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ORIGINAL ARTICLE

Association of maternal prepregnancy BMI with metabolomic profile across gestation

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BACKGROUND/OBJECTIVES: Elevated prepregnancy body mass index (pBMI) and excess gestational weight gain (GWG) constitute important prenatal exposures that may program adiposity and disease risk in offspring. The objective of this study is to investigate the influence of pBMI and GWG on the maternal metabolomic profile across pregnancy, and to identify associations with birth weight.

SUBJECTS/METHODS: This is a longitudinal prospective study of 167 nondiabetic women carrying a singleton pregnancy. Women were recruited between March 2011 and December 2013 from antenatal clinics affiliated to the University of California, Irvine, Medical Center. Seven women were excluded from analyses because of a diagnosis of diabetes during pregnancy. A total of 254 plasma metabolites known to be related to obesity in nonpregnant populations were analyzed in each trimester using targeted metabolomics. The effects of pBMI and GWG on metabolites were tested through linear regression and principle component analysis, adjusting for maternal sociodemographic factors, diet, and insulin resistance. A Bonferroni correction was applied for multiple comparison testing.

RESULTS: pBMI was significantly associated with 40 metabolites. Nonesterified fatty acids (NEFA) showed a strong positive association with pBMI, with specificity for mono-unsaturated and omega-6 NEFA. Among phospholipids, sphingomyelins with two double bonds and phosphatidylcholines containing 20:3 fatty acid chain, indicative of omega-6 NEFA, were positively associated with pBMI. Few associations between GWG, quality and quantity of the diet, insulin resistance and the maternal metabolome throughout gestation were detected. NEFA levels in the first and, to a lesser degree, in the second trimester were positively associated with birth weight percentiles.

CONCLUSIONS: Preconception obesity appears to have a stronger influence on the maternal metabolic milieu than gestational factors such as weight gain, dietary intake and insulin resistance, highlighting the critical importance of preconception health. NEFA in general, as well as monounsaturated and omega-6 fatty acid species in particular, represent key metabolites for a potential mechanism of intergenerational transfer of obesity risk.

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INTRODUCTION

The increasing prevalence of childhood obesity is of major concern because obese children are substantially more likely to be obese as adults, and to develop obesity-related diseases at earlier ages and of greater severity. Several environmental and genetic factors are described as risk factors for childhood obesity.[1] Maternal high-fat dietary intake and obesity during pregnancy are implicated in 'fetal programming' of offspring obesity.[2,3] Maternal prepregnancy body mass index (pBMI) is more strongly associated with excessive fetal growth and birth weight than hyperglycemia.[4] Different mechanisms have been discussed for this intergenerational cycle of obesity, including epigenetic modulations or *in utero* changes in the appetite control system,[4,5] that have been primarily investigated in animal models to date. Meanwhile, gestational alterations in the maternal and fetal metabolism among humans are not well understood and less studied.

Advances in metabolomics technology in recent years have greatly facilitated new insights into the study of human obesity and its underlying mechanisms.[6] However, significant alterations in maternal metabolism occur during pregnancy and even between pregnancy trimesters,[7] making comparisons with the nonpregnant state difficult or invalid. Although the impact of maternal obesity on adverse pregnancy and offspring outcomes is well documented, a more in-depth study of the maternal metabolome may highlight biomarkers of gestational metabolic disturbances and potential causal pathways for fetal programming of adult disease risks.[8] Metabolomics facilitates a detailed investigation of the metabolic state by determining single molecular species, for example, the determination of nonesterified fatty acids (NEFA)[9] and glycerophospholipids,[10] allows a differentiated view on fatty acid status. Such new insights among pregnant populations are important to assist our efforts in adapting nutrition, lifestyle or other factors in pregnancy for more favorable outcomes.

Although a few cross-sectional metabolomics studies have been conducted in pregnant cohorts, these have primarily focused on

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differentiating the metabolomics profile of healthy pregnant women compared with those with adverse pregnancy outcomes.[11–13] A recent study also depicted an association between maternal pBMI and lipid profile in early pregnancy.[14] Meanwhile, studies among nonpregnant populations have demonstrated variations in metabolomic profiles associated with dietary patterns[15,16] that may also hold importance in prenatal populations as raised maternal pBMI is associated with energy-dense, nutrient-poor diets in pregnancy.[17] We recently published the first study to longitudinally assess changes in maternal metabolomic profiles across a cohort of healthy pregnant women.[18] The objective of the present study was to advance this analysis by examining the nature and magnitude of the association between pBMI and gestational weight gain (GWG) and the maternal metabolomics profile across trimesters that is not accounted for by other potential determinants, for example, dietary quality (Alternate Healthy Eating Index adapted for pregnancy (AHEI-P)) and quantity (total energy intake), homeostatic model assessment of insulin resistance (HOMA-IR), maternal age and ethnicity. In addition, for metabolites demonstrating significance on multivariate analysis, we further investigated their associations with specific nutrient intakes considered to be important.

MATERIALS AND METHODS

This study is a secondary analysis of 167 nondiabetic women, recruited in their first trimester of pregnancy to a longitudinal, prospective birth cohort study at the University of California, Irvine, Development, Health and Disease Research Program. The study was approved by the University of California, Irvine Institutional Review Board and written, informed consent was obtained. Details of the inclusion criteria, follow-up visits in each trimester, metabolomics analysis of fasting plasma samples and handling/summarizing of metabolomics data have been previously described in detail.[18] The primary aim of the study was to look at associations between maternal–placental–fetal stress biology and infant adiposity, for which the study was powered. The Supplementary Materials and methods file provides a detailed description of the study conduct methodology for the current paper.

Statistical analysis

Statistical analyses were performed using IBM SPSS for Windows, version 22 (Chicago, IL, USA). Associations between trimester-specific GWG, trimester-specific dietary quality (AHEI-P) and quantity (total energy intake) as dependent variables and pBMI as the independent variable were assessed with linear models, adjusted for maternal race/ethnicity and age. Normality distributions of metabolomics data were explored through visual inspection of histograms and nonnormally distributed variables were log-transformed. Each subject's metabolite value and metabolic ratio indicator within each trimester was converted to a *z*-score. The sums of *z*-scores were computed for groups of related metabolites either according to dietary 'essentiality' (indispensable amino acids (AAs): leucine, isoleucine, valine, methionine, phenylalanine, tryptophan and threonine; or dispensable AAs: alanine, arginine, asparagine, aspartic acid, glutamine, glutamic acid, glycine, citrulline, ornithine, proline, serine, tyrosine (Tyr) and cysteine), chain length (short, medium and long-chain acylcarnitines (Carn)), or degree of saturation (saturated fatty acids, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) for NEFA, lyso-phosphatidylcholines (LPC), diacyl-linked phosphatidylcholines (PC.aa), acyl-alkyl-linked phosphatidylcholines (PC.ae) and sphingomyelines (SM.a)).

The associations between the continuous variables maternal pBMI and trimester-specific GWG with metabolite *z*-scores as the dependent variables within the same trimester were first assessed by a multivariate linear regression model, adjusting for AHEI-P, total energy intake, maternal age and ethnicity (Supplementary Table 1). A second model was used including the interaction term of GWG and BMI (Supplementary Table 2), but as no associations between the interaction effect and *z*-score metabolites was found, we focused our analysis on the first model. We additionally performed univariate analyses to depict the influence of pBMI on metabolites without adjusting for confounding variables, but results were very similar to the multivariate model (Supplementary Table 1). Finally, the potential for insulin resistance to mediate any observed significant associations of pBMI with metabolites was evaluated through a separate regression model in which trimester-specific HOMA-IR, pBMI and the interaction effect of pBMI and HOMA-IR were included as independent variables, whereas GWG and the dietary variables were not used (Supplementary Table 3). This separate regression model was required as we were limited to a maximum of six predictors in a regression by the sample number. To asses HOMA-IR associations with metabolite levels, in each trimester a linear regression model with the metabolites as dependent and HOMA-IR as independent variables was calculated, with adjustment for maternal age and ethnicity (Supplementary Table 4).

