Original Article
EPIDEMIOLOGY/GENETICS

Subclinical Inflammation During Third Trimester of Pregnancy Was Not Associated with Markers of the Metabolic Syndrome in Young Adult Offspring

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Objective: Growing evidence indicates that the metabolic syndrome (MS) is rooted in adverse exposures during fetal life. The aim of this study was to assess the possible associations between biomarkers of inflammation during third trimester of pregnancy and markers of MS in adult offspring.

Methods: High-sensitive C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleuki-6 (IL-6) were measured in serum samples obtained in gestational week 30. Offspring were clinically examined at age 20 years. Analyses based on 439 mother–offspring dyads were adjusted for maternal smoking during pregnancy, height, prepregnancy body mass index (BMI), education, and offspring's sex. Offspring MS markers included waist circumference, BMI, blood pressure, HOMA insulin resistance, and plasma levels of fasting glucose, triglycerides, cholesterol fractions, insulin, and leptin.

Results: The median level was 2.8 (interquartile range = 3.3) μg/ml for CRP, for TNF-α: 5.7 (3.2) pg/ml, for IL-1β: 0.5 (0.4) pg/ml, and for IL-6: 1.1 (0.7) pg/ml. Concentrations were not significantly associated with MS markers in the offspring. The results remained essentially unchanged after correction for potential confounding.

Conclusion: Markers for subclinical inflammation in third trimester in healthy women were not associated with components of MS in their adult offspring.

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Introduction

Mounting evidence suggests that adverse exposures in pregnancy, for instance obesity, diabetes and lifestyle factors, such as diet, smoking and physical activity, are involved in developmental programming of type 2 diabetes. Although low birth weight and diabetes in pregnancy represent the most well-established factors linking exposures during pregnancy with subsequent risk of diabetes and the metabolic syndrome (MS) among offspring (1), we lack knowledge about the distinct molecular mechanisms involved.

Subclinical inflammation is commonly present in patients with metabolic diseases, and inflammatory markers, such as CRP, TNF-α, IL-6, and IL-1β are associated with risk factors for chronic noncommunicable diseases, including type 2 diabetes (2-7). In addition, CRP and proinflammatory cytokines have been demonstrated to predict future development of type 2 diabetes (8,9). Interestingly, nondiabetic offspring of type 2 diabetic mothers were found to display elevated levels of CRP and inflammatory cytokines (10). Furthermore, it has been suggested that inflammation during pregnancy may play a role in the programming of obesity among offspring (11).

MS, which consists of the components central obesity, reduced HDL cholesterol and raised triglyceride levels, as well as raised blood pressure and plasma glucose levels (12), is associated with increased risk of cardiovascular disease and type 2 diabetes (13). Growing evidence links maternal MS, obesity, and diabetes during pregnancy with an increased risk among the offspring of developing diabetes

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and MS (14,15). Interestingly, a recent study found positive correlations between maternal and fetal levels of leptin and adiponectin and in addition a negative association between fetal leptin levels and fetal insulin sensitivity suggesting a possible maternal–fetal transmission of the propensity to obesity and insulin resistance (16). It is still unknown if markers of early or acute inflammation in pregnant women can predict the metabolic profile of the offspring later in life.

Pregnancy in itself is an inflammatory state (17,18). The level of inflammation in pregnancy has been associated with preeclampsia (19,20), preterm birth, and/or intrauterine growth restriction (21-23), conditions that may have adverse long-term consequences for health (24,25).

We do not know of any previous studies of the possible associations between inflammatory markers in pregnancy and the adult offspring's metabolic profile, and we, therefore, studied this potentially important issue in a unique Danish birth cohort with 20 years' follow-up.

