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Associations of maternal caffeine intake during pregnancy with abdominal and liver fat deposition in childhood

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Summary

Background: Maternal caffeine intake during pregnancy is associated with an increased risk of childhood obesity. Studies in adults suggest that caffeine intake might also directly affect visceral and liver fat deposition, which are strong risk factors for cardio-metabolic disease.

Objective: To assess the associations of maternal caffeine intake during pregnancy with childhood general, abdominal, and liver fat mass at 10 years of age.

Methods: In a population-based cohort from early pregnancy onwards among 4770 mothers and children, we assessed maternal caffeine intake during pregnancy and childhood fat mass at age 10 years.

Results: Compared with children whose mothers consumed <2 units of caffeine per day during pregnancy, those whose mothers consumed 4-5.9 and ≥6 units of caffeine per day had a higher body mass index, total body fat mass index, android/gynoid fat mass ratio, and abdominal subcutaneous and visceral fat mass indices. Children whose mothers consumed 4-5.9 units of caffeine per day had a higher liver fat fraction. The associations with abdominal visceral fat and liver fat persisted after taking childhood total body fat mass into account.

Conclusions: High maternal caffeine intake during pregnancy was associated with higher childhood body mass index, total body fat, abdominal visceral fat, and liver fat. The associations with childhood abdominal visceral fat and liver fat fraction were independent of childhood total body fat. This suggests differential fat accumulation in these depots, which may increase susceptibility to cardio-metabolic disease in later life.

KEYWORDS

body mass index, caffeine, childhood, liver fat, pregnancy, visceral fat

1 | INTRODUCTION

Caffeine is a methylxanthine that occurs naturally in several food products. Caffeine-containing beverages, including coffee and tea, are widely consumed by pregnant women. Caffeine crosses the placenta and enters the fetal circulation freely.[1] The activity of the principal enzyme in caffeine metabolism, cytochrome CYP1A2, decreases progressively during pregnancy and is absent in placenta and fetus.[2-4] As

a consequence, fetal exposure to caffeine is prolonged and might adversely influence the development of organ systems. Consumption of caffeine-containing beverages during pregnancy has been related to an increased risk of fetal death, impaired fetal growth, and low birth weight.[5-9] In addition to these short-term outcomes, maternal caffeine intake during pregnancy may also influence long-term offspring body fat development. We previously observed among 7857 mothers and their children from the Netherlands that high maternal caffeine intake during pregnancy was associated with a higher childhood body mass index and total body fat mass at the age of 6 years.[10] Similarly, studies among 615, 50 943, and 558 mothers and children from the United States, Norway, and Ireland, respectively, observed that any maternal caffeine intake during pregnancy was associated with an increased risk of obesity in childhood.[11-13]

In contrast, consumption of caffeine-containing beverages by non-pregnant adults seems to have beneficial effects on body fat accumulation and the risks of several diseases.[14-19] Previous studies suggest that consumption of caffeine-containing beverages is associated with lower visceral fat accumulation and lower risks of non-alcoholic fatty liver disease (NAFLD), possibly by influencing blood concentrations of adiponectin and pro-inflammatory cytokines.[15-19] Previous research suggests that blood concentrations of adipokines and cytokines in pregnant women are related to childhood body fat development.[20-22] However, it is not known whether maternal caffeine intake during pregnancy is also related to offspring abdominal and liver fat accumulation. Thus far, only animal studies have shown that in utero exposure to caffeine increases intra-hepatic fat content and the susceptibility to NAFLD.[23,24] As visceral and liver fat accumulation are related to the development of hypertension, type 2 diabetes, NAFLD, and the metabolic syndrome independent of excess body fat per se,[25,26] it is important to obtain further insight into whether maternal caffeine intake during pregnancy differentially affects offspring visceral and liver fat deposition.

Therefore, in a population-based cohort among 4770 mothers and children from early pregnancy onwards, we assessed the associations of maternal caffeine intake during pregnancy with childhood general, abdominal, and liver fat at the age of 10 years, with the main focus on abdominal and liver fat.

2 | METHODS

2.1 | Study design

This study was embedded in the Generation R Study, a prospective population-based cohort study from early pregnancy onwards performed in Rotterdam, the Netherlands.[27] The study was approved by the Medical Ethical Committee of the Erasmus Medical Center, University Medical Center, Rotterdam (MEC 198.782/2001/31). Written informed consent was obtained from all mothers at enrolment in the study. The response rate at birth was 61%. Of the 8879 mothers that were prenatally included in the study, 8097 had information available on caffeine intake during pregnancy. Of their children, 7900 were singleton and live born. Of these children, 4770 participated in body composition follow-up measurements at 10 years of age and were included in the analyses (Figure S1).

