Association of maternal pre-pregnancy BMI with metabolomic profile across gestation

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Running Title: Maternal BMI and gestational metabolomic profiles

Key Terms: Metabolomics; Pregnancy; Maternal Obesity; Fetal Programming; Fatty acids

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Disclosure statement: The authors declare no conflict of interest.

Financial Support:

The work reported herein was carried out with partial financial support from the Commission of the European Communities, the 7th Framework Programme, contract FP7-289346-EARLY NUTRITION, and European Research Council Advanced Grant ERC-2012-AdG – No. 322605 META-GROWTH. This paper does not necessarily reflect the views of the Commission and in no way anticipates the future policy in this area. Additional support is gratefully acknowledged from the National Competence Network on Obesity, Grant Nr. 01 GI 0825, German Ministry of Education and Research, Berlin; the University of Munich Innovative Research Priority Project MC-Health (sub-project I); and the US National Institute of Health Grants R01 HD-060628, RO1 HD-065825, R01 MH-091351 and R21 RDK-098765. The funders had no role in study design data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Background/Objectives: Elevated pre-pregnancy BMI (pBMI) and excess gestational weight gain (GWG) constitute important prenatal exposures which may program adiposity and disease risk in offspring. However, the biological mechanisms underlying these fetal programming pathways remain unclear. The objective of this study is to investigate the influence of pBMI and GWG on the maternal metabolomic profile across pregnancy trimesters, and to identify potential causal pathways for offspring adiposity.

Subjects/Methods: This is a longitudinal prospective study of 167 non-diabetic women carrying a singleton pregnancy. Women were recruited between March 2011 - December 2013 from antenatal clinics affiliated to the University of California Medical Center in Orange County, California. Seven women were excluded from analyses due to a diagnosis of diabetes during their pregnancies. A total of 254 plasma metabolites known to be related to obesity in non-pregnant populations were analyzed in each trimester using targeted metabolomics. The effects of pBMI and GWG on these metabolites were tested through linear regression and principle component analysis, adjusting for maternal diet, maternal insulin resistance, age, and race/ethnicity. A Bonferroni correction was applied for multiple comparison testing.

Results: pBMI was significantly associated with 40 metabolites. Non-esterified 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.

Conclusion: Pre-conception 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 pre-conception health. Mono-unsaturated and omega-6 fatty acids represent key metabolites for a potential mechanism of intergenerational transfer of obesity risk.

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 pre-pregnancy BMI (pBMI) is more strongly associated with excessive fetal growth and birthweight 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] which 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 to 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 to the non-pregnant state difficult or invalid. While 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 non-esterified fatty acids[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.

While a few cross-sectional metabolomics studies have been conducted in pregnant cohorts, these have primarily focused on differentiating the metabolomics profile of healthy pregnant women compared to

those with adverse pregnancy outcomes.[11-13] A recent study also depicted an association between maternal pre-pregnancy body mass index (BMI) and lipid profile in early pregnancy.[14] Meanwhile, studies among non-pregnant populations have demonstrated variations in metabolomic profiles associated with dietary patterns,[15, 16] which may also hold importance in prenatal populations as raised maternal BMI 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 GWG and the maternal metabolomics profile across trimesters, that is not accounted for by other potential determinants, e.g. dietary quality (Alternate Healthy Eating Index adapted for pregnancy (AHEI-P)) and quantity (total energy intake), insulin resistance (HOMA-IR), maternal age and ethnicity. Additionally, 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 non-diabetic women, recruited in their first trimester of pregnancy to a longitudinal, prospective birth cohort study at the University of California, Irvine (UCI), Development, Health and Disease Research Program. The study was approved by the UCI 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 Supplemental 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. Associations between trimester-specific GWG, dietary quality (Alternate Healthy Eating Index adapted for pregnancy (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 non-normally 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 AA: leucine, isoleucine, valine, methionine, phenylalanine, tryptophan, threonine, or dispensible AA: alanine, arginine, asparagine, aspartic acid, glutamine, glutamic acid, glycine, citrulline, ornithine, proline, serine, tyrosine, cysteine), chain length (short, medium and long-chain Carn), or degree of saturation (saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) for NEFA, lyso-PL, PCaa, PCae, and SMa).