Methods

The Danish Fetal Origin Cohort 1988

Nine hundred and sixty-five out of 1,212 eligible women with singleton pregnancies were recruited for a birth cohort study in Denmark in 1988 (26). By means of a self-administered questionnaire completed by the women prior to the antenatal visit in gestation week 30, information about medical history as well as socioeconomic factors was gained and supplemented by an interviewer-guided questionnaire following the antenatal visit. Provided consent was given, a blood sample was taken and processed under-standardized conditions. The vials were kept on ice and within an hour separated into serum, plasma, and erythrocytes that were frozen and stored at -20°C. Further information about the women's health, medical history, anthropometry, and birth outcomes was extracted from hospital records and from the Danish Medical Birth Registry as well as from the records kept by the midwives and general practitioners. Moreover, screening for gestational diabetes mellitus (GDM) was done with fasting glucose measurements in woman who were obese, had a family history of diabetes mellitus, GDM in a previous pregnancy, a previous delivery of an infant above 4,500*g*, previous stillbirth, age above 38 years, or glucosuria in the current pregnancy. When two independent fasting capillary plasma glucose values were above 4.6 mmol/l, the woman was referred to an OGTT.

Offspring follow-up

During 2008 and 2009, mothers and offspring were contacted and offspring invited to answer a web-based questionnaire, including inquiries on current health and lifestyle. For instance, they were asked how many hours per day, on a typical weekday, they were physically active. Possible answers were a) more than 3 h per day, b) 2-3 h per day, c) 1-1.5 h per day, d) 0-0.5 h per day, e) none, and f) don't know. The question was repeated for a typical Sunday. From these two questions, a combined variable with four levels of activity, covering physical activity during the whole week, was created.

All potential participants were asked to participate in a clinical examination. The participants were examined between 8:00 AM and 12:30 PM after an overnight fast. Height, weight, and waist circumference were measured. After 7 min of rest, blood pressure was measured three times in the horizontal position using an automatic blood pressure device (OMRON M6 Comfort HEM-7000-E). The average value of the last two measurements was used in the analyses. A venous blood sample was drawn and immediately centrifuged and frozen at -80°C.

From a total number of 965 women, we traced 894 offspring. The mother–offspring dyads that were not traced consisted of mothers and children with an incorrect personal identification number, mothers, and children, who had died or were abroad, or with unknown addresses. A total of 684 subjects (77% of the eligible population) participated in the follow-up study by filling out the questionnaire, providing information on the offspring's level of physical activity. Of these 439 attended the clinical examination and constituted the study population.

Exposure variables

Analyses of the biomarkers from third trimester of pregnancy were done on frozen serum samples in a central laboratory at Department of Internal Medicine and Cardiovascular Research Institute Maastricht, Maastricht University Medical Centre (by C.G.S). The possibility of measuring biomarkers on the 20-year-old serum samples was first tested in a pilot study on nine representative samples. The serum concentration (mean [±SD]) of CRP was 2.1 (±2.0) μg/ml, of IL-6 1.8 (±2.0) pg/ml, and of TNF-α 6.6 (±2.4) pg/ml, respectively. Furthermore, IL-1β was measured in all the serum samples at a mean concentration of 0.6 (±0.5) pg/ml. As reference value, we used data of The Amsterdam Growth and Health Longitudinal Study (AGAHLS). The AGAHLS is a longitudinal cohort study initiated in 1977. Details have been described elsewhere (27). Plasma biomarkers in blood samples from follow-up examinations were determined after storage of serum samples during 10 years at -80°C. Only data of women (~36 years, $n = 199$) was used as reference in pilot study. In these women, the concentration of CRP (median [IQR]) was 0.9 (0.4-2.9) μg/ml, of IL-6 2.4 (1.6-3.9) pg/ml, and of TNF-α (mean ± SD) 9.0 ± 3.1 pg/ml, respectively. From this pilot study, we concluded that we were able to measure CRP, TNF-α, IL-1β, and IL-6 in serum samples obtained in gestational week 30 after 20 years of storage, and that the absolute serum levels of CRP, TNF-α, and IL-6 in serum samples were comparable with measurements done on samples which were stored for 10 years at -80°C.