2.2 | Maternal caffeine intake during pregnancy

As described previously, information on maternal caffeine intake from coffee and tea during pregnancy was obtained by postal questionnaires in the first, second, and third trimesters of pregnancy.[7,10] Response rates for these questionnaires were 91%, 80%, and 77%, respectively.[7,10] Mothers who reported to drink any coffee or tea were asked how many cups of coffee or tea they consumed on average per day and what type of coffee or tea they consumed (caffeinated, decaffeinated, or a combination of both). According to standard values for caffeine content, a regular coffee serving (125 mL) in the Netherlands contains ~90 mg caffeine, decaffeinated coffee contains ~3 mg, and black tea contains ~45 mg.[28] To calculate the total caffeine intake in each trimester, the type of coffee or tea was weighted according to its caffeine content (caffeinated coffee = 1, caffeinated and decaffeinated coffee = 0.5, decaffeinated coffee = 0, caffeinated tea = 0.5, caffeinated and decaffeinated tea = 0.25, decaffeinated tea = 0, herbal tea = 0, and green tea = 0.5).[7] Thus, in our analyses, each unit of caffeine intake reflects caffeine exposure based on one cup of caffeinated coffee (90 mg caffeine).[10] Based on data availability, total caffeine intake was categorized into categories of <2, 2-3.9, 4-5.9, and ≥6 units per day (equivalent to <180, 180-359, 360-539, and ≥540 mg per day, respectively). For the main analyses using caffeine intake during the full pregnancy, caffeine intake of the trimesters was averaged.

2.3 | Childhood body fat mass

At the age of 10 years, we measured height and weight without shoes and heavy clothing and calculated body mass index (kg/m^2). We created age- and sex-adjusted standard deviation scores (SDS) of body mass index using a Dutch reference chart.[29] In addition, we defined childhood overweight/obesity according to the International Obesity Task Force cut-offs.[30] We measured total and regional body fat mass using dual-energy X-ray absorptiometry (DXA) (iDXA, General Electrics–Lunar, 2008, Madison, Wisconsin).[31] Android/gynoid fat mass ratio was calculated and used as a measure of body fat distribution comparable with waist/hip ratio.[31] Abdominal and organ fat were measured in a subgroup by magnetic resonance imaging (MRI), as described previously.[27] Briefly, all children were scanned using a 3.0 Tesla MRI (Discovery MR750w, General Electric Healthcare, Milwaukee, Wisconsin). The MRI protocol included an axial 3-point Dixon sequence for fat and water separation (IDEAL IQ) for liver fat measurements. This technique also enables the generation of liver fat fraction images.[32] An axial abdominal scan from lower liver to pelvis and a coronal scan centred at the head of the femurs were also performed with a 2-point Dixon acquisition

(LavaFlex). The obtained fat scans were analysed by the Precision Image Analysis company (PIA, Kirkland, Washington), using the sliceOmatic (TomoVision, Magog, Canada) software package. All extraneous structures and any image artefacts were removed manually.[33] Total subcutaneous and visceral fat volumes ranged from the dome of the liver to the superior part of the femoral head. Fat masses were obtained by multiplying the total volumes by the specific gravity of adipose tissue, 0.9 g/mL. Liver fat fraction was determined by defining four regions of interest of at least 4 cm^2 in the central portion of the hepatic volume. Subsequently, the mean signal intensities were averaged to generate an overall mean liver fat fraction estimation. To create fat measures independent of child's height, we estimated the optimal adjustment by log-log regression analyses and subsequently divided total and subcutaneous fat mass by $height^3$ (total body fat mass index and subcutaneous fat mass index) and visceral fat mass by $height^4$ (visceral fat mass index, Methods S1).[34,35]

2.4 | Covariates

Information on maternal age, pre-pregnancy body mass index, parity, ethnicity, educational level, and folic acid supplementation use was obtained by questionnaire at enrolment in the study. Smoking and alcohol intake during pregnancy were repeatedly assessed by questionnaire. Information on gestational diabetes, gestational hypertensive disorders (gestational hypertension and pre-eclampsia), date of birth, child's sex, and birth weight was obtained from midwife and hospital registries. Average television watching time was assessed by questionnaire at the age of 10 years.

2.5 | Statistical analysis

First, we performed a non-response analysis comparing participants included the analysis with those lost to follow up at the age of 10 years. Second, we assessed the associations of maternal caffeine intake during pregnancy with childhood general fat measures and the risk of overweight/obesity at age 10, using linear and logistic regression models. Third, we assessed the associations of maternal caffeine intake during pregnancy with childhood abdominal subcutaneous and visceral fat mass indices and liver fat fraction, using linear regression models. Non-normally distributed outcome variables were log-transformed. To enable comparison of effect estimates across the different outcomes, we calculated SDS for each of the outcomes. The models were first adjusted for child's age and sex only (basic models). Next, we additionally adjusted the models for maternal ethnicity, education, smoking during pregnancy, alcohol consumption during pregnancy, folic acid supplementation use, and childhood television watching time (confounder models). These confounders were selected based on existing literature, associations with the exposure and outcome in the study sample, and a change in effect estimates of >10%. Maternal age, pre-pregnancy body mass index, parity, and gestational hypertensive disorders were also considered, but were not associated with either the exposure or the outcome or did not change the effect estimates with >10% and were therefore not included in the models. To explore whether any observed associations of caffeine intake during pregnancy with the outcomes were mediated by gestational age at birth and birth weight, we added these variables to the confounder models (mediator models). We performed tests for trend by adding the categorized caffeine intake variable to the models as continuous variable. Fourth, to further explore whether maternal caffeine intake during pregnancy was specifically associated with childhood abdominal fat mass and liver fat fraction, independent from total body fat mass, we used conditional regression analyses. We created measures of childhood abdominal subcutaneous fat mass, abdominal visceral fat mass, and liver fat fraction that are independent of total body fat mass by regressing these detailed childhood fat measures on childhood total body fat mass index. The standardized residuals from these models were used as an outcome for the regression models focused on the associations of maternal caffeine intake during pregnancy with conditional childhood abdominal and liver fat measures.[36] Fifth, to identify potential critical periods, we assessed the associations of trimester-specific maternal caffeine intake with childhood general, abdominal, and liver fat using linear regression models. As sex differences in childhood body fat development have been reported,[37,38] we tested for interactions between maternal caffeine intake during pregnancy and child's sex, but these interaction terms were not statistically significant (P values > .05). Missing values of covariates (maximum percentage of missing values: 20.8%) were imputed using Multiple Imputation, and pooled results from five imputed datasets were reported. All statistical tests were two-sided, with a significance threshold of 0.05. The analyses were performed using the Statistical Package for the Social Sciences version 24.0 (IBM Corp, Armonk, New York, USA) and R version 3.3.4 (R Foundation for Statistical Computing).

