It is possible that maternal smoking during pregnancy is associated with increased IMT but that this change in IMT does not translate to an increase in AI.

Strengths and limitations

Our study's strength is our data which includes detailed prenatal and perinatal information along with extensive data on lifestyle, socioeconomic, demographic and physical characteristics of mothers and adult offspring at age 32. There are several limitations to our study. Our sample size was small. Assuming a type I error of 0.05 and a power of 0.80, we should have been able to detect a true difference of 66% in the mean AI and a true difference of 11% in the mean RHI of offspring of mothers who smoked during pregnancy compared to offspring of mothers who didn't smoke. Maternal smoking during pregnancy was determined from maternal interviews shortly after birth. Due to social undesirability, women might have underreported their smoking status during pregnancy leading to misclassification of our exposure and a bias toward the null. A meta-analysis by Patrick *et al* showed that, when compared to biochemical assessments of smoking, the sensitivity of self-reported smoking was 87% and the specificity 89%[24]. Nevertheless, smoking during pregnancy is an even less acceptable as a social habit than smoking in general and women may be less inclined to admit to smoking during pregnancy. Our results were similar when, in a sensitivity analysis, we added the mothers who reported in the

post-partum interview that they had stopped smoking before the pregnancy to the 'smoking during pregnancy' group (supplement table 1). Fathers' smoking status during pregnancy was reported by the mother shortly after birth rather than being obtained directly from the fathers. However, in a recent addition to the JPS1 dataset, 784 fathers were interviewed regarding their smoking status during pregnancy and their spouses smoking status. The agreement percent between reported paternal smoking status from the fathers and the mothers was 93% and the k estimate was 0.68 (Hagit Hochner, personal communication).

Conclusion

We found little evidence supporting an association between maternal smoking during pregnancy and offspring AI and RHI in young adulthood. Nor was there evidence supporting an association between any parental smoking or smoking of both parents and offspring AI and RHI. Further study with a larger sample size is recommended.

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Tables

Table 1: Maternal and offspring characteristics stratified by maternal smoking status during pregnancy, from the Jerusalem Perinatal Family Follow-Up Study (JPS1), 2007-2009

	Maternal smoking during pregnancy					
	yes			no		
Maternal characteristics¶	Raw data	weighted*	n (53)	Raw data	weighted*	n (347)
Age at birth, *yrs*	26.5 (5.4)	25.9 (5.1)	53	28.3 (11.8)	27.6 (5.4)	347
Pre-pregnancy BMI, kg/m^2	22.9 (3.7)	21.3 (2.5)	53	24.5 (4.1)	22.1 (3.1)	343
Ethnicity, *%*			53			347
Israel	17.0	17.4		11.8	8.3	
Other Middle East	24.5	23.8		29.4	31.0	
North Africa	35.9	31.0		23.6	20.5	
Ashkenazi	22.6	27.8		35.2	40.2	
Education, *yrs*	10.6 (3.1)	11.2 (2.9)	52	11.8 (3.3)	12.1 (3.2)	336
SES			53			347
low	41.5	50.5		19.0	16.2	
medium	37.7	35.7		39.2	36.0	
high	20.8	13.8		41.8	47.8	
Paternal smoking, *% ever*	73.6	80.2	53	36.1	33.8	347
Offspring characteristics¶						
Birth weight, *gr*	3241 (596)	3110 (382)	53	3426 (605)	3291 (439)	347
Gestational length, *week*	39.7 (1.8)	39.7 (1.5)	51	39.8 (1.6)	39.7 (1.5)	344
Gender, *% male*	45.3	46.8	53	56.0	52.5	347
BMI at age 17, kg/m^2	22.0 (3.7)	21.7 (2.9)	52	21.9 (3.5)	21.3 (3.2)	283
Current BMI, kg/m^2	27.4 (4.2)	25.8 (3.2)	53	28.6 (4.0)	26.3 (3.3)	340
Ever smoking, *% yes*	46.2	49.1	52	38.5	34.5	343
Heart rate, *bpm*	70.7 (10.0)	69.3 (10.3)	53	68.6 (9.8)	68.3 (8.4)	345
Systolic BP, *mmHg*	105.2 (12.3)	102.7 (11.4)	53	108.4 (13.0)	106.1 (13.2)	345
Diastolic BP, *mmHg*	70.8 (8.1)	69 .6 (7.9)	53	72.9 (8.9)	71.8 (9.0)	345
Glucose, *mmol/L*	85.0 (38.7)	80.9 (21.9)	51	80.3 (15.1)	80.0 (11.4)	339
Triglycerides, *mmol/L*	119.8 (72.2)	113.8 (58.0)	52	110.1 (66.5)	105.7 (68.8)	339
Cholesterol, *mmol/L*	190.0 (36.2)	185.9 (25.0)	52	186.7 (36.5)	187.1 (34.7)	339
HDL, *mmol/L*	50.9 (14.5)	47.6 (10.8)	52	50.0 (15.5)	50.9 (15.1)	
LDL, *mmol/L*	115.1 (32.5)	115.6 (23.3)	52	114.6 (32.0)	115.4 (31.4)	337
Waist, *cm*	87.5 (11.9)	88.6 (11.4)	53	88.6 (14.3)	85.6 (14.1)	345
Waist-hip ratio	0.84 (0.08)	0.84 (0.07)	53	0.84 (0.07)	0.83 (0.07)	345
RHI†	1.78 (0.52)	1.64 (0.47)	53	1.76 (0.54)	1.72 (0.45)	342
AI‡	-5.35 (13.79)	-7.13 (13.31)	53	-7.68 (12.14)	-7.27 (12.62)	346

