Inter-Generational Link of Obesity in Term and Preterm Births: Role of Maternal Plasma Acylcarnitines

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Abstract

BACKGROUND/OBJECTIVES—Acylcarnitines, intermediates of fatty acid oxidation, are known to be involved in obesity and insulin resistance. Since maternal prepregnancy overweight or obesity (OWO) is a recognized major risk factor for offspring OWO, we hypothesized that maternal plasma acylcarnitines may play a role in inter-generational OWO.

SUBJECTS/METHODS—This study included 1402 mother-child pairs (1043 term, 359 preterm) recruited at birth from 1998–2013 and followed prospectively up to age 18 years at the Boston Medical Center. The primary outcomes were child OWO defined as BMI $\geq 85^{th}$ percentile for age and sex. The primary exposures were maternal prepregnancy OWO defined as BMI ≥ 25 and maternal acylcarnitine levels measured in plasma samples collected soon after delivery using liquid chromatography–tandem mass spectrometry (LC-MS) in a targeted manner.

RESULTS—Approximately 40% of the children in this study were OWO by age 5. Maternal OWO had a significant association with childhood OWO, both in term and preterm births. β-hydroxybutyryl-carnitine (C4-OH) levels were significantly and positively associated with child OWO among term births after adjustment for potential confounders and multiple-comparisons. Children born to OWO mothers in the top tertile C4-OH levels were at highest risk of OWO: OR=3.78 (95%: 2.47, 5.79) as compared with those born to non-OWO mothers in the lowest tertile (P for interaction of maternal OWO and C4-OH= 0.035). In a four-way decomposition of mediation/interaction analysis, we estimated that C4-OH levels explained about 27% (se=0.08) of inter-generational OWO risk (P=0.001). In contrast, these associations were not observed in preterm births.

CONCLUSIONS—In this U.S. urban low-income birth cohort, we provide further evidence of the inter-generational link of OWO and reveal the differential role of C4-OH in explaining the inter-generational obesity between term and preterm births. Further investigations are warranted to better understand and prevent the inter-generational transmission of OWO.

Introduction

In the U.S., despite tremendous research and intervention efforts to understand and prevent the obesity epidemic, important challenges remain,[1] particularly related to the escalating epidemic of child and adolescent obesity.[2] While most pediatric obesity intervention programs have focused on older children, two recent interventions conducted in infants and toddlers and among preschool children[3, 4] have stressed the importance of intervening as early in life as possible. Indeed, there is growing recognition that childhood obesity may origin *in utero*,[5] as evidenced by the influence of the intrauterine environment in the risk of later life obesity.[6–8] It is well-established that infants born to overweight or obese mothers are more likely to become obese in childhood,[9–11] which predisposes these

children to long-term adverse health outcomes. This inter-generational link of overweight or obesity (OWO) amplifies the obesity epidemic from one generation to the next. Thus, understanding and breaking this vicious inter-generational cycle is critically important to halt or reverse the persistent obesity epidemic.

The precise mechanisms underlying the inter-generational link of OWO remain to be elucidated. While genetic inheritance may play a role, its ability to explain the inter-generational link of OWO is very limited.[12] Another possibility is that maternal obesity alters the *in utero* metabolic environment. A study conducted among siblings showed that children born before maternal bariatric surgery were at a much greater risk of developing obesity than their siblings who were born after the surgery.[8] These results suggest that maternal obesity may create an obesogenic intrauterine environment that puts the developing fetus at risk of future obesity; however, more insights are needed to understand the mechanistic pathways underlying the link between mothers' and their children's obesity risk.

Maternal obesity is characterized as insulin resistance, which may have an important role in obesity-related developmental programming.[13] Although studies have identified dysregulation of fatty acid oxidation in obesity and insulin resistance,[14] it is unknown if dysregulation of fatty acid oxidation may contribute to inter-generational obesity. One simple way to determine derangement in fatty acid oxidation is to profile blood acylcarnitines, intermediates of fatty acid oxidation.[15, 16] Indeed, increased blood acylcarnitines have been linked to metabolic disorders in reproductive age women[15] and in pregnant women.[17] It is possible that maternal elevated acylcarnitines may lead to an adverse *in utero* metabolic environment for the fetus and to their subsequently poor metabolic health, although such a hypothesis has not been examined in prospective birth cohort studies.

To fill this knowledge gap, we prospectively examined associations of maternal plasma acylcarnitine profiles with offspring OWO risk among mothers with and without OWO in the Boston Birth Cohort. We were particularly interested in understanding whether maternal plasma acylcarnitines mediate or modulate the effect of maternal OWO on offspring OWO and whether the associations differ between term and preterm births. A key difference between these groups is that the latter group either missed or had a shortened third trimester due to premature delivery, thus, they spent less time exposed to maternal metabolites during the third trimester, a critical period for fetal weight gain.