3 | RESULTS

3.1 | Participants' characteristics

Table 1 shows that, of the 4770 women included, 2780 (58.3%), 1583 (33.2%), 329 (6.9%), and 78 (1.6%) consumed <2 units, 2-3.9 units, 4-5.9 units, and ≥6 units of caffeine per day, respectively, during pregnancy. Women who had higher caffeine intakes were older and were more likely to be higher educated, multiparous, and from European descent. They used less often folic acid supplementation and smoked and consumed alcohol more often during pregnancy. Table S1 shows that, as compared with women included in the analyses, those lost to follow up had slightly lower caffeine intakes and a lower pre-pregnancy BMI, were younger, were more often multiparous, and were less often from European descent. These women used folic supplementation less often, smoked more often, and consumed alcohol less often during pregnancy.

TABLE 1 Characteristics of the mothers and their children

	Total Group	<2 Units	2-3.9 Units	4-5.9 Units	≥6 Units	*P* Value
	N = 4770	N = 2780 (58.3%)	N = 1583 (33.2%)	N = 329 (6.9%)	N = 78 (1.6%)	
Maternal characteristics						
Caffeine intake during pregnancy, median (95% range), units	1.6 (0.0-5.2)	1.0 (0.0-1.8)	2.6 (2.0-3.8)	4.5 (4.0-5.7)	6.7 (6.0-10.3)	<.001
Age, median (95% range), y	31.2 (20.4-39.6)	30.4 (19.6-39.1)	32.0 (21.9-40.1)	32.9 (23.5-39.8)	33.7 (23.2-40.8)	<.001
Pre-pregnancy BMI, median (95% range), kg/m^2	22.6 (18.1-34.5)	22.6 (18-34.6)	22.4 (18.3-34.1)	22.8 (18.5-34.5)	22.7 (18.4-31.5)	.242
Education, N (%)						
Primary	340 (7.4)	233 (8.7)	87 (5.6)	13 (4.0)	7 (9.2)	<.001
Secondary	1944 (42.2)	1247 (46.7)	543 (35.3)	116 (36.1)	38 (50.0)	
Higher	2324 (50.4)	1191 (44.6)	910 (59.1)	192 (59.8)	31 (40.8)	
Parity, nulliparous (%)	2793 (58.8)	1715 (62.0)	873 (55.4)	168 (51.1)	37 (47.4)	<.001
Ethnicity, European (%)	3113 (65.8)	1624 (59.1)	1163 (73.8)	264 (80.7)	62 (80.5)	<.001
Folic acid supplementation use, Yes (%)	2955 (78.2)	1663 (75.9)	1039 (81.7)	216 (82.4)	37 (67.3)	<.001
Smoking during pregnancy, Yes (%)	1065 (24.3)	493 (19.3)	412 (28.3)	116 (38.3)	44 (58.7)	<.001
Alcohol consumption during pregnancy, Yes (%)	2495 (57.5)	1271 (50.2)	969 (67.3)	203 (69.0)	52 (70.3)	<.001
Gestational hypertensive disorders, Yes (%)	272 (5.9)	166 (6.2)	80 (5.2)	22 (6.8)	4 (5.3)	NA
Gestational diabetes, Yes (%)	46 (1.0)	26 (1.0)	15 (1.0)	5 (1.6)	0 (0.0)	NA
Child characteristics						
Males, No. (%)	2361 (49.5)	1410 (50.7)	732 (46.2)	176 (53.5)	43 (55.1)	.009
Gestational age at birth, median (95% range), wk	40.1 (35.9-42.3)	40.1 (35.5-42.3)	40.3 (36.4-42.3)	40.3 (36.0-42.4)	40.3 (34.5-42.4)	.023
Birth weight, median (95% range), g	3460 (2262-4480)	3450 (2250-4430)	3490 (2368-4520)	3530 (2392-4534)	3390 (1999-4263)	.007
Gestational age adjusted birth weight, mean (SD)	−0.1 (1.0)	−0.1 (1.0)	0.0 (1.0)	0.0 (1.1)	−0.3 (1.1)	.027
Ever breastfeeding, Yes (%)	3627 (92.6)	2063 (92.3)	1244 (93.3)	266 (92.7)	54 (91.5)	NA
Introduction of solid foods, before 6 mo (%)	2800 (89.1)	1569 (88.7)	979 (89.4)	214 (90.7)	38 (90.5)	NA
Age at 10-y follow-up measurement, median (95% range), y	9.7 (9.4-10.7)	9.7 (9.3-10.8)	9.7 (9.4-10.7)	9.7 (9.4-10.4)	9.7 (9.3-10.3)	.650
Television watching, More than 2 h/d (%)	1168 (30.9)	717 (33.3)	346 (26.8)	80 (28.7)	25 (40.3)	<.001
Body mass index at 10 y, median (95% range), kg/m^2	17.0 (14.0-24.8)	17.0 (14.0-25.1)	16.8 (14.1-24.2)	17.2 (14-24.1)	17.8 (14.3-24.0)	<.001
Overweight, N (%)	862 (3.7)	556 (16.2)	231 (11.8)	54 (13.8)	21 (22.0)	NA
Total body fat mass, median (95% range), kg	8.5 (4.5-22.0)	8.6 (4.5-22.2)	8.4 (4.5-21.7)	8.5 (4.8-21.9)	9.7 (4.6-20.9)	.044
Android/gynoid fat mass ratio, median (95% range)	0.2 (0.2, 0.5)	0.2 (0.2, 0.5)	0.2 (0.2, 0.5)	0.2 (0.2, 0.5)	0.3 (0.2, 0.5)	.023
Abdominal subcutaneous fat, median (95% range), kg	1.3 (0.6, 5.4)	1.3 (0.6, 5.4)	1.3 (0.6, 4.9)	1.4 (0.7, 4.7)	1.5 (0.8, 6.2)	.017
Abdominal visceral fat, median (95% range), kg	0.4 (0.2, 1.0)	0.4 (0.2, 1.0)	0.4 (0.2, 0.9)	0.4 (0.2, 0.9)	0.5 (0.2, 1.2)	.002
Liver fat fraction, median (95% range), %	2.0 (1.2, 5.2)	2.0 (1.2, 5.3)	2.0 (1.3, 4.6)	2.1 (1.4, 5.4)	2.1 (1.3, 8.4)	.444