Concentrations of CRP, TNF-α, IL-6, and IL-1β were measured by 4-plex multiarray electrochemiluminescense detection platforms of MesoScaleDiscovery (MesoScaleDiscovery, Gaithersburg, MD, USA, www.mesoscale.com) (28). This system uses multiarray plates fitted with multielectrodes per well with each electrode being coated with a different capture antibody. For the present study, two 4-plex assays (plates fitted with four electrodes per well, i.e., four separate well spots with a different capture antibody bound to each) was used and we selected data of CRP, TNF-α, IL-6, and IL-1β. The assay procedure follows that of a classic sandwich ELISA with any of the analytes of interest captured on the relevant electrode. These captured analytes were, in turn, detected by a secondary, analyte-specific, ruthenium-conjugated antibody, which is capable of emitting light after electrochemical stimulation. This method minimizes nonspecific signals as the stimulation mechanism (electricity) is decoupled from the signal (light). Each sample was analyzed in

duplicate on the same array plate. The intra- and interassay coefficients of variation (CV) for the platform of MSD were, for CRP, 4.6% and 5.8%; for TNF-α: 4.5% and 8.5%, for IL-6: 6.0% and 15.6%, and for IL-1β: 13.3% and 18.0%. The variables were analyzed as continuous variables and as supplementary analyses in quintiles based on the samples of all women in the cohort ($n = 894$).

Outcome markers for MS

Primary outcome variables were chosen among the continuous variables inherent in the definition of MS, i.e., waist circumference (cm), fasting levels of plasma glucose (mmol/l), triglycerides (mmol/l), and HDL cholesterol (mmol/l), as well as systolic and diastolic blood pressure measurements (mm Hg). In addition, the primary outcome variables were supplemented with secondary variables associated with MS, including BMI (kg/m^2), plasma levels of LDL and total cholesterol (mmol/l), concentrations of fasting plasma insulin (pmol/l), and leptin (μg/l), as well as HOMA-IR. Waist circumference and BMI were determined solely by means of data from the clinical examinations (439 subjects) to eliminate the possibility of under-reported data on BMI and waist circumference.

Offspring biomarkers

Offspring biomarkers were all measured in Denmark. Blood samples were obtained during 2008 and 2009 and immediately frozen at −80°C. Plasma glucose was measured during the examination immediately after blood sampling using bedside equipment. Serum triglycerides and cholesterol fractions (total cholesterol, LDL, and HDL) were continuously measured according to standard methods at Aalborg Hospital in several rounds until the beginning of 2010. Insulin, leptin, and adiponectin were analyzed at the Medical Research Laboratories, Aarhus University Hospital in January 2010. Serum leptin concentrations were determined by a time-resolved immunofluorometric assay based on commercially available reagents and recombinant human leptin as standard (29). Plasma insulin concentrations were determined using a commercial ELISA kit. Insulin resistance was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR) by means of the formula: fasting glucose (mmol/l) × fasting insulin (mU/l)/22.5 (30).

Statistical analyses

Baseline characteristics of pregnant women either with participating or nonparticipating offspring were tested for differences by χ^2 test. Median levels of maternal biomarkers were tested for trends across covariate categories using Kruskal–Wallis test. Associations between maternal levels of biomarkers and offspring outcome variables were examined by multivariate linear regression analyses. Waist circumference was adjusted for BMI using the residual method (31), which provides a measure of waist circumference uncorrelated with BMI. Owing to skewed distributions, all outcome variables except adjusted waist circumference and blood pressure were log-transformed.

We *a priori* decided to include the following covariates: maternal height (continuous, 3% missing), education (five categories, 5% missing), smoking (yes or no, 5% missing), and prepregnancy BMI (continuous, 3% missing). All maternal biomarker measurements were above detection levels. Three outliers among the biomarkers (CRP ≥112 mg/l; Il-6 ≥60 pg/ml, and Il-1β ≥18 pg/ml) were excluded from the analyses as well as observations with missing covariate values ($n = 50$ [11%]).

Maternal height and prepregnancy BMI were included as these variables are possible determinants of anthropometric and metabolic measures in the offspring. Maternal education and smoking were included to account for potential social and lifestyle confounding.

As a supplementary analysis, imputation of missing values ($n = 50$) was used to test if the same conclusion could be reached with

TABLE 1 Characteristics of pregnant women in the birth cohort dependent on their offspring's participation in the follow-up[a]