Trimester-specific metabolites were further analyzed for their associations with sex- and gestational age-specific birth weight percentiles[19] adjusting for ethnicity (Supplementary Table 5).

To address the issue of multiple comparisons, a Bonferroni correction was applied for the testing of 254 metabolites, sums and ratios at 3 different time points (corrected significance level: $P < 0.000197$). Significant results were also visualized using Manhattan plots, where the $\log_{10}(P)$ values (y axis) are plotted for each metabolite (x axis) and the sign is used to indicate the direction of the relationship, created using R statistical software, version 3.0.2 (Vienna, Austria) or Microsoft Excel 2010, version 14.0.7151.5001 (Redmond, WA, USA). Individual lipid metabolites found to be significantly associated with pBMI or GWG were further investigated for their association with specific nutrient intakes of interest in a linear model, adjusted for pBMI, GWG, ethnicity and age (Supplementary Table 6).

Finally, principal component analysis of all metabolites was performed with R statistical software, version 3.0.1. The received principle components were considered dependent variables in a linear regression model to examine the association with pBMI, adjusted for trimester-specific GWG, total energy intake, AHEI-P score, maternal age and maternal ethnicity as well as HOMA-IR and birth weight percentile.

RESULTS

Maternal characteristics of the study population are presented in Table 1. All women delivered healthy term babies; the mean ± s.d. gestational age at delivery was 39.4 ± 1.4 weeks, and mean birth weight at delivery was 3.36 kg. Of the women, 42% of were classified as overweight or obese and mean pBMI was similar between Hispanic and non-Hispanic women (26.4 vs 25.4 kg m^{-2} respectively, $P = 0.302$). Diet quality (AHEI-P score) showed a small nonsignificant increase with advancing gestation, but there with large variation among the cohort (Table 1). Trimester-specific GWG and total GWG were strongly negatively associated with pBMI ($P < 0.001$), whereas HOMA-IR was strongly positively associated with pBMI in each trimester ($P < 0.001$ in trimesters 1 and 2, $P = 0.004$ in trimester 3). Prepregnancy BMI was not associated with total energy intake ($P = 0.291$, 0.053, 0.057), but inversely related to AHEI-P ($P = 0.013$, < 0.001, 0.010) in trimester 1, 2 and 3, respectively. Maternal age and ethnicity had no influence on total energy intake and AHEI-P.

Metabolomic analysis

A total of 254 metabolites were quantified. Within the multivariate model, the separate effects of each independent variable associated with individual metabolites at each time point are presented in Supplementary Table 1. As markers of overall dietary intake, neither dietary quantity (energy intake) nor quality (AHEI-P) were independently associated with any metabolite (Figure 1). Similarly, GWG exerted minimal influence on the metabolome either alone (Figure 1 and Supplementary Table 1) or when considering its interaction with pBMI (Supplementary Table 2). However, pBMI demonstrated several strong significant and independent associations in both the univariate and multivariate models (Figure 1). A total of 40 significant associations were found between pBMI with metabolites across all trimesters, whereas only a few significant associations were found with GWG (3), age (2) and ethnicity (4), and none with AHEI-P and total energy intake.

Table 1. Population demographics, anthropometry and dietary intakes ($N = 160$)

		Mean (s.d.)	
Prepregnancy weight (kg)		68.8 (16.8)	
Height (cm)		163.1 (6.8)	
Prepregnancy BMI (kg m^{-2})		25.9 (6.0)	
Age at recruitment (years)		27.7 (5.4)	
GWG from prepregnancy to trimester 1 (kg)		1.8 (2.9)	
GWG from prepregnancy to trimester 2 (kg)		5.0 (3.9)	
GWG from prepregnancy to trimester 3 (kg)		10.2 (5.3)	
Total GWG from prepregnancy until delivery (kg)		14.4 (6.7)	
	Trimester 1	Trimester 2	Trimester 3
Dietary intakes			
Total energy (kcal)	1762.4 (372.2)	1810.7 (412.7)	1793.5 (420.7)
AHEI-P score	54.9 (11.3)	56.2 (10.7)	56.6 (11.3)
Insulin resistance			
HOMA-IR	2.89 (2.08)	2.63 (1.42)	3.67 (2.73)
		N (%)	
BMI category			
Underweight (< 18.5 kg m^{-2})		6 (3.7)	
Normal weight (18.5–24.9 kg m^{-2})		83 (51.6)	
Overweight (25.0–29.9 kg m^{-2})		37 (23.0)	
Obese (30.0–39.9 kg m^{-2})		27 (16.8)	
Morbidly obese (>40 kg m^{-2})		4 (2.5)	
Hispanic ethnicity		68 (42.2)	
White Hispanic		51 (75.0)	
Asian Hispanic		2 (2.9)	
Other Hispanic		12 (17.6)	
Multi-race Hispanic		3 (4.4)	
Non-Hispanic ethnicity		92 (57.1)	
White non-Hispanic		68 (73.9)	
Black non-Hispanic		4 (4.3)	
Asian non-Hispanic		12 (13.0)	
Multi-race non-Hispanic		3 (3.3)	

Abbreviations: AHEI-P, Adaptive Healthy Eating Index for Pregnancy; BMI, body mass index; GWG, gestational weight gain; HOMA-IR, homeostatic model assessment of insulin resistance.

Association of pBMI and GWG with metabolites

The majority of NEFA metabolites in trimesters 1 and 2 were significantly positively associated with pBMI, as well as the stearoyl-CoA desaturase-1 (SCD) enzyme activity ratios (Figure 2 and Supplementary Table 1). However, the omega-3 long-chain LC-PUFA C20:5 (eicosapentanoic acid) and C22:6 (docosahexanoic acid) were not significantly associated with pBMI in any trimester. In trimester 3, after Bonferroni correction is applied, the associations of the omega-6 long-chain PUFA C20:3 (dihomo-γ-linolenic acid), C20:4 (arachidonic acid) and C22:4 (adrenic acid), and the ratio of C16:1 to C16:0 were still significant. The only AAs significantly associated with pBMI were asparagine (negatively associated in trimester 3) and glutamic acid (positively associated in trimester 2) (Table 2). The branched-chain AAs (leucine, isoleucine, valine) and the aromatic AAs (phenylalanine, Tyr) showed a positive trend, but no significant associations to pBMI in trimester 1. None of the acylcarnitines or acylcarnitine ratios showed associations with pBMI after Bonferroni correction (Supplementary Table 1), but β-hydroxybutyric acid was positively associated with pBMI in trimester 3. Among the phospholipid subgroups, the SM.a class demonstrated a strong positive association with pBMI in trimester 1 only (Table 2), particularly among SM.a containing two double bonds, most likely containing 18:1 and an additional MUFA species, and those with a 36-carbon chain length. However, these associations disappeared by the second trimester. Among phosphatidylcholines, a few species showed a positive association with pBMI in the first trimester: PC.aa.C30.3, PC.aa.C32.3 and PC.aa.C38.3. In trimester 3, PC.aa.C42.6, PC.ae.C40.0, PC.ae.C42.0 and asparagine were the only metabolites negatively associated with pBMI. The only significant positive influence of trimester-specific GWG on metabolites was observed for α-ketoglutaric acid (α-KG) in trimesters 1 and 3, as well as SM.a.C30.1 in trimester 1 after Bonferroni correction (Table 2 and Supplementary Table 1). In trimester 2, α-KG acid showed the same tendency, but did not reach the corrected significance level. All metabolites, which were significantly associated with pBMI, were also investigated in a separate regression model including an interaction effect of HOMA and pBMI, but no significant associations were found (Supplementary Table 3). Associations between HOMA-IR with metabolites were also weak. In the first trimester, Tyr, PC.aa.C30.0, PC.aa.C32.1 and SM.a.C36.1 were positively associated with HOMA-IR, whereas glutamic acid and α-KG were positively associated in the second trimester (Supplementary Table 4). In the last trimester, no associations were found between any metabolite and HOMA-IR.