Note: Values represent mean (SD), median (95% range) or number of participants (valid %). One unit of caffeine represents the equivalent of one cup of coffee (90 mg of caffeine). NA: Chi-square test not available as a result of low expected cell counts.

3.2 | Maternal caffeine intake during pregnancy and childhood general body fat mass

Figure 1 shows that in the confounder model, as compared with children whose mothers consumed <2 units of caffeine per day during pregnancy, those whose mothers consumed 4-5.9 and ≥6 units of caffeine per day during pregnancy had a higher body mass index (differences: 0.12 standard deviation [SD] [95% confidence interval (CI), 0.01-0.24] and 0.24 [95% CI, 0.01-0.47], respectively), total body fat mass index (differences: 0.14 SD [95% CI, 0.04-0.25] and 0.22 [95% CI, 0.02-0.43], respectively), and android/gynoid fat mass ratio (differences: 0.16 SD [95% CI, 0.05-0.27] and 0.22 [95% CI, 0.01-0.44], respectively) at the age of 10 years (exact differences are given in Table S2). A dose-response relationship was present for each of these outcomes (*P*-values for trend < .05). Children whose mothers consumed ≥6 units of caffeine per day also tended to have a higher risk of overweight/obesity (odds ratio: 1.59 [95% CI, 0.92-2.75], Figure 2). Results from the basic model were similar (Table S3). Additional adjustment for gestational age at birth and birth weight did not change the results (Table S4). Table S5 shows that no trimester-specific associations were present, but rather that associations were similar across pregnancy.

3.3 | Maternal caffeine intake during pregnancy and childhood abdominal fat mass and liver fat fraction

Figure 3A shows that in the confounder model, as compared with children whose mothers consumed <2 units of caffeine per day during pregnancy, those whose mothers consumed 4-5.9 and ≥6 units of caffeine per day during pregnancy had a higher abdominal subcutaneous fat mass index (differences: 0.15 SD [95% CI, −0.01 to 0.30] and 0.35 SD [95% CI, 0.04-0.65], respectively) and a higher abdominal visceral fat mass index (differences: 0.14 SD [95% CI, −0.03 to 0.30] and 0.43 SD [95% CI, 0.11-0.76], respectively). Children whose mothers consumed 4-5.9 units of caffeine per day also had a higher liver fat fraction, as compared with those whose mothers consumed <2 units per day during pregnancy (difference: 0.20 SD [95% CI, 0.04-0.36]); exact differences are given in Table S6). A dose-response relationship was present for each of the outcomes (*P* values for trend < .05). Results from the basic model were similar (Table S7). Additional adjustment for gestational age at birth and birth weight did not influence the observed estimates (Table S8). Table S9 shows that the associations for each trimester separately were comparable with those for the full pregnancy.