	Mothers with nonparticipating offspring ($n = 526$)	Mothers with participating offspring ($n = 439$)	
	Percent	Percent	*P* value
Height (cm)			0.90
−159	8	8	
160-164	21	21	
165-169	35	33	
170-174	24	27	
175-	12	12	
Education			0.002
None	18	12	
Vocational	28	23	
Bachelor	40	44	
Academic	14	20	
BMI (kg/m^2)			0.51
<18.6	11	9	
18.6 to <25	78	82	
25 to <30	7	6	
30-	4	3	
Smoking	43	37	0.09
Nulliparous	56	60	0.19
CRP			0.07
Lowest quintile	20	20	
Mid quintile	20	21	
Highest quintile	23	17	
TNF-α			0.82
Lowest quintile	20	20	
Mid quintile	19	21	
Highest quintile	21	19	
IL-6			0.62
Lowest quintile	20	20	
Mid quintile	21	19	
Highest quintile	21	19	
IL-1β			0.67
Lowest quintile	20	19	
Mid quintile	20	21	
Highest quintile	22	18	

[a]Differences between the two groups of women are reported as *P* value from χ^2 test for measure of association.

TABLE 2 Associations between maternal inflammatory markers and covariates[a]

		Level of maternal inflammatory markers in each covariate category							
		CRP		TNF-α		IL-6		IL-1β	
Covariate	Category	Median ± IQR	*P* trend	Median ± IQR	*P* trend	Median ± IQR	*P* trend	Median ± IQR	*P* trend
Maternal height (cm)	−159	2.6 ± 3.4	0.77	4.9 ± 2.9	0.34	1.1 ± 0.6	0.77	0.4 ± 0.3	0.23
	160-164	3.3 ± 4.8		5.6 ± 2.5		1.1 ± 0.7		0.5 ± 0.5	
	165-169	2.9 ± 3.6		5.5 ± 3.2		1.2 ± 0.9		0.5 ± 0.5	
	170-174	2.9 ± 3.2		5.8 ± 3.0		1.1 ± 0.7		0.5 ± 0.5	
	175-	3.1 ± 4.0		5.6 ± 3.3		1.2 ± 0.7		0.5 ± 0.5	
Prepregnancy BMI	1-18.5	2.1 ± 3.2	<0.0001	5.4 ± 3.1	0.06	1.1 ± 0.6	0.02	0.5 ± 0.6	0.39
	18.6-24.9	2.9 ± 3.4		5.5 ± 2.9		1.1 ± 0.8		0.5 ± 0.5	
	25-29.9	5.2 ± 9.5		5.7 ± 3.0		1.2 ± 0.8		0.6 ± 0.5	
	30-	8.8 ± 7.2		7.1 ± 2.6		1.3 ± 1.0		0.5 ± 0.3	
Maternal smoking	No	2.8 ± 3.7	0.12	5.7 ± 3.2	0.32	1.1 ± 0.7	0.05	0.5 ± 0.5	0.54
	Yes	3.3 ± 3.8		5.6 ± 2.7		1.2 ± 0.8		0.5 ± 0.5	
Maternal education	None	3.8 ± 4.9	0.0001	5.8 ± 3.3	0.31	1.2 ± 1.0	0.54	0.5 ± 0.5	0.66
	Vocational	3.3 ± 4.4		5.5 ± 2.9		1.1 ± 0.8		0.5 ± 0.5	
	Bachelor	2.8 ± 3.4		5.7 ± 3.1		1.1 ± 0.6		0.5 ± 0.5	
	Academic	2.2 ± 2.9		5.6 ± 2.5		1.1 ± 0.8		0.5 ± 0.5	
Offspring's sex	Male	3.0 ± 3.6	0.45	5.7 ± 3.1	0.28	1.1 ± 0.7	0.76	0.5 ± 0.5	0.23
	Female	2.9 ± 3.9		5.5 ± 2.9		1.1 ± 0.8		0.5 ± 0.4	

[a]Trends of mean levels of maternal inflammatory markers across covariates in categories are reported as *P* trend from Kruskal-Wallis test. Biomarker concentrations and distributions according to covariates are based on all traced women in the cohort ($n = 894$). Distribution of biomarker levels according to offspring sex is furthermore restricted to the dyads, which include the 688 offspring answering the web-based questionnaire.

increased statistical power. Missing values on the continuous variables were imputed by median imputation, while missing values on the categorical variables were imputed by mode imputation. For maternal BMI and height, we thus used the medians 20.9 kg/m^2 and 168 cm, and for smoking and education we used the most frequent observation, which was 'no' and 'other education'. Furthermore, to unmask potential associations between maternal inflammatory markers and metabolic outcomes in the offspring that might be confounded by maternal BMI and smoking, we conducted supplementary analyses with maternal prepregnancy BMI and smoking excluded from the statistical model. These results are included in Table 3. In addition, two supplementary analyses were conducted with offspring physical activity and BMI, respectively, included in the model to eliminate the possible influence by these determinants on the levels of offspring MS markers and thus to reduce the variance of the outcome variables. Moreover, exposure levels in women diagnosed with GDM were compared with healthy pregnant women.