¶ Continuous variables presented as mean (SD) † RHI – reactive hyperemic index ‡ AI – augmentation index
* Values were weighted to account for stratified sampling of mother-child pairs based on offspring birth weight and maternal pre-pregnancy BMI

Table 2: Associations of maternal smoking during pregnancy and RHI[†] or AI[‡]

	mean difference associated with maternal smoking	95% CI		p	n
All offspring					
RHI					
Unadjusted	-0.071	-0.274	0.132	0.493	389
Adjusted[*]	-0.021	-0.278	0.237	0.875	361
AI					
Unadjusted	0.138	-5.716	5.992	0.963	394
Adjusted[*]	-0.469	-6.294	5.355	0.874	366
Offspring whose fathers did not smoke during pregnancy					
RHI					
Unadjusted	0.008	-0.222	0.237	0.949	227
Adjusted[*]	0.014	-0.246	0.275	0.913	218
AI					
Unadjusted	4.693	-5.722	15.108	0.376	230
Adjusted[*]	3.472	-3.953	10.897	0.358	219

[†] RHI – reactive hyperemic index [‡] AI – augmentation index

[*] Adjusted for gender, gestational week, maternal age at birth, maternal education, mother's ethnic origin, SES, and offspring smoking

Table 3: Associations of parental smoking during pregnancy and RHI[†] or AI[‡]

	mean difference associated with maternal smoking	95% CI		p	n
Maternal or paternal smoking during pregnancy (either parent)					
RHI					
Unadjusted	-0.067	-0.211	0.077	0.363	389
Adjusted[*]	-0.042	-0.194	0.111	0.592	361
AI					
Unadjusted	-2.553	-6.754	1.648	0.233	394
Adjusted[*]	-2.931	-6.997	1.134	0.157	366
Maternal and paternal smoking during pregnancy (both parents)[¶]					
RHI					
Unadjusted	-0.112	-0.360	0.136	0.376	253
Adjusted[*]	-0.068	-0.418	0.283	0.705	236
AI					
Unadjusted	-2.446	-8.990	4.097	0.462	255
Adjusted[*]	-2.119	-9.851	5.613	0.590	238

[†] RHI – reactive hyperemic index [‡] AI – augmentation index

[*] Adjusted for gender, gestational week, maternal age at birth, maternal education, mother's ethnic origin, SES, and offspring smoking

¶ Subjects were compared to offspring who were not exposed to smoking during pregnancy by either parent.

Supplements

Calculating RHI and AI

RHI = (Indexed PAT ratio * Baseline correction factor)

where the Indexed PAT ratio = (RH occluded 90s-120s +RH occluded 120s-150s) / (RH control 90s-120s + RH control 120s-150s)

The RH for each time period above is the mean pulse wave amplitude (PWA) of post occlusion section divided by the mean PWA of baseline region of interest.

Occluded = test arm, the arm on which the cuff is placed; Control = control arm

The Baseline correction factor is based on the observation that subjects with an initial high signal have a smaller potential for dilation even if their endothel is healthy (their artery is already dilated to much of its capacity). The higher the baseline signal is, the more weight a given increase in amplitude receives.

AI = (P2-P1)/P1

where P2 is the pressure at the 2nd peak in systole (the reflected wave from the periphery) and P1 is the pressure at the 1st peak in systole (the systolic contraction of the ventricle). The result is further normalized to heart rate of 75bpm.

Supplement tables

Supplement table 1: Associations of maternal ever smoking vs. never smoking and RHI[†] or AI[‡]

	mean difference associated with maternal smoking	95% CI		p	n
All offspring					
RHI					
Unadjusted	-0.099	-0.275	0.078	0.272	387
Adjusted[*]	-0.073	-0.289	0.142	0.502	359
AI					
Unadjusted	0.600	-4.566	5.765	0.820	392
Adjusted[*]	0.135	-5.179	5.450	0.960	364

[†] RHI – reactive hyperemic index [‡] AI – augmentation index

[*] Adjusted for gender, gestational week, maternal age at birth, maternal education, mother's ethnic origin, SES, and offspring smoking