Figure 3B shows that after conditioning on total body fat mass index to assess the effects of maternal caffeine intake during pregnancy on childhood abdominal fat and liver fat fraction independent of childhood total body fat, maternal caffeine intake during pregnancy of ≥6 units and 4-5.9 units per day remained associated with abdominal visceral fat mass index and liver fat fraction, respectively (differences: 0.32 SD [95% CI, 0.00-0.64] and 0.20 SD [95% CI, 0.04-0.36]). A significant dose-response relationship remained also present for these outcomes (*P* values for trend < .05). No associations were present with childhood abdominal subcutaneous fat mass index conditioned on childhood total body fat mass index.

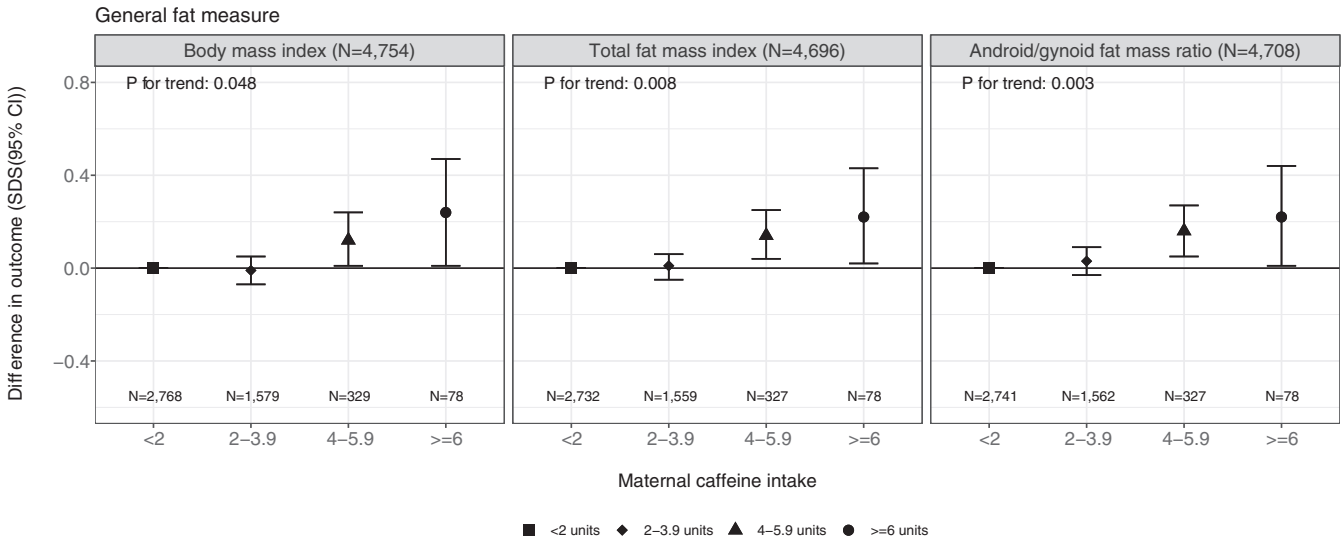

FIGURE 1 Associations of maternal caffeine intake during pregnancy with childhood general body fat mass. Values are regression coefficients (95% confidence intervals) from the confounder models that reflect the difference in childhood body mass index, total body fat mass index, android/gynoid fat mass ratio in children of mothers who consumed 2-3.9, 4-5.9, and ≥6 units of caffeine per day, as compared with those whose mothers consumed <2 units of caffeine per day. One unit of caffeine represents the equivalent of one cup of coffee (90 mg). The models are adjusted for child's sex, child's age at follow-up measurement, maternal ethnicity, maternal education, maternal smoking, maternal alcohol use, folic acid supplementation, and television watching time. *P* values for trend were obtained from models in which the categorized caffeine intake variable (<2, 2-3.9, 4-5.9, and ≥6 units) was entered as continuous variable

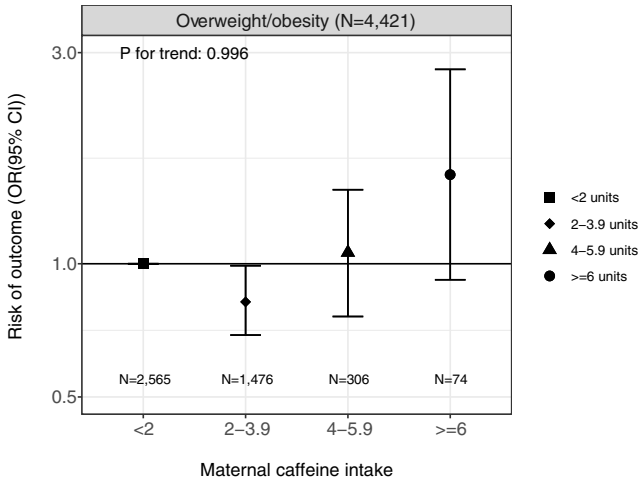

FIGURE 2 Associations of maternal caffeine intake during pregnancy with the risk of childhood overweight/obesity. Values are odds ratios (95% confidence intervals) from the confounder models that reflect the risk of overweight/obesity in children of mothers who consumed 2-3.9, 4-5.9, and ≥6 units of caffeine per day, as compared with those whose mothers consumed <2 units of caffeine per day. One unit of caffeine represents the equivalent of one cup of coffee (90 mg). The models are adjusted for child's sex, child's age at follow-up measurement, maternal ethnicity, maternal education, maternal smoking, maternal alcohol use, folic acid supplementation, and television watching time. *P* values for trend were obtained from models in which the categorized caffeine intake variable (<2, 2-3.9, 4-5.9, and ≥ 6 units) was entered as continuous variable