Principle component analysis

The first 10 principle components explained 75.1%, 75.0% and 74.6% of the variation of the metabolites in trimester 1, 2 and 3, respectively. Among these, principle component 2 was most strongly associated with pBMI in trimesters 1 and 2 (Table 3) and

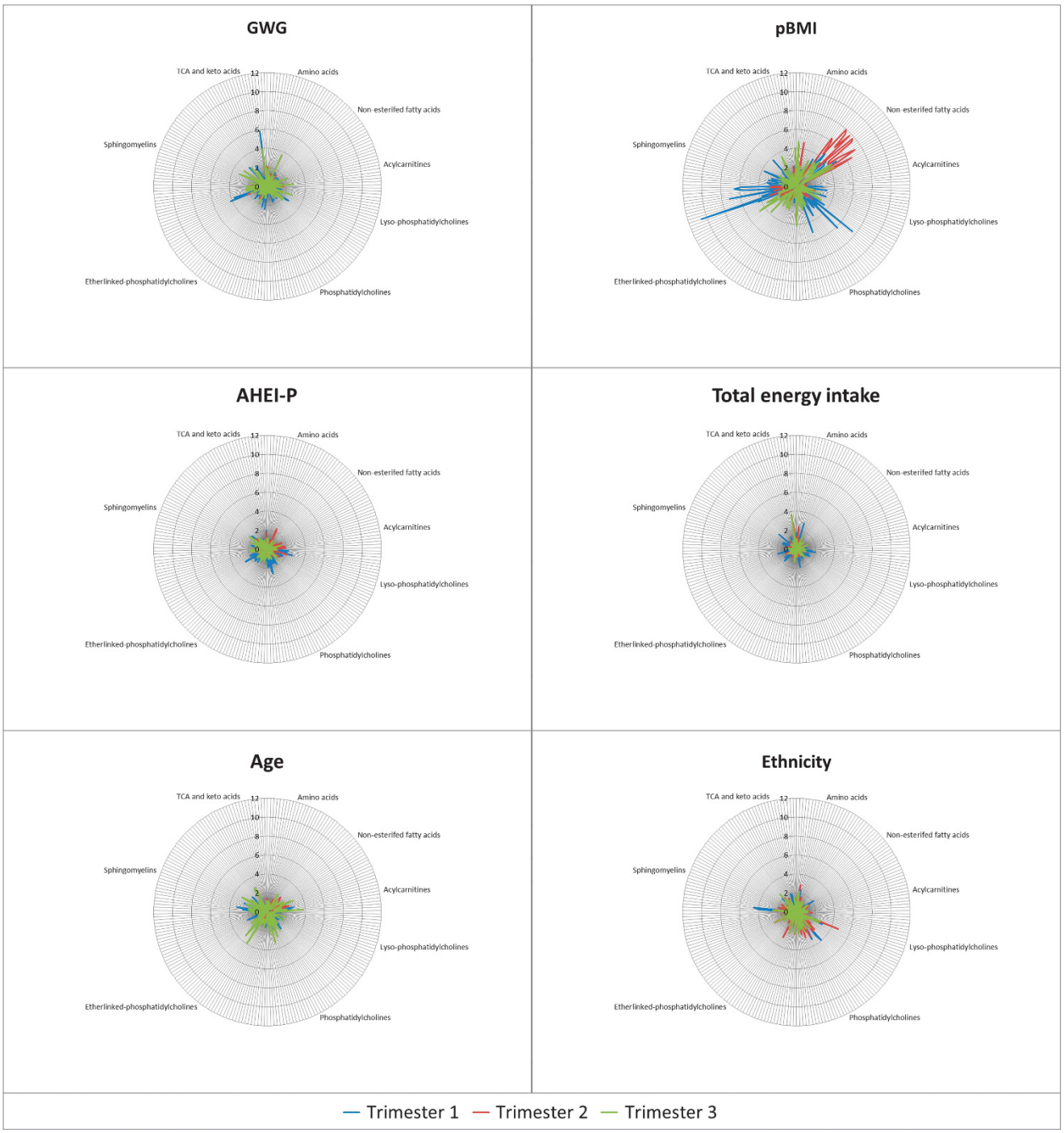

Figure 1. Associations of GWG, pBMI, AHEI-P, total energy intake, maternal age and maternal ethnicity to all metabolites at each trimester. Negative log-transformed *P*-values are plotted for each metabolite arranged by metabolite groups. Higher values represented in the outer circles present a higher association between metabolite and predictor. *P*-values were calculated by linear regression models with pBMI, trimester-specific gestational weight gain, total energy intake, AHEI-P, maternal age and maternal ethnicity as independent variables. Bonferroni corrected *P*-value was 0.000197 (− log10(*P*-value) = 3.71).

was primarily weighted by NEFA in both trimesters (Supplementary Table 7), particularly saturated, monounsaturated and n-6 NEFA. Principle component 6, mainly composed of amino acids, was associated with HOMA-IR in the first trimester ($P = 7.92E-05$).

Association of metabolites with birth weight percentile

Several metabolites showed significant associations with birth weight percentile before correction for multiple testing (Table 4 and Supplementary Table 5). Specifically, NEFA in trimester 1, and to a lesser extent in trimester 2, were positively associated, as was principle component 2 in trimester 2. Meanwhile, trimester 3 LPC species with 18 carbon atoms showed a negative association to birth weight percentile (LPC.a.C18.0, LPC.a.C18.1, LPC.a.C18.2, LPC.a.C18.3, LPC.e.C18.0 and LPC.e.C18.1). However, none of these associations remained statistically significant after Bonferroni correction.

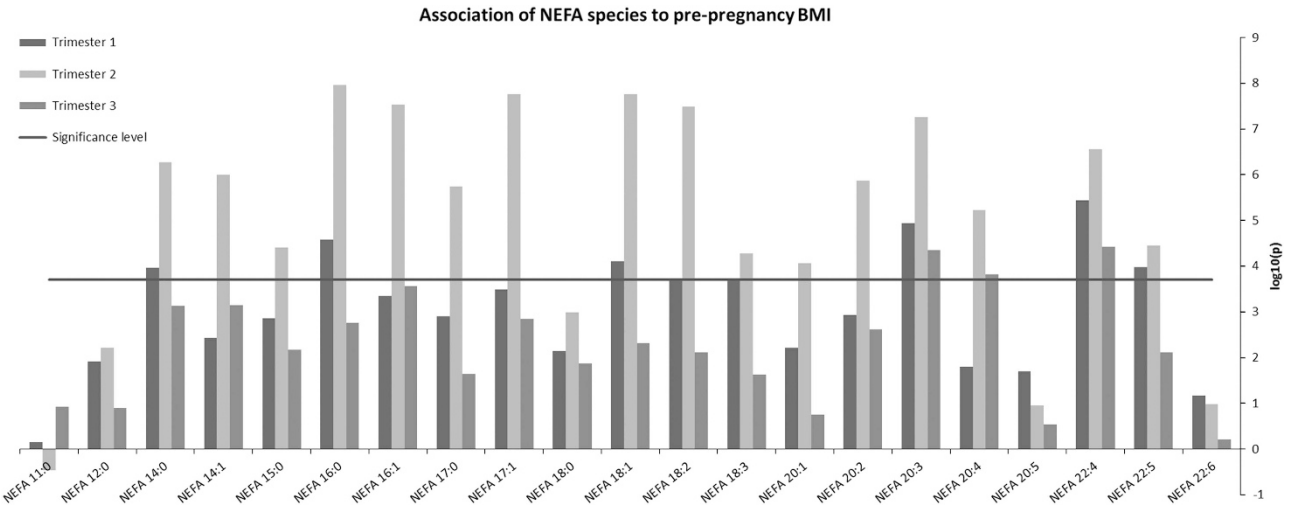

Figure 2. Associations of pBMI to NEFA species at each trimester. Negative log-transformed *P*-values are plotted for each NEFA species. *P*-values were calculated by linear regression models with pBMI as independent variable adjusted for trimester-specific gestational weight gain, total energy intake, AHEI-P, maternal age and maternal ethnicity. Straight line, Bonferroni corrected *P*-value was 0.000197 ($-\log_{10}$(*P*-value) = 3.71).