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 (Supplemental Table 1). A second model was used including the interaction term of GWG and BMI (Supplemental Table 2), but since 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 (Supplemental 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 HOMA-IR, pBMI and the interaction effect of pBMI and HOMA-IR were included as independent variables, while GWG and the dietary variables were not used (Supplemental Table 3). This separate regression model was required since we were limited to a maximum of six predictors in a regression by the sample number.

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 point (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 3.0.1, or Excel 2010, version 14.0.7151.5001. 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 (Supplemental Table 4)

Finally, Principal Component Analysis (PCA) 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.

Results

Maternal characteristics of the study population are presented in Table 1. All women delivered healthy term babies; the mean ± standard deviation gestational age at delivery was 39.4±1.4 weeks, and mean birth weight at delivery was 3.36 kg. 42% of women 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). Trimester specific GWG and total GWG were strongly negatively associated with pBMI (p<0.001), while HOMA-IR was strongly positively associated with pBMI in each trimester (p<0.001 in trimester 1 and 2, p=0.004 in trimester 3). Pre-pregnancy 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.

<u>Metabolomic analysis</u>

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 Supplemental 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 (Supplemental Table 1, Figure 1) or when considering its interaction with pBMI (Supplemental 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, while only a few significant associations were found with GWG (3), AHEI-P (0), total energy intake (0), age (2) and ethnicity (4).

Association of pre-pregnancy BMI and GWG with metabolites

The majority of NEFA metabolites in trimester 1 and trimester 2 were significantly positively associated with pBMI, as well as the SCD enzyme activity ratios (Figure 2, Supplemental Table 1). However, the omega-3 long-chain polyunsaturated fatty acids (LC-PUFA) C20:5 (eicosapentanoic acid (EPA)) and C22:6 (docosahexanoic acid (DHA)) were not significantly associated with pBMI in any trimester. In trimester 3, after Bonferroni correction is applied, the associations of the omega-6 LC-PUFA C20:3 (dihomo-gamma-linolenic acid (DGLA)), C20:4 (arachidonic acid (ARA)), and C22:4 (adrenic acid), and the ratio of C16:1 to C16:0 were still significant. The only AA significantly associated with pBMI were asparagine (negatively associated in trimester 3) and glutamic acid (positively associated in trimester 2) (Table 2). The branched-chain AA (leucine, isoleucine, valine) and the aromatic AA (phenylalanine, tyrosine) 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 (Supplemental Table 1), but beta-hydroxybutyric acid was positively associated with pBMI in trimester 3. Among the phospholipid sub-groups, 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 (Figure 3). 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.40.0, 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 alpha-ketoglutaric acid (α-KG) in trimester 1 and 3, as well as SM.a.C30.1 in trimester 1 after Bonferroni correction (Table 2, Supplemental 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 (Supplemental Table 3).

Principle component analysis

The first ten 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 was primarily weighted by NEFA in both trimesters (Supplemental Table 5), particularly saturated, monounsaturated and n-6 NEFA.

Dietary analysis

Single lipid metabolites significantly associated with pBMI were also related to specific dietary fat intakes (Supplemental Table 4). None of the associations were significant after correction for multiple testing. Only NEFA 20:4 (trimester 1&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[19] and offspring development including adverse cardio-metabolic profile, increased birthweight and greater adiposity,[20, 21] as well as mental health outcomes.[22] Various potential mechanisms

202 including epigenetic changes, alterations in the reward system, central control of food choice and intake,
203 changes in hormonal levels such as leptin and ghrelin, or placental adaptations for transfer of nutrients to
204 the developing fetus are involved in these processes.[23] While these concepts of 'fetal programming' of
205 offspring disease risk are subject to ongoing investigation, significant further characterization of the
206 underlying mechanisms is required in order to identify possible targets for intervention strategies during
207 pregnancy that may successfully interrupt the intergenerational cycles of obesity.[5]

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

217 Despite recent studies in non-pregnant populations reporting altered metabolomics profiles associated
218 with specific dietary intake patterns,[15, 16] total energy intake and AHEI-P, a validated measure of dietary
219 quality in pregnancy, had no impact and did not alter the significant associations of pBMI with the
220 metabolome. Furthermore, none of the dietary parameters were related to any metabolite and additional
221 analyses, relating specific dietary intake of fat or fat components to lipid metabolites also showed no
222 significant association. Thus, these results support the notion that the maternal metabolome is
223 predominantly influenced by obesity and less by dietary intake during pregnancy or by GWG. While it is
224 possible that longer-term pre-pregnancy dietary habits influence the maternal metabolome during
225 gestation, this has yet to be investigated.