4 | DISCUSSION

In this population-based prospective cohort study, high maternal caffeine intake during pregnancy was associated with higher childhood general body fat mass, abdominal fat mass, and liver fat fraction at the age of 10 years. The associations of high maternal caffeine intake with childhood abdominal visceral fat mass and liver fat fraction seemed to be independent from childhood total body fat mass.

4.1 | Interpretation of main findings

Caffeine-containing beverages are frequently consumed during pregnancy. Increasing evidence suggests that maternal caffeine intake during pregnancy might be related to long-term offspring body fat development.[10-12,39] We previously showed among 7857 6-year-old children from the same cohort as the current study that maternal caffeine intake during pregnancy of ≥4 units per day was associated with a higher body mass index and total body fat mass. Maternal caffeine intake during pregnancy of ≥6 units per day was also associated with a higher android/gynoid fat mass ratio, reflecting a central body fat accumulation.[10] A study among 272 mother-child pairs from Brazil observed that any caffeine intake by women with an uncomplicated pregnancy was associated with a higher offspring sum of skinfold thicknesses at age 3 months.[39] A study among 50 943 participants from Norway showed that any caffeine intake during pregnancy was associated with an increased risk of childhood overweight at ages 3 and 5, whereas at 8 years, this association was only present for high caffeine intakes.[12] In an Irish study among 558 mother-child pairs, higher maternal caffeine intake during pregnancy was associated with higher risks of overall and central obesity at the ages of 5 and 9 years.[13] In line with these previous studies, we observed in the current study that higher maternal caffeine intake during pregnancy was associated with higher body mass index, total body fat mass, and android/gynoid fat mass ratio at the age of 10 years, as indicated by significant tests for trend. The strongest effects were present for maternal caffeine intake during pregnancy 4 or more units per day. For instance, as compared with children whose mothers consumed <2 units of caffeine per day, children whose mothers consumed ≥6 units of caffeine per day during their pregnancy had a 0.24 SD higher body mass index, corresponding to a difference of approximately 0.7 kg/m^2. These effect sizes are comparable with those observed for well-known determinants of childhood body mass index, such as maternal pre-pregnancy overweight and smoking during pregnancy.[40-42] These associations were similar across the trimesters of pregnancy. Mothers with high caffeine intakes during pregnancy also had higher alcohol intakes and smoked more often during their pregnancies. However, associations were present across the full range of maternal caffeine intake, and adjusting the models for these lifestyle-related factors did not influence the results. We therefore do not consider it likely that the observed associations can be fully explained by differences in these factors. Thus, these findings suggest that maternal caffeine intake throughout pregnancy has long-term consequences for offspring body fat development, as reflected by higher total body fat mass and a central body fat distribution.

Studies in adults suggest that consumption of caffeine-containing beverages might also be associated with abdominal and ectopic fat deposition, although the direction of these associations might be different from the direction of the associations of maternal caffeine intake during pregnancy with offspring body fat development.[15-19] A study among 364 Japanese men showed inverse associations of coffee consumption with visceral fat mass and visceral to