Dietary analysis

Single lipid metabolites significantly associated with pBMI were also related to specific dietary fat intakes (Supplementary Table 6). None of the associations were significant after correction for multiple testing. Only NEFA 20:4 (trimester 1 and 2) and 20:5 (trimester 2) were negatively associated with total fat intake without Bonferroni correction.

DISCUSSION

We present the first study depicting the longitudinal influence of pBMI on the maternal metabolome across gestation. Entering pregnancy with an elevated BMI can significantly impact pregnancy complications[20] and offspring development including adverse cardiometabolic profile, increased birth weight and greater adiposity,[21,22] as well as mental health outcomes.[23] Various potential mechanisms including epigenetic changes, alterations in the reward system, central control of food choice and intake, changes in hormonal levels such as leptin and ghrelin or placental adaptations for transfer of nutrients to the developing fetus are involved in these processes.[24] Although these concepts of 'fetal programming' of offspring disease risk are subject to ongoing investigation, significant further characterization of the underlying mechanisms is required in order to identify possible targets for intervention strategies during pregnancy that may successfully interrupt the intergenerational cycles of obesity.[5]

Our findings reveal distinct and independent associations between maternal pBMI and various NEFA and phospholipid species, although only limited associations with AAs were detected. Although pBMI was our primary predictor of interest, we also sought to investigate the potential for GWG, HOMA-IR and dietary intake throughout gestation to exert an independent and/or combined effect on metabolomic profiles alongside pBMI. Interestingly, our results reveal minimal influence of HOMA-IR and GWG on any of the analyzed metabolites. Only SM 30.1 and α-KG were significantly associated with GWG. To support tissue synthesis associated with fetal growth, maternal AAs are generally spared from degradation during pregnancy. Decreased AA oxidation and transamination may explain the observed elevation in α-KG that would otherwise be metabolized to glutamate in transamination processes.

Despite recent studies in nonpregnant populations reporting altered metabolomics profiles associated with specific dietary intake patterns,[15,16] total energy intake and AHEI-P, a validated measure of dietary quality in pregnancy, had no impact and did not alter the significant associations of pBMI with the metabolome. Furthermore, none of the dietary parameters were related to any metabolite and additional analyses, relating specific dietary intake of fat or fat components to lipid metabolites also showed no significant association. Thus, these results support the notion that the maternal metabolome is predominantly influenced by obesity and less by dietary intake during pregnancy or by GWG. Although it is possible that longer-term prepregnancy dietary habits influence the maternal metabolome during gestation, this has yet to be investigated. Furthermore, we note that metabolites that were observed to significantly change between the trimesters, including branched-chain amino acids (BCAA), threonine, n-3 NEFA and acylcarnitines,[18] were not related to any determinant studied in this cohort. Thus, we conclude that normal physiological changes in metabolism occurring during pregnancy, such as placental metabolite transfer or ketone body synthesis, have a stronger influence on the studied metabolome and its alterations compared with genetic (ethnicity), environmental (diet) or biophysical/metabolic (GWG, pBMI, HOMA-IR) factors. In general, both approaches, change in pregnancy and influence of exposure, have to be considered separately and changes in metabolites during pregnancy could not be related to exposures.

Among all analyzed metabolites, the NEFA species showed the strongest positive associations with pBMI, demonstrated in both univariate modeling and principal component analysis. A relation between the total concentration of NEFA in the maternal circulation during pregnancy and occurrence of gestational diabetes mellitus has been previously described.[25] In general, women with higher pBMI exhibit larger fat depots before pregnancy in the adipose tissue, the major source of NEFA.[26] Hence, the normal physiological accumulation of fat in the first two trimesters[7] may be spared in obese women through less GWG compared with nonobese pregnant women.[20] Unchanged or potentially augmented insulin sensitivity in the first half of healthy pregnancy promotes an anabolic state, with enhanced lipogenesis in adipose tissue,[27] as the insulin-inhibiting effect on the hormone sensitive lipoprotein lipase is increased.[28] However, it appears that entering pregnancy in the obese state disturbs this normal anabolic activity through early-gestational insulin resistance.[25]