Methods

Study sample

This study included 1402 mother-child pairs from the Boston Birth Cohort, a predominantly urban, low-income minority birth cohort, enrolled and followed at the Boston Medical Center, Boston, MA, as described previously.[18] In the parent study, enrollment occurred within 2–3 days after delivery, and all eligible mothers completed a questionnaire interview and participated in a blood draw. Twins or triplets and newborns with major birth defects were excluded. Postnatal follow-up of the Boston Birth Cohort children has been ongoing

since 2004. A standardized questionnaire was used to assess postnatal demographic, infant feeding, and environmental information.[19] The study protocol was approved by the Institutional Review Boards of Boston University Medical Center and Johns Hopkins Bloomberg School of Public Health. All study mothers provided written informed consent.

As illustrated in the flowchart (Figure S1), of the 3163 mother-child pairs who were enrolled from 1998–2013 at birth and followed prospectively, 1410 mothers had measured plasma acylcarnitine profiles. Of those, we excluded 8 children who had missing BMI data at 2–18 years of age. The final sample for this study included 1402 mother-child pairs. The comparison of maternal demographic characteristics between the analytic and excluded samples is presented in Table S1. The analytic sample was comparable to the excluded sample, except that the analytic sample had a slightly higher percentage of Black mothers and a lower percentage of smokers.

Perinatal and postnatal variables

Maternal prepregnancy weight and height assessments were based on questionnaire data. Maternal BMI was calculated as weight in kilograms divided by height in meters squared, and then categorized into two groups: non-OWO ($<25 kg/m^2$) and OWO ($\geq$ 25 kg/m^2). Educational attainment was classified into two categories: high school and below or college and above. Maternal report of smoking during pregnancy was categorized as never smoker, intermittent, or continuous smoker.[20] Maternal race/ethnicity was grouped into Black, Hispanic, or Other (which included Caucasian, Asian, Pacific Islander, more than one race, and other). Pregnancy complications, Cesarean section and birthweight was abstracted from the electronic medical record (EMR). Maternal diabetes status was classified as nondiabetic, gestational diabetes, and pre-existing diabetes.[18] Hypertensive disorders were identified by the presence of chronic/gestational hypertension, preeclampsia/eclampsia or HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets).[18] Gestational age was determined by the first day of the last menstrual period and early prenatal ultrasonographic results,[20] and further grouped into term birth ($\geq$ 37 weeks) and preterm birth (<37 weeks). Information regarding infant breastfeeding history was primarily assessed by age 2 years at pediatric well-child follow-up visits, and grouped into exclusively breastfeeding, exclusively formula feeding, or both breast and formula feeding.[19]

Definitions of overweight or obesity (OWO) in childhood

Child weight and height were measured by medical staff during pediatric well-child visits and retrieved from the EMR. The measures taken at the last visits for each age group were used in this study. BMI *z*-scores and percentiles were calculated using U.S. national reference data.[21] OWO was defined as BMI $\geq$ 85th percentile of age and gender.

Assessments of maternal plasma acylcarnitines

Plasma acylcarnitines were profiled at the Broad Institute, MA, USA, using liquid chromatography–tandem mass spectrometry (LC-MS) in a targeted manner. Acylcarnitines were identified by their m/z, retention time, and through a comparison to library entries of purified known standards. We assessed 27 acylcarnitine species (Table S2). In this study, we

included 23 acylcarnitine species; 4 species were removed because of a high fraction of missing values (>10%) or high coefficient of variance (CV >25%).

Ascertainment of child metabolic biomarkers

We assessed child plasma insulin (a marker of insulin resistance)[18] and leptin (a marker of adiposity),[22] using sandwich immunoassays based on flow metric xMAP technology on Luminex 200 machines (Luminex Corp., Austin, TX) with an inter-assay CV of 4.0% and 4.5%, respectively.[18] Adiponectin was measured by ELISA with an inter-assay CV of <5.8%. Adiponectin/leptin ratio were calculated and used as a marker of insulin sensitivity. [23]

Statistical analysis

Because the measures of acylcarnitine species were relative values, to compare the effect of individual acylcarnitine species, we performed inverse normal transformation of each acylcarnitine species concentration as established by previous studies.[24] The transformation would allow for better interpretation of effect size (1 unit = 1 sd change in acylcarnitine) and it would minimize the effect of potential outliers if exists. As a first step, we performed multiple linear regression analyses and identified acylcarnitine species which were associated with both maternal OWO and child OWO. We adjusted for multiple testing by using the Bonferroni procedure. Two-sided P values <0.002 (0.05/23) were considered statistically significant.