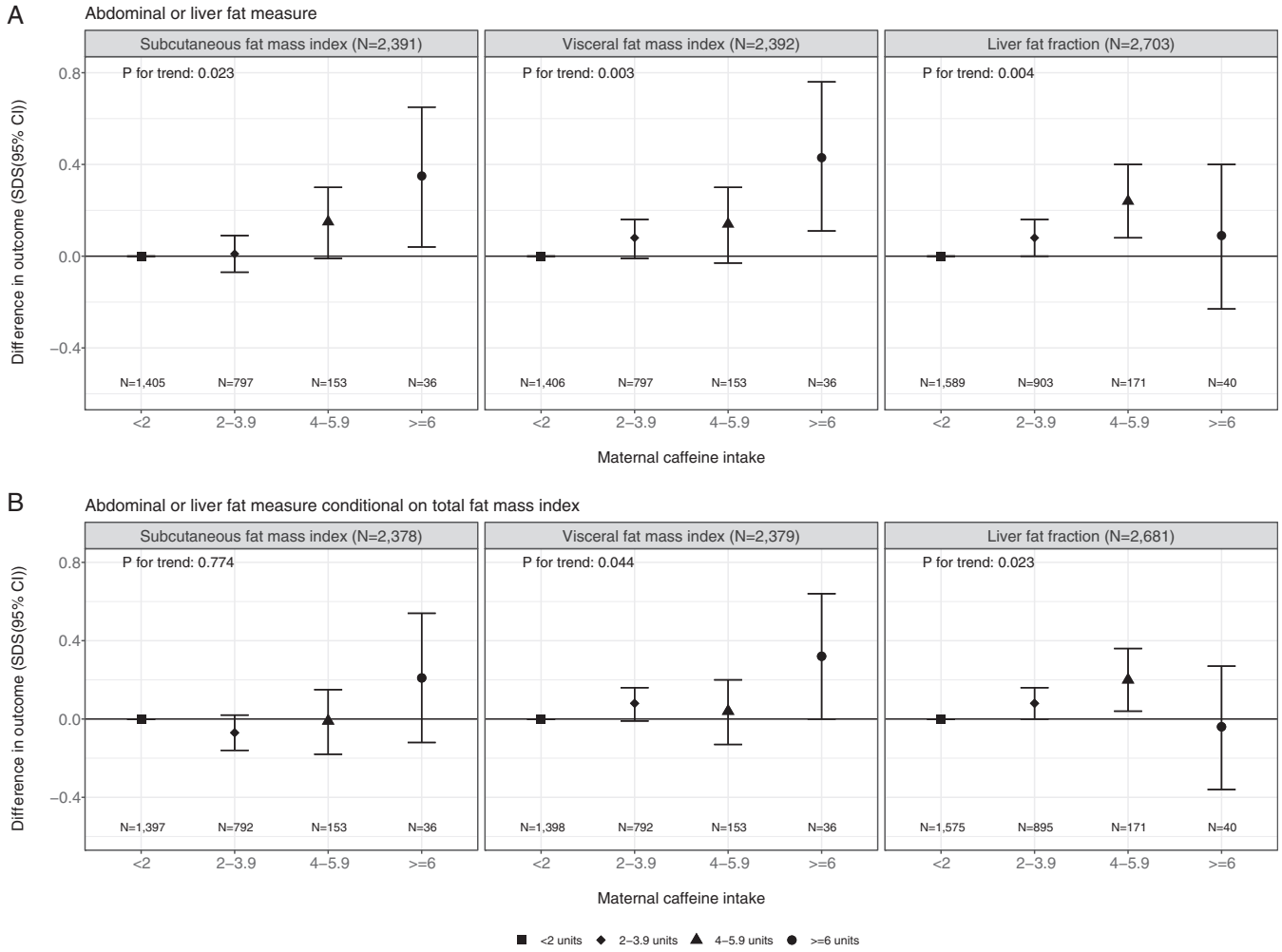

FIGURE 3 Associations of maternal caffeine intake during pregnancy with childhood abdominal fat mass and liver fat fraction. Values are regression coefficients (95% confidence intervals) from the confounder models that reflect the difference in (A) childhood outcomes in SDS and (B) childhood outcomes in standardized residuals in children of mothers who consumed 2-3.9, 4-5.9, and ≥6 units of caffeine per day, as compared with those whose mothers consumed <2 units of caffeine per day. One unit of caffeine represents the equivalent of one cup of coffee (90 mg). The models are adjusted for child's sex, child's age at follow-up measurement, maternal ethnicity, maternal education, maternal smoking, maternal alcohol use, folic acid supplementation, and television watching time. *P* values for trend were obtained from models in which the categorized caffeine intake variable (<2, 2-3.9, 4-5.9, and ≥6 units) was entered as continuous variable

subcutaneous fat mass ratio.[15] A meta-analysis of five studies showed that the risk of NAFLD was 30% lower in participants who consumed coffee as compared with those who did not.[18] It is not known whether caffeine intake by pregnant women is also related to offspring fat deposition in these specific fat depots. Only one study, among 7857 participants from our cohort from the Netherlands, showed that maternal caffeine intake during pregnancy was not associated with pre-peritoneal fat mass measured by abdominal ultrasound at age 6, which was used as proxy of visceral fat.[10] In the current study, we observed that higher maternal caffeine intake during pregnancy was associated with higher childhood abdominal subcutaneous fat mass, abdominal visceral fat mass, and liver fat fraction measured by MRI at age 10. This inconsistency might be explained by differences in measures of abdominal visceral fat mass. Pre-peritoneal fat mass provides an estimation of abdominal visceral fat mass, while MRI provides more precise measures and is the gold standard for the measurement of intra-abdominal and organ fat deposition.[33] Also, the associations of maternal caffeine intake during pregnancy might only become apparent at older childhood ages. The results for each of the trimesters separately were comparable with those for the full pregnancy. The associations with abdominal visceral fat mass and liver fat fraction persisted after taking total body fat mass into account. This suggests that maternal caffeine intake throughout pregnancy might differentially affect fat deposition in these depots in the offspring, independent of their total body fat development. As visceral and liver fat accumulation are related to the development of cardio-metabolic disease independently of total body fat, these children might be at risk of later cardio-metabolic disease.[25,26] The associations with abdominal subcutaneous fat mass were not independent from total body fat mass.

This might be explained by subcutaneous fat being the main compartment of fat storage across the full body. Thus, maternal caffeine intake throughout pregnancy might affect offspring visceral and liver fat deposition, independent from their total amount of body fat.