A preliminary test for interaction of sex showed significant interaction with several, but not all of the studied associations. Therefore, the statistical analyses were performed for all offspring, while adjusting for offspring sex, and subsequently also for males and females separately to investigate potential differences between the sexes. Changes in outcome variables for 5 units increment in levels of IL-6, and IL-1β and for 10 units increment in TNF-α and CRP are presented as absolute changes for waist circumference and blood pressure and as relative changes for BMI and biomarkers. These measures of association are expressed as 'difference per 5 units increment (95% CI)' and 'ratio per 5 units increments (95% CI)', respectively, and equivalent for 10 units increment. Associations were considered statistically significant at the 5% level. All analyses were performed using the SAS GLM procedure (Version 9.3; SAS Institute, Cary, NC).

Results

Mothers of participating offspring were more often normal weight and nonsmokers and had a higher education compared with mothers of nonparticipating offspring (Table 1). Participation was not associated with the levels of inflammatory markers. Higher prepregnancy BMI was associated with higher levels of CRP and IL-6 and borderline significantly associated with higher levels of TNF-α. Also, higher levels of CRP were associated with lower education (Table 2). No significant associations were found with the other covariates included in the model: maternal height and smoking, and the offspring's sex.

Analyses of pairwise linear associations between the four biomarkers were done by means of Spearman's correlations. Apart from CRP and IL-1β, the biomarkers were significantly correlated although the strength of the correlations was rather modest ($r \sim 0.10$-0.25).

In the analyses of biomarker levels in the pregnant women and markers of MS in their offspring we found no associations with either the primary or the secondary outcome variables (Table 3).

TABLE 3 MS markers in the offspring dependent on their mothers' levels of inflammatory markers in third trimester[a]