Table 2. Significant associations of pBMI and GWG with metabolites

Metabolite	*GWG*		*pBMI*		*AHEI-P*		*Total energy*		*Age*		*Ethnicity*		*Adjusted* R^2	*Overall P-value*
	β	P-*value*	*β*	P-*value*	*β*	P-*value*	*β*	P-*value*	*β*	P-*value*	*β*	P-*value*		
Trimester 1														
NEFA 14:0	−0.027	3.86E−01	0.064	**1.09E−04**	−0.006	5.14E−01	0.000	7.60E−01	0.022	1.69E−01	−0.067	7.09E−01	0.156	**1.53E−04**
NEFA 16:0	−0.050	8.97E−02	0.065	**2.67E−05**	−0.005	5.00E−01	0.000	9.24E−01	0.036	1.85E−02	−0.292	8.30E−02	0.252	**2.21E−07**
NEFA 18:1	−0.061	4.53E−02	0.062	**7.83E−05**	−0.001	8.78E−01	0.000	7.56E−01	0.030	5.64E−02	−0.210	2.25E−01	0.213	**3.55E−06**
NEFA 18:2	−0.061	4.41E−02	0.059	**1.90E−04**	0.002	8.31E−01	0.000	7.49E−01	0.019	2.25E−01	−0.303	8.33E−02	0.194	**1.35E−05**
NEFA 20:3	−0.015	6.15E−01	0.071	**1.15E−05**	0.004	6.46E−01	0.000	7.78E−01	0.018	2.47E−01	−0.311	7.58E−02	0.189	**1.87E−05**
NEFA 22:4	−0.048	1.13E−01	0.074	**3.63E−06**	0.004	6.54E−01	0.000	9.88E−01	0.011	4.83E−01	−0.145	4.03E−01	0.211	**4.09E−06**
NEFA 22:5	−0.047	1.38E−01	0.064	**1.05E−04**	0.009	3.13E−01	0.000	6.17E−01	0.028	9.02E−02	0.044	8.08E−01	0.154	**1.80E−04**
PC.aa.C30.3	0.021	4.73E−01	0.090	**2.56E−08**	0.016	5.25E−02	0.000	6.04E−02	0.015	3.43E−01	−0.302	7.83E−02	0.255	**1.70E−07**
PC.aa.C32.3	0.024	4.28E−01	0.081	**6.73E−07**	0.009	2.69E−01	0.000	2.65E−01	0.017	2.87E−01	−0.177	3.13E−01	0.191	**1.64E−05**
PC.aa.C38.3	0.073	1.75E−02	0.073	**7.31E−06**	0.009	2.86E−01	0.000	9.70E−01	0.023	1.54E−01	−0.408	2.16E−02	0.201	**8.44E−06**
SM.a.C30.1	0.119	**6.45E−05**	0.062	**4.74E−05**	0.020	1.21E−02	0.000	4.94E−01	0.042	5.84E−03	−0.019	9.10E−01	0.281	**2.32E−08**
SM.a.C32.1	0.101	1.16E−03	0.062	**1.11E−04**	0.012	1.57E−01	0.000	4.51E−01	0.035	2.70E−02	−0.124	4.81E−01	0.193	**1.41E−05**
SM.a.C32.2	0.105	2.01E−04	0.103	**2.48E−11**	0.019	1.40E−02	0.000	2.79E−01	0.024	9.89E−02	−0.125	4.30E−01	0.354	**5.44E−11**
SM.a.C33.2	0.072	3.94E−02	0.071	**1.70E−04**	0.018	7.47E−02	0.000	8.12E−01	0.013	4.88E−01	−0.253	2.22E−01	0.149	1.18E−03
SM.a.C34.2	0.034	2.57E−01	0.087	**6.78E−08**	0.014	8.12E−02	−0.001	3.60E−02	0.020	1.83E−01	−0.332	5.33E−02	0.255	**1.73E−07**
SM.a.C36.1	−0.014	6.47E−01	0.077	**1.95E−06**	0.005	5.49E−01	0.000	3.90E−01	0.018	2.46E−01	−0.268	1.25E−01	0.210	**4.50E−06**
SM.a.C36.2	−0.017	5.71E−01	0.082	**2.88E−07**	0.012	1.48E−01	0.000	9.81E−02	0.014	3.66E−01	−0.235	1.72E−01	0.231	**1.02E−06**
SM.a.C36.3	−0.010	7.47E−01	0.078	**1.37E−06**	0.008	3.66E−01	0.000	2.49E−01	0.023	1.44E−01	−0.254	1.45E−01	0.214	**3.37E−06**
SM.a.C42.3	0.005	8.66E−01	0.063	**1.83E−04**	0.013	1.33E−01	0.000	1.30E−01	0.024	1.53E−01	−0.327	7.60E−02	0.140	4.12E−04
Alpha-Ketoglutaric acid	0.079	**1.30E−06**	0.019	2.28E−01	−0.087	6.22E−01	0.087	4.85E−03	−0.005	5.49E−01	0.000	2.38E−01	0.212	**4.35E−06**
Trimester 2														
Glutamic acid	0.009	6.90E−01	0.067	**2.20E−05**	−0.006	4.87E−01	0.000	6.08E−01	0.021	1.58E−01	−0.145	3.84E−01	0.132	2.83E−04
NEFA 14:0	0.035	1.13E−01	0.080	**5.38E−07**	0.007	4.08E−01	0.000	7.86E−01	0.020	1.80E−01	0.060	7.16E−01	0.162	**4.06E−05**
NEFA 14:1	0.046	3.32E−02	0.076	**1.00E−06**	0.002	8.20E−01	0.000	5.66E−01	0.021	1.42E−01	−0.146	3.68E−01	0.161	**4.37E−05**
NEFA 15:0	0.024	4.10E−01	0.066	**3.96E−05**	0.000	9.73E−01	0.000	8.94E−01	0.032	3.99E−02	−0.077	6.46E−01	0.147	2.55E−04
NEFA 16:0	−0.004	8.39E−01	0.084	**1.08E−08**	−0.003	6.44E−01	0.000	9.55E−01	0.036	8.36E−03	−0.147	3.31E−01	0.271	**6.67E−09**
NEFA 16:1	0.020	3.23E−01	0.084	**2.93E−08**	−0.002	8.07E−01	0.000	1.23E−01	0.031	2.67E−02	−0.237	1.27E−01	0.232	**1.99E−07**
NEFA 17:0	0.009	6.77E−01	0.074	**1.80E−06**	−0.006	4.20E−01	0.000	4.09E−01	0.030	4.08E−02	−0.065	6.92E−01	0.176	**1.29E−05**
NEFA 17:1	0.011	5.77E−01	0.084	**1.73E−08**	−0.005	4.93E−01	0.000	2.67E−01	0.034	1.54E−02	−0.225	1.42E−01	0.250	**3.94E−08**
NEFA 18:1	−0.016	4.10E−01	0.082	**1.71E−08**	−0.007	3.27E−01	0.000	3.88E−01	0.029	3.41E−02	−0.014	9.24E−01	0.263	**1.33E−08**
NEFA 18:2	−0.019	3.31E−01	0.081	**3.23E−08**	−0.004	5.61E−01	0.000	7.01E−01	0.020	1.41E−01	−0.168	2.64E−01	0.253	**3.19E−08**
NEFA 18:3	0.008	7.02E−01	0.063	**5.23E−05**	−0.003	7.12E−01	0.000	9.49E−01	0.016	2.77E−01	−0.039	8.14E−01	0.105	1.82E−03
NEFA 20:1	−0.006	7.88E−01	0.061	**8.71E−05**	−0.011	1.77E−01	0.000	2.26E−01	0.030	4.69E−02	−0.098	5.50E−01	0.143	**1.43E−04**
NEFA 20:2	−0.004	8.41E−01	0.074	**1.35E−06**	−0.004	6.25E−01	0.000	2.64E−01	0.017	2.47E−01	−0.303	5.79E−02	0.194	**3.59E−06**
NEFA 20:3	−0.004	8.52E−01	0.083	**5.52E−08**	0.005	4.90E−01	0.000	9.21E−01	0.021	1.44E−01	−0.178	2.61E−01	0.216	**6.33E−07**
NEFA 20:4	0.005	8.22E−01	0.072	**5.90E−06**	0.011	1.59E−01	0.000	6.15E−01	0.022	1.43E−01	0.186	2.61E−01	0.148	**1.10E−04**
NEFA 22:4	0.006	7.65E−01	0.087	**2.80E−07**	0.008	3.34E−01	0.000	8.25E−01	0.015	3.03E−01	−0.055	7.35E−01	0.172	**2.04E−05**
NEFA 22:5	0.006	7.78E−01	0.065	**3.59E−05**	0.013	1.16E−01	0.000	4.40E−01	0.032	3.32E−02	0.094	5.72E−01	0.143	**1.44E−04**
Saturated NEFA	0.129	2.73E−01	0.371	**1.31E−05**	−0.002	9.72E−01	0.000	7.65E−01	0.157	5.06E−02	−0.200	8.23E−01	0.141	**1.72E−04**
Monounsaturated NEFA	0.078	4.14E−01	0.373	**1.17E−07**	−0.018	6.07E−01	0.001	2.53E−01	0.146	2.61E−02	−0.839	2.47E−01	0.213	**9.13E−07**
Polyunsaturated NEFA	0.087	5.77E−01	0.504	**3.24E−05**	0.077	1.91E−01	−0.001	5.98E−01	0.210	4.95E−02	−0.635	5.98E−01	0.140	2.96E−04
Ratio 18:1/18:0	−0.028	1.48E−01	0.073	**3.45E−07**	0.009	1.97E−01	0.001	3.84E−03	0.025	5.91E−02	0.012	9.33E−01	0.256	**2.37E−08**
Ratio 16:1/16:0	0.024	2.65E−01	0.074	**1.49E−06**	−0.006	4.71E−01	0.001	3.29E−03	0.014	3.36E−01	−0.026	8.70E−01	0.170	**2.20E−05**

Table 2. (Continued)

Metabolite	*GWG*		*pBMI*		*AHEI-P*		*Total energy*		*Age*		*Ethnicity*		*Adjusted* R^2	*Overall P-value*
	β	P-*value*	*β*	P-*value*	*β*	P-*value*	*β*	P-*value*	*β*	P-*value*	*β*	P-*value*		
Trimester 3														
Asparagine	0.002	9.09E−01	−0.063	**1.87E−05**	0.013	7.94E−02	0.000	8.58E−02	−0.006	6.77E−01	0.411	1.34E−02	0.200	**2.91E−06**
NEFA 20:3	0.009	5.50E−01	0.059	**4.52E−05**	0.001	8.64E−01	0.000	6.00E−01	0.038	1.12E−02	−0.047	7.72E−01	0.145	**1.46E−04**
NEFA 20:4	−0.006	7.31E−01	0.056	**1.50E−04**	−0.004	5.70E−01	0.000	8.90E−01	0.037	1.60E−02	0.341	4.18E−02	0.178	**1.53E−05**
NEFA 22:4	−0.009	5.76E−01	0.066	**3.71E−05**	−0.005	5.47E−01	0.000	6.59E−01	0.034	2.76E−02	0.197	2.32E−01	0.184	**1.07E−05**
Ratio 16:1/16:0	0.000	9.82E−01	0.062	**4.85E−05**	−0.004	5.95E−01	0.000	5.24E−01	0.010	5.41E−01	0.219	2.00E−01	0.122	7.25E−04
PC.aa.C42.6	0.000	9.88E−01	−0.062	**6.42E−05**	−0.004	6.06E−01	0.000	9.39E−01	0.022	1.80E−01	−0.004	9.84E−01	0.103	2.25E−03
PC.ae.C40.0	−0.021	1.97E−1	−0.057	**1.55E−04**	−0.009	2.57E−01	0.000	7.19E−01	0.049	2.08E−03	0.280	1.00E−01	0.157	**6.33E−05**
PC.ae.C42.0	−0.022	2.46E−01	−0.077	**2.42E−05**	−0.004	6.29E−01	0.000	2.81E−01	0.039	1.79E−02	0.194	3.03E−01	0.196	**5.37E−05**
Alpha-Ketoglutaric acid	0.057	**9.23E−05**	0.018	2.43E−01	−0.013	9.38E−01	0.062	2.36E−04	−0.002	7.44E−01	0.000	2.79E−02	0.147	2.03E−04
Beta-Hydroxybutyric acid	0.039	1.05E−02	0.063	**8.52E−05**	−0.026	8.80E−01	−0.029	8.10E−02	−0.003	6.63E−01	0.000	3.42E−01	0.173	**4.14E−05**