Among all analyzed metabolites, the NEFA species showed the strongest positive associations with pBMI, demonstrated in both the univariate modeling and the PCA. A relation between the total concentration of NEFA in the maternal circulation during pregnancy and occurrence of GDM has been previously described.[24] In general, women with higher pBMI exhibit larger fat depots before pregnancy in the adipose tissue (AT), the major source of NEFA.[25] Hence, the normal physiological accumulation of fat in the first two trimesters[7] may be spared in obese women through less GWG compared to non-obese pregnant women.[19] Unchanged or potentially augmented insulin sensitivity in the first half of healthy pregnancy promotes an anabolic state, with enhanced lipogenesis in AT,[26] as the insulin-inhibiting effect on the hormone sensitive lipoprotein lipase is increased.[27] However, it appears that entering pregnancy in the obese state disturbs this normal anabolic activity through early-gestational insulin resistance.[24] Despite this, our analysis of the BMI – HOMA-IR interaction with the metabolome did not reveal significant associations beyond those already identified with pBMI alone. 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.[26] We have recently reported that plasma NEFA concentrations do not significantly change across trimesters despite the late-gestation expected increase in lipolysis,[18] which 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 beta-hydroxybutyric acid to be elevated with higher pBMI in trimester 3, indicating a higher rate of fasting-induced ketogenesis in obese women, perhaps due to elevated NEFA supply following late-pregnancy induced lipolysis. Nevertheless, elevated NEFA levels have been found to be strong predictors of elevated birthweight, overweight and increased body fat in the infant,[28, 29] thereby representing a potential metabolic pathway for the programming of offspring adiposity in obese pregnancy. In general, maternal lipids are associated with excessive fetal growth independent of GDM status, which may explain the stronger influence of pBMI on offspring growth compared to maternal hyperglycemia.[4]

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 which remained positively associated to pBMI in trimester 3, while 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, which has been implicated to influence BMI during the first 10 years of life.[30] The n-6 arachidonic acid (20:4) is the main precursor of eicosanoids enhancing the differentiation of adipose precursor cells into adipocytes, which is particularly related to linoleic acid intake.[31] In a study of rats, linoleic acid intake over four generations increased adipose tissue mass compared to a control diet, although caloric intake was the same.[32] This NEFA was among the strongest related to pBMI in the second trimester in the present results. Moon et al. 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.[33] Furthermore, excessive n-6 FA intake and insufficient n-3 intake has been reported as the most important risk factor associated with fetal programming.[34] Thus, there is a convincing body of evidence emerging to suggest that maternal n-6 NEFA or n-6 FA in the AT represent metabolomic biomarkers for trans-generational 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 stearoyl-CoA desaturase-1 (SCD-1) enzyme, which 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,[35] possibly due to a switch in fat metabolism from the catabolic to the anabolic state.[36] The higher SCD-1 rate may affect maternal metabolism and promote further esterification and lipid accumulation in the muscle and the liver rather than oxidation.[35] Increased intra-cellular lipids are associated with insulin resistance.[37] 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]

Additionally, lipid accumulation in fetal muscle and liver will also promote the development of a pro-inflammatory 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 which are part of the lipoproteins.[38] 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 non-pregnant subjects.[39-41] However, the biological significance of these results requires further exploration, as does the effect of monounsaturated lipid species on fetal or infant outcome.

Among the other phospholipid metabolites, it stands out that PC with three double bonds were positively associated to pBMI in trimester 1, which is 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 20:3 and LPC 16:1 showed the strongest association to pBMI among all LPC. The omega-6 FA 20:3 (DGLA), is a known FA related to obesity.[42, 43] A previous study showed a positive correlation between PC containing FA 20:3 in the maternal circulation and offspring adiposity.[44] 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 GDM,[45] and cord blood FA 20:3 was negatively related to later insulin resistance.[46] Summarized, we have identified that raised pBMI is associated with elevated levels of lipids containing n-6 species or MUFA, which may emerge from a high-fat diet and elevated SCD-1 activity. These metabolites therefore represent the strongest candidates responsible for the transfer of maternal obesity to the offspring.