		Ratio or difference* (95% CI)							
		Maternal CRP		Maternal TNF-α		Maternal IL-6		Maternal IL-1β	
		2.79 μg/ml (±3.27)		5.67 pg/ml (±3.16)		1.11 pg/ml (±0.67)		0.48 pg/ml (±0.42)	
Offspring MS markers	Mean (±SD)		*P* value		*P* value		*P* value		*P* value
Fasting glucose	4.9 mmol/l (±1.1)	0.99 (0.98, 1.01)	0.47	0.99 (0.96, 1.01)	0.26	0.96 (0.91, 1.01)	0.12	0.97 (0.91, 1.02)	0.25
Adjust 1		1.00 (0.98, 1.01)	0.70	0.98 (0.96, 1.00)	0.06	0.97 (0.93, 1.02)	0.29	0.98 (0.93, 1.03)	0.44
Adjust 2		1.00 (0.99, 1.01)	0.75	0.98 (0.96, 1.00)	0.06	0.97 (0.93, 1.02)	0.29	0.98 (0.93, 1.03)	0.46
Triglycerides	0.9 mmol/l (±1.5)	0.98 (0.91, 1.04)	0.48	0.93 (0.83, 1.04)	0.21	0.87 (0.68, 1.11)	0.25	1.17 (0.90, 1.52)	0.25
Adjust 1		0.97 (0.90, 1.03)	0.29	0.94 (0.84, 1.06)	0.30	0.83 (0.65, 1.06)	0.13	1.14 (0.88, 1.48)	0.33
Adjust 2		0.97 (0.90, 1.03)	0.31	0.94 (0.84, 1.05)	0.27	0.83 (0.65, 1.06)	0.14	1.15 (0.89, 1.49)	0.29
HDL cholesterol	1.4 mmol/l (±1.2)	1.01 (0.98, 1.05)	0.44	1.00 (0.94, 1.07)	0.92	0.97 (0.85, 1.11)	0.68	1.04 (0.91, 1.20)	0.55
Adjust 1		1.01 (0.97, 1.04)	0.70	1.02 (0.96, 1.08)	0.57	0.93 (0.82, 1.05)	0.26	1.02 (0.89, 1.16)	0.82
Adjust 2		1.01 (0.97, 1.04)	0.64	1.02 (0.96, 1.08)	0.50	0.93 (0.83, 1.06)	0.27	1.02 (0.90, 1.16)	0.75
LDL cholesterol	2.4 mmol/l (±1.3)	1.00 (0.95, 1.05)	0.93	1.02 (0.94, 1.11)	0.62	1.04 (0.87, 1.23)	0.67	1.19 (0.99, 1.42)	0.07
Adjust 1		0.99 (0.95, 1.04)	0.71	1.03 (0.95, 1.11)	0.50	1.02 (0.85, 1.21)	0.86	1.17 (0.98, 1.40)	0.09
Adjust 2		0.99 (0.94, 1.04)	0.66	1.02 (0.95, 1.11)	0.56	1.02 (0.86, 1.21)	0.85	1.17 (0.98, 1.41)	0.08
Total cholesterol	4.3 mmol/l (±1.2)	1.00 (0.97, 1.03)	0.98	1.00 (0.95, 1.06)	0.96	0.99 (0.88, 1.11)	0.85	1.12 (0.99, 1.27)	0.08
Adjust 1		0.99 (0.96, 1.02)	0.65	1.01 (0.96, 1.07)	0.66	0.96 (0.86, 1.07)	0.45	1.10 (0.98, 1.23)	0.12
Adjust 2		1.00 (1.00, 1.00)	0.66	1.01 (0.96, 1.07)	0.68	0.96 (0.86, 1.07)	0.47	1.10 (0.98, 1.24)	0.10
Systolic blood pressure*	109.9 mm Hg (±10.6)	0.92 (−0.80, 2.65)	0.29	1.96 (−1.04, 4.95)	0.20	−6.63 (−13.0, −0.24)	0.04	−0.52 (−7.39, 6.36)	0.88
Adjust 1		0.73 (−0.68, 2.14)	0.31	0.99 (−1.43, 3.40)	0.42	−5.80 (−11.0, −0.61)	0.03	0.27 (−5.27, 5.81)	0.92
Adjust 2		0.69 (−0.71, 2.09)	0.33	1.02 (−1.38, 3.43)	0.40	−5.86 (−11.0, −0.74)	0.03	0.00 (−5.49, 5.48)	1.00
Diastolic blood pressure*	65.7 mm Hg (±6.7)	0.97 (−0.13, 2.06)	0.08	0.22 (−1.69, 2.13)	0.82	−0.49 (−4.57, 3.60)	0.81	3.11 (−1.25, 7.47)	0.16
Adjust 1		0.47 (−0.61, 1.55)	0.40	0.40 (−1.46, 2.25)	0.67	−1.87 (−5.86, 2.12)	0.36	2.29 (−1.95, 6.53)	0.29
Adjust 2		0.37 (−0.71, 1.45)	0.50	0.23 (−1.63, 2.09)	0.81	−1.96 (−5.93, 2.02)	0.33	2.07 (−2.17, 6.30)	0.34
Waist circumference*	81.6 cm (±6.0)	0.10 (−0.67, 0.87)	0.81	0.89 (−0.46, 2.23)	0.19	−0.69 (−3.77, 2.39)	0.66	−0.69 (−3.77, 2.39)	0.66
Adjust 1		0.22 (−0.51, 0.95)	0.56	0.62 (−0.64, 1.87)	0.34	−0.20 (−3.09, 2.68)	0.89	−0.20 (−3.09, 2.68)	0.89
Adjust 2		0.14 (−0.57, 0.85)	0.70	0.42 (−0.81, 1.64)	0.50	0.05 (−2.57, 2.68)	0.97	−0.52 (−3.31, 2.28)	0.72
BMI	22.2 kg/m^2 (±1.1)	0.99 (0.97, 1.01)	0.48	1.02 (0.98, 1.06)	0.26	0.92 (0.85, 1.00)	0.04	1.06 (0.98, 1.16)	0.15
Adjust 1		0.98 (0.96, 1.00)	0.12	1.02 (0.98, 1.06)	0.30	0.91 (0.84, 0.98)	0.01	1.06 (0.97, 1.15)	0.19
Adjust 2		0.98 (0.96, 1.00)	0.10	1.02 (0.98, 1.05)	0.35	0.91 (0.84, 0.98)	0.01	1.06 (0.97, 1.15)	0.19
HOMA-IR	1.2 (±1.6)	0.97 (0.90, 1.04)	0.36	1.05 (0.92, 1.20)	0.45	0.92 (0.69, 1.21)	0.54	1.00 (0.74, 1.36)	0.97
Adjust 1		0.95 (0.89, 1.03)	0.20	1.06 (0.93, 1.21)	0.39	0.89 (0.67, 1.18)	0.41	0.99 (0.74, 1.34)	0.97
Adjust 2		0.95 (0.88, 1.02)	0.17	1.03 (0.90, 1.18)	0.62	0.88 (0.66, 1.16)	0.36	0.97 (0.72, 1.31)	0.84
Insulin	39.5 pmol/l (±1.5)	0.98 (0.91, 1.05)	0.54	1.04 (0.92, 1.18)	0.50	0.95 (0.73, 1.23)	0.70	1.12 (0.85, 1.48)	0.41
Adjust 1		0.96 (0.90, 1.04)	0.31	1.05 (0.93, 1.19)	0.41	0.91 (0.70, 1.19)	0.50	1.10 (0.83, 1.45)	0.50
Adjust 2		0.96 (0.89, 1.03)	0.24	1.03 (0.91, 1.16)	0.63	0.91 (0.70, 1.17)	0.46	1.08 (0.82, 1.42)	0.58
Leptin	6.7 μg/l (±3.3)	0.97 (0.80, 1.18)	0.75	0.89 (0.63, 1.25)	0.51	1.12 (0.54, 2.32)	0.76	1.77 (0.81, 3.84)	0.15