Abbreviations: AHEI-P, Adaptive Healthy Eating Index for Pregnancy; GWG, gestational weight gain; NEFA, nonesterified fatty acids; pBMI, prepregnancy body mass index; PC.aa, phosphatidylcholines; PC.ae, ether-linked phosphatidylcholines; SM.a, sphingomyelins. β-Coefficients (β) and *P*-values for pBMI and GWG were calculated by linear regression models with pBMI and GWG as independent variables adjusted for total energy intake, Alternate Healthy Eating Index adapted for pregnancy (AHEI-P), maternal age and maternal ethnicity. Overall *P*-value for the model was calculated with analysis of variance (ANOVA). Bold values indicate *P*-value < 0.000197.

Despite this, our analysis of the pBMI–HOMA-IR interaction with the metabolome did not reveal significant associations beyond those already identified with pBMI alone. Furthermore, HOMA-IR was not associated with any NEFA in pBMI-independent models. This may suggest that various obesity-induced metabolic and hormone fluctuations, rather than insulin resistance alone, may contribute to the normal enhanced lipolysis in late gestation. Furthermore, the effect of pBMI on NEFA disappears in the third trimester, when fat mobilization is known to occur to support the period of accelerated fetal growth.[27] We have recently reported that plasma NEFA concentrations do not significantly change across trimesters despite the late-gestation expected increase in lipolysis[18] that may be attributed to increased rates of fasting-induced ketogenesis or transfer to the fetus. Thus, it is possible that similar rates of lipolysis and/or NEFA utilization occur in late gestation among all women regardless of pBMI. We found β-hydroxybutyric acid to be elevated with higher pBMI in trimester 3, indicating a higher rate of fasting-induced ketogenesis in obese women, perhaps because of elevated NEFA supply following late-pregnancy induced lipolysis. In general, maternal lipids are associated with excessive fetal growth independent of gestational diabetes mellitus status, and this may explain the stronger influence of pBMI on offspring growth compared with maternal hyperglycemia.[4] Nevertheless, elevated NEFA levels have been found to be strong predictors of elevated birth weight, overweight and increased body fat in the infant.[29,30] In line with this published evidence, in the current study we found NEFA species in the first trimester and the principle component representing NEFA in the second trimester to be associated with infant birth weight. Given that these NEFA are also strongly influenced by the preconceptional obesity state, these metabolites represent a potential metabolic pathway for the programming of offspring adiposity in obese pregnancy. Thus, these findings strongly indicate the need for preconception women's health interventions, particularly among those overweight and obese, rather than initiating interventions during pregnancy.

The present study significantly adds to the current literature by also investigating single NEFA species related to pBMI. In the second trimester, pBMI influenced the monounsaturated NEFA 14:1, 16:1, 17:1 and 18:1, as well as those dominated by the omega-6 (n-6) isomer: 20:3, 20:4 and 22:4. The n-6 NEFA were the only NEFA that remained positively associated to pBMI in trimester 3, whereas there was minimal association of n-3 NEFA to pBMI across all trimesters. These results suggest that the fetuses of obese women are exposed to higher ratio of n-6/n-3 FA that has been implicated to influence BMI during the first 10 years of life.[31] The n-6 arachidonic acid (20:4) is the main precursor of eicosanoids enhancing the differentiation of adipose precursor cells into adipocytes that is particularly related to linoleic acid intake.[32] In a study of rats, linoleic acid intake over four generations increased adipose tissue mass compared with a control diet, although caloric intake was the same.[33] This NEFA was among the strongest related to pBMI in the second trimester in the present results. Moon *et al.*[34] showed that maternal n-6 status in late pregnancy was related to greater fat mass in the offspring at 4 and 6 years of age. Furthermore, excessive n-6 FA intake and insufficient n-3 intake has been reported as the most important risk factor associated with fetal programming.[35] Thus, there is a convincing body of evidence emerging to suggest that maternal n-6 NEFA or n-6 FA in the adipose tissue represent metabolomic biomarkers for transgenerational transfer of obesity.

We additionally identified that the ratios of NEFA 16:1 to 16:0 and 18:1 to 18:0 were significantly related to pBMI. This indicates upregulation of the SCD-1 enzyme that metabolizes saturated fatty acids to monounsaturated fatty acids, and is also reflected in the SM species. Elevated SCD-1 activity has previously been associated with obesity,[36] possibly because of a switch in fat metabolism from the catabolic to the anabolic state.[37] The higher