The limited findings related to AA in the current study are in contrast to previous non-pregnancy studies which reported significant positive associations of BCAA, sulfur-containing AA or aromatic AA with obesity.[6, 47] Although the usual relation of AA to obesity or insulin resistance is not seen in the present study, these results are in agreement with stable levels of these AA observed across pregnancy trimesters despite the normal gestation-induced progressive insulin resistance.[18] A possible explanation is placental uptake of AA and transfer to the fetus for protein synthesis,[48] particularly in the case of BCAA which are used for placental nitrogen supply. However, the highly significant associations 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 AA.[49] Kuc et al. reported lower levels of asparagine in pre-eclamptic pregnant women.[50] Glutamate and aspartate are the only AA which are not actively transported across the placenta[48] and glutamate from the fetal circulation is taken up into the placenta.[51] Thus, higher maternal levels of glutamate are not depleted via fetal transport similar to other AA but likewise, higher maternal glutamate levels may not influence fetal growth. However, higher glutamate levels may affect asparagine synthesis, since asparagine synthetase, the key enzyme in biosynthesis of asparagine, generates both glutamate and asparagine.[52]

Besides some AA, short-and long-chain Carn are often related to obesity and insulin resistance,[47, 53] 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,[26] beta-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 to obesity may become less apparent during pregnancy due to normal metabolic adaptations throughout gestation.

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 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 pre-pregnancy metabolomics data limits our interpretation of pregnancy effects on the association of pBMI and the maternal metabolome.

In summary, this is the first study to our knowledge to demonstrate an association between pre-pregnancy BMI and a pattern of metabolites related to obesity, which differs from non-pregnant 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.

Supplementary Material

Supplementary information is available at the International Journal of Obesity's website.

Acknowledgements

We thank Franca Kirchberg (Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children's Hospital, University of Munich) who supported the statistical data analysis and Stefanie Winterstetter (Division of Metabolic and Nutritional Medicine, Dr. von Hauner Children's Hospital, University of Munich) who prepared the plasma samples for LC-MS/MS analysis.

Author contributions

CH: Performed quality control, statistical data analysis, and data interpretation. Wrote the manuscript.

KLL: : Performed statistical data analysis, and data interpretation. Wrote the manuscript.

OU: : Performed laboratory analysis and quality control. Contributed reagents/materials/analysis tools Revised the manuscript.

CB: Designed research studies. Revised the manuscript.

PDW: Designed research studies. Revised the manuscript.

BK: Designed research studies. Contributed reagents/materials/analysis tools. Revised the manuscript

SE: Designed research studies. Revised the manuscript.

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Figure Legends

Figure 1 Associations of gestational weight gain (GWG), pre-pregnancy BMI (pBMI), Alternate Healthy Eating Index adapted for pregnancy (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 pre-pregnancy BMI, 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); AA, Amino acids; NEFA- Non-esterified fatty acids; LPC, lyso-phosphatidylcholine; PCaa, phosphatidylcholines; PCae, alkyl-linked phosphatidylcholines; SM, sphingomyelins; CA- Carboxylic acids.

Figure 2 Associations of pre-pregnancy BMI to non-esterified fatty acid (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 pre-pregnancy BMI as independent variable adjusted for trimester specific gestational weight gain, total energy intake, Alternate Healthy Eating Index adapted for pregnancy, maternal age and maternal ethnicity. Straight line, Bonferroni corrected p-value was 0.000197 ($-\log_{10}$(p-value)= 3.71).

Figure 3 Associations of pre-pregnancy BMI to sphingomyelins (SM.a) species in the first trimester. Negative log transformed p-values are plotted for each SM.a species. P-values were calculated by linear regression models with pre-pregnancy BMI as independent variable adjusted for trimester specific gestational weight gain, total energy intake, Alternate Healthy Eating Index adapted for pregnancy, maternal age and maternal ethnicity. Dashed-dotted line, Bonferroni corrected p-value of 0.000197; Dotted line, uncorrected p-value of 0.05.