The mechanisms underlying the observed associations are not well known. Studies in adults have suggested that consumption of caffeine-containing beverages might increase adiponectin concentrations and decrease concentrations of pro-inflammatory cytokines, subsequently influencing visceral and liver fat masses.[15,19] Although high maternal adiponectin concentrations during pregnancy have been related to a higher risk of childhood obesity,[20] the role of adipokines and cytokines in the association of maternal caffeine intake during pregnancy with offspring fat deposition is unknown. We speculate that caffeine intake by pregnant women may affect adiponectin concentrations and the pro-inflammatory state, which may affect fetal nutrient supply and subsequently lead to developmental adaptations in adipose tissue. Alternatively, animal studies have suggested that in utero exposure to caffeine may overexpose the developing fetus to glucocorticoids, leading to an altered development of the HPA-axis.[43,44] High glucocorticoid concentrations have been related to increased central obesity. In addition, the concentration of glucocorticoid receptors is higher in visceral adipose tissue as compared with other fat depots, possibly resulting in differential fat deposition in these depots.[45] Rats exposed to caffeine in utero had increased intrahepatic fat concentrations and increased susceptibility to NAFLD,[23,24] possibly by similar mechanisms. Finally, the associations might be explained by confounding by unhealthy lifestyle factors that are shared within families. However, a negative control analysis among 50 943 participants showed stronger associations for maternal caffeine intake during pregnancy with the risk of childhood overweight at the age of 3 years, as compared with those for paternal caffeine intake at the time of their partners pregnancy.[12] Similarly, in another recent negative control analysis among 558 participants, maternal caffeine intake, but not paternal caffeine intake, was associated with childhood body mass index and waist circumference at ages 5 and 10 years.[13] These results suggest that an intra-uterine programming mechanism might at least partly underlie these associations. Further studies are needed to disentangle the mechanisms underlying the associations of maternal caffeine intake and childhood abdominal and liver fat deposition.

Our results are consistent with those of previous studies and further highlight the importance of limiting maternal caffeine intake during pregnancy with respect to its potential adverse effects on long-term body fat development in the offspring. The current recommendations for maternal caffeine intake during pregnancy range between 200 and 300 mg per day and are based on the risks of adverse pregnancy and birth outcomes.[46-48] The most pronounced effects observed in our study were for caffeine intakes above these guidelines. However, the dose-response relationship in our and previous studies[5,6,8-13] suggest that the adverse effects of maternal caffeine intake with respect to both pregnancy outcomes and long-term body fat development are not restricted to high caffeine intakes, but increase across the range of maternal caffeine intake. A review of randomized controlled trials had insufficient evidence to confirm that avoiding caffeine consumption during pregnancy is beneficial with respect to adverse pregnancy outcomes.[49] Based on our findings and findings from other observational studies, further adequately powered randomized controlled trials are needed to assess whether avoiding caffeine consumption during pregnancy improves both pregnancy and long-term offspring health outcomes, as compared with current recommendations. Our findings and findings from other observational studies need to be incorporated in future guidelines regarding maternal caffeine consumption during pregnancy, and these guidelines need to further emphasize potential beneficial effects on offspring health outcomes by further reducing caffeine intake during pregnancy below the current recommendations.

4.2 | Strengths and limitations

This study was embedded in a large population-based cohort from early pregnancy onwards, enabling us to prospectively study the associations of interest. Of all participants with information on maternal caffeine intake during pregnancy, 39.6% did not participate in the follow-up measurements at age 10. This non-response might have led to biased estimates if the associations of interest differ between participants included and lost to follow up. This seems unlikely as only a minor difference in maternal caffeine intake was observed between these groups. However, the selection towards a higher educated, healthier population might have affected the generalizability of our results. Maternal caffeine intake might have been underreported, possibly leading to misclassification of the caffeine intake categories and underestimation of the effect estimates. In accordance with the Dutch Nutrition Centre, we assumed that coffee and tea were consumed in cups of 125 mL.[46] This might have differed between participants, which may have led to some misclassification of maternal caffeine intake. We only had data available about caffeine intake from coffee and tea and not from other sources, such as soft drinks, energy drinks, chocolate, and medications. However, at the time of data collection (2002-2006), coffee and tea accounted for 70% and 26%, respectively, of all caffeine consumed.[50] We had data available on many possible confounders. However, residual confounding might still be present, for example, by maternal and child's physical activity and dietary habits.

5 | CONCLUSIONS

Our results suggest that high maternal caffeine intake during pregnancy is associated with higher general body fat, abdominal subcutaneous and visceral fat mass and liver fat fraction in childhood. The associations of maternal caffeine intake during pregnancy with childhood visceral fat mass and liver fat seem to be largely independent from childhood total body fat mass. This suggests differential fat accumulation in these depots, which may increase susceptibility to cardiometabolic disease.

CONFLICT OF INTEREST STATEMENT

No conflict of interest was declared.

AUTHORS' CONTRIBUTIONS

E.V., M.E.H., and R.G. designed and conducted the research and wrote the paper. E.V. and M.E.H. performed the statistical analysis. V.W.V.J. and E.H.G.O. coordinated data acquisition and critically reviewed and revised the manuscript. E.V. and R.G. had primary responsibility for the final content. All authors approved the final manuscript and agree to be accountable for all aspects of the work.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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