Table 3. PCA-derived factors and association with pBMI and GWG

Trimester	*Principle component*	*Proportion of variance*	*Cumulative variance*	*GWG*		*pBMI*		*AHEI-P*		*Total energy*		*Age*		*Ethnicity*		*Adj* R^2	P-*value*
				β	P-*value*	*β*	P-*value*	*β*	P-*value*	*β*	P-*value*	*β*	P-*value*	*β*	P-*value*		
Trimester 1	1	34.5%	34.5%	0.559	6.17E−02	0.395	1.27E−02	0.170	6.03E−02	−0.002	4.69E−01	0.398	1.88E−02	−2.641	1.40E−01	0.160	1.61E−03
	2	11.1%	45.6%	0.183	2.25E−01	−0.347	**2.87E−05**	0.041	3.65E−01	0.000	7.10E−01	−0.102	2.30E−01	2.102	2.18E−02	0.334	**2.20E−07**
	3	6.2%	51.7%	−0.030	8.35E−01	−0.071	3.39E−01	−0.023	5.85E−01	0.000	7.65E−01	−0.045	5.71E−01	0.314	7.12E−01	−0.049	9.41E−01
	4	5.1%	56.8%	−0.066	5.98E−01	−0.093	1.57E−01	−0.050	1.82E−01	0.000	8.51E−01	0.041	5.58E−01	−0.435	5.60E−01	−0.013	5.66E−01
	5	4.5%	61.3%	−0.031	7.65E−01	−0.099	7.30E−02	0.048	1.29E−01	−0.001	1.37E−01	0.011	8.53E−01	2.343	3.04E−04	0.225	**8.09E−05**
	6	3.8%	65.1%	−0.102	3.32E−01	−0.029	5.97E−01	0.027	3.89E−01	−0.002	1.33E−02	−0.075	2.07E−01	0.195	7.57E−01	0.055	9.24E−02
	7	3.0%	68.1%	0.109	2.49E−01	0.111	2.69E−02	−0.003	9.15E−01	0.001	3.80E−01	−0.084	1.13E−01	0.980	8.51E−02	0.046	1.20E−01
	8	2.6%	70.7%	−0.108	2.12E−01	0.086	6.01E−02	−0.011	6.64E−01	−0.001	2.63E−01	0.062	2.02E−01	0.647	2.14E−01	0.079	4.05E−02
	9	2.4%	73.1%	−0.030	7.31E−01	−0.052	2.54E−01	0.002	9.46E−01	0.001	2.51E−01	−0.002	9.71E−01	−0.301	5.62E−01	−0.032	7.91E−01
	10	2.0%	75.1%	0.007	9.25E−01	0.066	9.72E−02	−0.013	5.71E−01	0.001	9.03E−02	−0.063	1.40E−01	0.844	6.35E−02	0.067	6.09E−02
Trimester 2	1	38.6%	38.6%	−0.187	5.84E−01	0.028	9.01E−01	0.160	9.71E−02	−0.006	3.09E−02	0.173	3.34E−01	−1.131	5.68E−01	0.012	3.16E−01
	2	9.1%	47.7%	−0.100	4.51E−01	−0.462	**4.91E−07**	−0.007	8.50E−01	−0.001	2.88E−01	−0.065	3.45E−01	1.496	5.26E−02	0.295	**8.08E−07**
	3	6.0%	53.7%	0.080	5.12E−01	−0.103	1.93E−01	0.038	2.65E−01	0.000	8.76E−01	0.131	4.28E−02	1.881	8.96E−03	0.189	2.67E−04
	4	5.1%	58.8%	0.082	4.86E−01	0.114	1.39E−01	0.004	8.96E−01	−0.001	1.56E−01	0.024	6.95E−01	−1.683	1.52E−02	0.086	2.57E−02
	5	4.1%	62.9%	0.035	7.61E−01	0.101	1.77E−01	0.017	5.91E−01	0.000	8.59E−01	0.025	6.75E−01	−1.169	8.13E−02	0.006	3.68E−01
	6	3.3%	66.2%	0.041	6.73E−01	−0.065	3.07E−01	−0.059	3.27E−02	0.001	1.27E−01	0.032	5.34E−01	−1.081	5.69E−02	0.028	1.98E−01
	7	2.6%	68.7%	−0.013	8.63E−01	0.131	1.10E−02	−0.023	3.01E−01	0.000	6.13E−01	0.135	1.25E−03	−0.479	2.88E−01	0.176	4.94E−04
	8	2.3%	71.0%	−0.082	2.63E−01	0.067	1.59E−01	−0.007	7.36E−01	0.000	6.13E−01	−0.093	1.64E−02	0.551	1.93E−01	0.065	5.66E−02
	9	2.2%	73.3%	0.032	6.98E−01	−0.010	8.56E−01	−0.028	2.25E−01	0.001	3.06E−01	0.014	7.39E−01	0.476	3.18E−01	−0.029	7.77E−01
	10	1.7%	75.0%	0.054	4.53E−01	−0.061	1.93E−01	−0.051	1.24E−02	0.000	5.10E−01	0.014	7.17E−01	−0.034	9.34E−01	0.017	2.76E−01
Trimester 3	1	37.7%	37.7%	−0.283	4.05E−01	−0.202	2.59E−01	0.205	3.17E−02	−0.003	2.43E−01	0.306	8.68E−02	−2.446	2.28E−01	0.042	1.31E−01
	2	9.4%	47.1%	0.014	9.30E−01	0.206	1.52E−02	0.025	5.70E−01	0.000	8.64E−01	0.177	3.57E−02	0.651	4.92E−01	0.082	3.19E−02
	3	6.1%	53.2%	0.226	6.10E−02	−0.128	4.37E−02	0.046	1.73E−01	−0.002	6.61E−02	−0.026	6.74E−01	2.120	3.75E−03	0.208	**1.23E−04**
	4	4.8%	58.1%	−0.063	6.03E−01	0.061	3.43E−01	−0.006	8.52E−01	0.000	8.26E−01	0.019	7.70E−01	−0.817	2.61E−01	−0.022	6.87E−01
	5	4.2%	62.2%	−0.081	4.45E−01	−0.065	2.45E−01	0.035	2.42E−01	0.000	7.76E−01	0.102	7.00E−02	1.477	2.18E−02	0.125	5.82E−03
	6	3.4%	65.6%	0.017	8.62E−01	0.066	2.07E−01	−0.027	3.27E−01	0.001	2.03E−01	0.056	2.79E−01	−0.390	5.11E−01	0.000	4.29E−01
	7	2.6%	68.2%	0.015	8.65E−01	0.031	5.02E−01	0.035	1.55E−01	−0.001	3.89E−01	−0.030	5.16E−01	−0.334	5.27E−01	−0.026	7.29E−01
	8	2.5%	70.7%	0.138	1.10E−01	0.077	8.94E−02	0.008	7.35E−01	0.000	9.95E−01	−0.035	4.31E−01	−0.235	6.46E−01	−0.008	5.13E−01
	9	2.1%	72.7%	−0.029	6.89E−01	0.002	9.56E−01	−0.014	4.99E−01	0.000	4.79E−01	−0.059	1.30E−01	0.975	2.89E−02	0.016	2.84E−01
	10	1.8%	74.6%	−0.045	5.01E−01	−0.122	7.24E−04	0.008	6.81E−01	0.002	1.61E−03	−0.007	8.30E−01	−0.680	8.87E−02	0.192	2.71E−04

Abbreviations: AHEI-P, Adaptive Healthy Eating Index for Pregnancy; GWG, gestational weight gain; pBMI, prepregnancy body mass index; PCA, principal component analysis. β-Coefficients (β) and *P*-values for pBMI and GWG were calculated by linear regression models with pBMI and GWG as independent variables adjusted for total energy intake, AHEI-P, maternal age and maternal ethnicity. Overall *P*-value for the model was calculated with analysis of variance (ANOVA). Bold values indicate *P*-value < 0.000197.

Table 4. Significant associations of metabolites with birth weight percentile

Metabolite	*Trimester 1*		*Trimester 2*		*Trimester 3*		*Metabolite*	*Trimester 1*		*Trimester 2*		*Trimester 3*	
	β	P-*value*	*β*	P-*value*	*β*	P-*value*		*β*	P-*value*	*β*	P-*value*	*β*	P-*value*
Glutamic acid	6.132	**0.018**	3.489	0.171	4.264	0.083	NEFA 12:0	5.500	**0.036**	1.342	0.594	1.453	0.574
Carn.a.C8.1	6.018	**0.020**	3.387	0.166	1.094	0.668	NEFA 14:0	5.587	**0.034**	3.441	0.204	2.669	0.323
LPC.a.C18.0	−1.042	0.692	−2.455	0.315	−5.726	**0.032**	NEFA 14:1	6.451	**0.015**	1.443	0.579	1.032	0.694
LPC.a.C18.1	−2.663	0.315	−3.046	0.226	−5.137	**0.046**	NEFA 15:0	6.044	**0.019**	5.678	**0.031**	1.998	0.444
LPC.a.C18.2	−4.330	0.090	−2.964	0.237	−5.099	**0.045**	NEFA 16:0	6.546	**0.014**	5.798	**0.037**	2.321	0.370
LPC.a.C18.3	1.194	0.643	0.000	1.000	−5.510	**0.027**	NEFA 16:1	5.988	**0.025**	3.715	0.164	3.254	0.222
LPC.e.C18.0	−0.813	0.765	−1.303	0.599	−5.866	**0.023**	NEFA 17:0	6.685	**0.012**	7.427	**0.008**	3.067	0.243
LPC.e.C18.1	1.716	0.626	−2.371	0.441	−6.292	**0.049**	NEFA 17:1	6.110	**0.019**	6.032	**0.032**	2.907	0.265
PC.aa.C18.0	−2.870	0.394	−6.961	**0.027**	−6.252	0.065	NEFA 18:1	6.712	**0.010**	5.232	0.063	3.630	0.163
PC.aa.C42.6	−1.717	0.514	−2.860	0.251	−5.672	**0.024**	NEFA 18:2	5.367	**0.040**	4.236	0.137	1.935	0.464
PC.aa.C44.12	−2.600	0.327	−2.162	0.404	−5.274	**0.040**	NEFA 18:3	6.463	**0.012**	2.975	0.252	3.461	0.191
SM.a.C21.0	−0.711	0.794	−1.905	0.448	−6.416	**0.013**	NEFA 20:1	5.878	**0.026**	3.194	0.233	4.023	0.129
SM.a.C21.2	−5.216	**0.044**	−1.812	0.475	−4.755	0.060	NEFA 20:2	5.648	**0.036**	3.249	0.250	2.715	0.307
SM.a.C31.1	3.492	0.184	−0.402	0.872	−5.685	**0.029**	NEFA 20:3	6.368	**0.018**	4.757	0.103	3.363	0.214
SM.a.C38.4	5.313	**0.048**	4.057	0.110	−3.522	0.160	NEFA 20:4	6.086	**0.021**	3.368	0.229	2.530	0.359
β-Hydroxybutyric acid	5.952	**0.026**	1.947	0.450	3.785	0.135	NEFA 20:5	6.956	**0.014**	3.071	0.260	0.732	0.789
Malic acid	6.480	**0.016**	−4.748	0.063	−1.059	0.690	NEFA 22:4	6.165	**0.024**	3.313	0.220	3.434	0.198
PC2	1.061	0.110	−1.371	**0.037**	−0.878	0.166	NEFA 22:5	7.139	**0.008**	2.578	0.376	2.440	0.375