TABLE 3. (continued).

		Ratio or difference* (95% CI)							
		Maternal CRP		Maternal TNF-α		Maternal IL-6		Maternal IL-1β	
		2.79 μg/ml (±3.27)		5.67 pg/ml (±3.16)		1.11 pg/ml (±0.67)		0.48 pg/ml (±0.42)	
Offspring MS markers	Mean (±SD)		*P* value		*P* value		*P* value		*P* value
Adjust 1		0.89 (0.78, 1.02)	0.10	1.02 (0.80, 1.29)	0.88	0.76 (0.46, 1.27)	0.30	1.36 (0.79, 2.33)	0.27
Aadjust 2		0.88 (0.77, 1.01)	0.07	0.98 (0.77, 1.24)	0.86	0.75 (0.45, 1.25)	0.28	1.32 (0.77, 2.26)	0.32

[a]Shown are the differences per 5 units increment in IL-6 and IL-1β and per 10 units increment in CRP and TNF-α in the outcome variables waist circumference and systolic and diastolic blood pressure (indicated by *) and ratio for all other log transformed outcome variables. The results are presented as crude associations, as associations adjusted for maternal smoking and prepregnancy BMI (kg/m^2) (adjust 1) and as associations fully adjusted for prepregnancy BMI (kg/m^2), height (cm), smoking, education and offspring sex (adjust 2). n CRP = 389, n TNF-α = 390, n IL-6 = 389, n IL-1β = 389. The table also gives the median ± interquartile range (IQR) for the four inflammatory markers and the means (95% CI) for offspring outcome variables.

The results did not show significant differences between crude associations, associations adjusted by maternal smoking and prepregnancy BMI or associations fully adjusted for potential confounding. However, higher levels of IL-6 were associated with lower systolic blood pressure in the offspring (difference per 5 units increment in IL-6 −5.86 mm Hg [95% CI: −11.0, −0.74] in the fully adjusted analysis). This association persisted in the subgroup of female offspring (difference −6.62 mm Hg [95% CI −12.4, −0.89]). However, supplementary analyses with the exposure variable in quintiles did not show any associations, neither in the combined analyses nor in analyses stratified by offspring sex (data not shown). No other associations were detected in the combined analyses or in the subgroup of females or males. These null findings were supported by analyses of crude correlations (Spearman) between these inflammatory markers and the offspring outcome variables (data not shown).