Abbreviations: LPC.a., lysophosphatidylcholines; NEFA, nonesterified fatty acids; PC.aa, phosphatidylcholines; PC.ae, ether-linked phosphatidylcholines; SM.a, sphingomyelins. β-Coefficients (β) and *P*-values for trimester-specific metabolite levels and principle components were calculated by linear regression models with metabolite as independent variable adjusted for maternal ethnicity. Bold values indicate *P*-value < 0.05.

SCD-1 rate may affect maternal metabolism and promote further esterification and lipid accumulation in the muscle and the liver rather than oxidation.[36] Increased intracellular lipids are associated with insulin resistance.[38] On the other hand, MUFA can be transferred to the fetus and drive lipogenesis rather than lipid oxidation, resulting in larger fat depots in the fetus and higher birth weight infants, a known risk factor for childhood obesity.[3] In addition, lipid accumulation in fetal muscle and liver will also promote the development of a proinflammatory state and insulin resistance in the offspring.[2,4]

The increased concentration of SM species associated with raised BMI also suggests an enhanced SM biosynthesis that is part of the lipoproteins.[39] It could be speculated that SM or ceramides, intermediate products of SM biosynthesis, may contribute to the development of insulin resistance in obese pregnant women and thus contribute to elevated glucose and insulin supply to the placenta and the fetus. However, the relation of SM to pBMI only occurs in the first trimester and disappears with advancing gestation. Thus, the SM association may be attributed to the obese state of the women independent of pregnancy, as supported by previous publications among nonpregnant subjects.[40–42] Among the other phospholipid metabolites, it stands out that PC with three double bonds were positively associated to pBMI in trimester 1, in line with our results for NEFA 20:3. The PC.aa. C30.3, C32.3 and C38.3 contain FA 20:3 at sn-2 position and FA 10:0, 12:0 and 18:0 at sn-1, respectively. Despite not reaching statistical significance, LPC.a.C20:3 and LPC.a.C16:1 showed the strongest association to pBMI among all LPC. The omega-6 FA 20:3 (dihomo-γ-linolenic acid), is a known FA related to obesity.[43,44] A previous study showed a positive correlation between PC containing FA 20:3 in the maternal circulation and offspring adiposity.[45] In contrast, concentrations of PC species containing FA 20:3 were found to be lower in placenta of obese pregnant women, as well as women with gestational diabetes mellitus,[46] and cord blood FA 20:3 was negatively related to later insulin resistance.[47] Summarized, we have identified that raised pBMI is associated with elevated levels of lipids containing n-6 species or MUFA that may emerge from a high-fat diet and elevated SCD-1 activity. However, we found no associations between n-3 or n-6 NEFA or phospholipid species, or MUFA or SCD-1 activity ratios, with birth weight of the offspring, but have to consider that the largest depot of fatty acid in the human blood, the triacylglycerols, were not measured within our metabolomics platform. Furthermore, we interpret these results with caution given that birth weight is poorly associated with infant adipose stores, and that infant adiposity (that may be measured by skin-fold thicknesses or dual-energy X-ray absorptiometry imaging) has been highlighted as a stronger predictor of later child obesity risk.[48]

The limited findings related to AAs in the current study are in contrast to previous nonpregnancy studies that reported significant positive associations of BCAA, sulfur-containing AAs or aromatic AAs with obesity.[6,49] Although the usual relation of AAs to obesity is not seen in the present study, HOMA-IR was positively associated to Tyr and principle component 6, composed of AAs, in the first trimester only. BCAA and aromatic AAs, like Tyr, have been previously related to IR.[50] In a study with obese children, we have previously showed that Tyr rather than the BCAA are related to IR in the prehyperglycemic state.[51] The lower associations in trimesters 2 and 3 are in agreement with stable levels of these AAs observed across pregnancy trimesters despite the normal gestation-induced progressive IR.[18] A possible explanation is placental uptake of AAs and transfer to the fetus for protein synthesis,[52] particularly in the case of BCAA that are used for placental nitrogen supply. Thus, that normal pregnancy physiological changes are influencing the AA levels rather than IR. However, the highly significant associations with maternal pBMI observed for asparagine and glutamic acid are striking. Positive associations of glutamate and negative associations of asparagine with BMI were also found in Hispanic obese children, but along with other AAs.[53] Kuc *et al.*[54] reported lower levels of asparagine in pre-eclamptic pregnant women. Glutamate and aspartate are the only AAs that are not actively transported across the placenta[52] and glutamate from the fetal circulation is taken up into the placenta.[55] Thus, higher maternal levels of glutamate are not depleted via fetal transport similar to other AAs. However, higher glutamate levels may affect asparagine synthesis, as asparagine synthetase, the key enzyme in biosynthesis of asparagine, generates both glutamate and asparagine.[56]

Besides some AAs, short-and long-chain Carn are often related to obesity and IR,[49,57] but were also not significantly associated to pBMI or GWG in any trimester. As fatty acids become an increasingly important substrate for energy provision with advancing gestation,[27] β-oxidation rates may rise to provide acetyl-CoA for ketogenesis, particularly in the fasted state when glucose supply is low.[18] Thus, any potential relation of Carn and AA to obesity may become less apparent during pregnancy because of normal metabolic adaptations throughout gestation. This hypothesis may also explain the absence of an association between Carn and AAs with any of the investigated factors in this study.

This study has several notable strengths including the longitudinal design and metabolomic profiling among a large cohort of women with uncomplicated pregnancies but with a high obesity rate. Inclusion of GWG, dietary and insulin resistance data, among which parameters we observed wide interindividual variation, also facilitated consideration for behavioral and metabolic factors related to maternal obesity that could potentially moderate or exacerbate the associations between pBMI and the metabolome. However, the absence of prepregnancy metabolomics data limits our interpretation of pregnancy effects on the association of pBMI and the maternal metabolome. Furthermore, as this was a study of a healthy obstetric population among which women with a diagnosis of gestational diabetes were excluded, we cannot assume that similar metabolomics associations would occur in women with complicated pregnancies or adverse outcomes.

In summary, this is the first study to our knowledge to demonstrate an association between prepregnancy BMI and a pattern of metabolites related to obesity that differs from nonpregnant cohorts. The strong effect observed on NEFA and the different behavior of NEFA species may indicate key mechanisms in the transmission of maternal obesity to offspring. Further studies are required to replicate our novel findings and provide more detailed interpretation of the underlying mechanisms.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

CH: performed quality control, statistical data analysis and data interpretation and wrote the manuscript; KLL: performed statistical data analysis and data interpretation and wrote the manuscript; OU: performed laboratory analysis and quality control, contributed reagents/materials/analysis tools, and revised the manuscript; CB, PDW and SE: designed research studies and revised the manuscript;: BK: designed research studies, contributed reagents/materials/analysis tools and revised the manuscript.

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