The supplementary analyses with imputation of missing values (data not shown) or with maternal prepregnancy BMI and smoking excluded from the statistical model (Table 3) did not lead to changes in the results and neither did the adjustments for the offspring's ambient level of physical activity and BMI.

Among the 439 women included in the analyses, 11 (2.5%) were diagnosed with GDM. There was a small tendency towards higher levels of inflammatory markers in women with GDM than with no GDM; however, the differences were not significant nor related to offspring outcome variables.

Discussion

For the first time, we investigated whether markers of systemic subclinical inflammation in third trimester of pregnancy may be associated with the offspring's metabolic profile many years later. It is well known that diabetes, obesity, and insulin resistance in the mother are associated with inflammation, and that these metabolic conditions affect the growth and development of the fetus to various extents, with possible consequences for the future metabolic health (14,32,33). However, it is unknown whether inflammatory markers in healthy pregnant women are associated with MS in the offspring. The four markers of inflammation analyzed in this study are all considered to play some role in metabolism and vascular functions, and in particular TNF-α has been suggested as a possible central link between obesity, impaired glucose tolerance and type 2 diabetes (34). TNF-α has furthermore been suggested as a predictor of insulin resistance in normal pregnancies (35).

Nevertheless, this study does not demonstrate evidence that markers of low-grade inflammation in pregnancy are associated with any measurable unfavorable metabolic profile in young adult offspring. Four biomarkers of inflammation were carefully selected and studied individually. The rationale for selecting these four inflammatory markers was based on the knowledge of these markers being among the most important mediators of inflammation and their association with metabolic and vascular functions and diseases (20,35,36). To this end, we speculated that they could have distinct and possibly different roles in developmental programming of metabolism in the offspring despite being interrelated. Besides, the statistically significant association between higher maternal IL-6 levels and lower systolic blood pressure among female offspring only, none of the other

maternal inflammatory biomarkers turned out to be associated with the individual markers of MS in the offspring. The unexpected association between IL-6 plasma levels in the mother and systolic blood pressure in the offspring, observed when IL-6 was analyzed as a continuous variable, did not show up in the additional analyses with the levels of IL-6 divided into quintiles. Caution is warranted with respect to over-interpretation of this result due to the risk of a false-positive finding as a result of multiple comparisons. Indeed, the significance disappeared after correction for the number of comparisons performed.

The concentrations of the inflammatory markers measured in third trimester of pregnancy were comparable to cited values in the literature (37-39). In the first trimester, pregnancy is characterized by a proinflammatory phase, subsequently overtaken by an anti-inflammatory state during the third trimester (17,18). Still, detailed data on biomarker levels in third trimester of normal pregnancies are sparse. Since circulating biomarkers of systemic subclinical inflammation are highly dependent on factors, such as BMI, infections, smoking, medication, and alcohol consumption (40), the analyses were adjusted for prepregnancy BMI and smoking. This adjustment, however, did not affect the findings. Moreover, adjustment for the offspring's ambient level of physical activity and supplementary analyses with the exposure variables in quintiles did not change the results, which further supports the null findings of the study.

According to our a priori hypothesis, our clear null findings were somewhat surprising. Thus CRP, TNF-α, and IL-6 were associated with prepregnancy BMI (Table 2). However the women were generally healthy; thus, only 9% had a prepregnancy BMI $\geq$25 kg/m^2, and the observed levels of inflammatory markers were generally low (Table 3). Furthermore, they did not differ significantly in the 11 women with GDM. Of course, we cannot exclude the possibilities; first, that storage at -20°C for 20 years may have caused some degree of uncertainty to the measurement of exact levels of inflammatory markers, and secondly that the levels of inflammation in the pregnant women were too low to affect the metabolic development of their offspring. Regardless, subclinical inflammation is defined as subtle elevations of inflammatory markers within or only slightly above the normal range, supporting the biological relevance of the negative findings in this well powered and indeed phenotypically well characterized and extensive study material.

In conclusion, the results suggest that in a normal population of pregnant women, markers of inflammation in third trimester are not associated with markers of MS in offspring at the age of 20 years. Although these data need further validation from other studies, future studies should aim to identify other mechanisms linking subclinical inflammatory states during pregnancy to adverse metabolic outcomes in the offspring. O

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