In the subsequent analyses, we focused on maternal β-hydroxybutyryl-carnitine (C4-OH), which was strongly associated with both maternal OWO and child OWO. We first explored the relationships of C4-OH with gestational age and the outcomes of interest using smoothing plots (PROC LOESS). We then applied linear and logistic regression models to investigate C4-OH in relation to child BMI z-scores and OWO, respectively, adjusted for mother's age at delivery, educational attainment, race/ethnicity, parity, and smoking. To further quantify the dose-response associations between C4-OH and child outcomes, we estimated the coefficient of BMI *z*-score and odds ratio (OR) of child OWO risk by the C4-OH tertiles. In addition, we evaluated the joint association of maternal prepregnancy OWO and C4-OH tertiles with child OWO risk. We tested the interaction of maternal prepregnancy OWO (Yes vs. No) and C4-OH (middle and top tertiles vs. low tertile) on child's BMI *z*-scores and odds of OWO by adding a multiplicative term in the models. The reason is that given maternal BMI (on a continuous scale), children born to mothers with second and top tertiles of C4-OH levels had similar BMI z-scores and OWO risk, but clearly higher than those whose mother with lowest tertile levels; and this difference varied by maternal BMI with a threshold around 25kg/m^2 (Figure S2). The effect modifications were assessed with the likelihood ratio test using an a priori α value of 0.05.

To further characterize the role of C4-OH in inter-generational OWO, we performed mediation/interaction analysis using a four-way decomposition method which allows for four-way calibration of the total effect: controlled direct effect (CDE), reference interaction (INT$_{ref}$), mediated interaction (INT$_{ref}$), and pure indirect effect (PIE).[25] The CDE (the component due to neither mediation nor interaction) is the direct effect of the exposure on

the outcome without going through the mediator. The INT_{ref} (the component due to interaction but not mediation) is the interactive effect when the mediator is left to the level it would take in the absence of exposure. The INT_{med} is the component due both to mediation and interaction, and the PIE is the component due to mediation alone, and the total indirect effect is the sum of INT_{med} and PIE.

Finally, to examine the robustness of our results, we conducted a series of sensitivity analyses. We additionally adjusted for other covariates (Cesarean section, maternal diabetes, hypertensive disorders, fetal growth, and breastfeeding status). We also evaluated the associations of interest by children's age groups at outcome assessments, i.e., at age 2–5 years, 6–9 years, and 10–18 years, respectively, and among a subset where underweight mothers were removed. To further address potential unmeasured confounders, we conducted propensity score–matched sensitivity analyses.[26] To further establish a clear role for C4-OH, we performed similar analyses on the child's plasma insulin, leptin, and adiponectin/ leptin ratio among term births. All analyses were conducted using SAS software, version 9.4 (SAS Institute), and R software, version 3.4.1 (R Project for Statistical Computing). Both term and preterm births had >80% power testing the effects.

Results

The study sample consisted of 1402 mother-child pairs (1043 term and 359 preterm births), 710 (50.6%) were boys, 980 (69.9%) were Black, and 288 (20.5%) were Hispanic. The prevalence was 53.7% for maternal prepregnancy OWO and 43.4% for offspring OWO at age 2–18 years, respectively. Table 1 shows that mothers who had preterm births were older and had a higher proportion of smokers, diabetes, hypertensive disorders and Cesarean section. Term births and preterm births had similar rates of OWO from age 2 to18 years.

Acylcarnitines, maternal prepregnancy OWO, and child OWO at age 2–18 years

Maternal prepregnancy OWO was significantly associated with 13 of the 23 total acylcarnitines with a Bonferroni-corrected P value <0.002 (Table S3). These 13 acylcarnitines included short- (C4-OH), medium- (C6, C7, C8, C10, C10:2), and long-chain (C12, C12:1, C14:1, C14:2, C16, C18:1, C18:2) species. Eleven of these associations were still significant after adjustment for confounders (maternal age, education, race/ethnicity, smoking status, parity, and gestational age), including C4-OH, C6, C7, C8, C10, C10:2, C12, C12:1, C14:1, C14:2, and C18:2. However, only C4-OH was significantly associated with child OWO risk after adjustment for potential confounders (Table S4). Because among those 23 acylcarnitine species, C4-OH had the strongest association with both maternal and child OWO risk, we focused on C4-OH for the remaining analyses.

The relationship between maternal C4-OH and gestational age

Given the large variation of gestational age in this birth cohort, we had the opportunity to examine the relationship between maternal C4-OH levels and gestational age (Figure 1, Panel A). We found that the pattern differed between non-OWO and OWO mothers: over the range of gestational age from 24–42 weeks, C4-OH increased with gestational age among OWO mothers; in contrast, C4-OH decreased with gestational age among non-OWO

mothers. Noteworthy, the difference in C4-OH between non-OWO and OWO mothers became more pronounced as gestational age approached term (Figure 1, Panel A).

Joint effect of maternal OWO and C4-OH on child OWO risk

The associations between C4-OH with child OWO risk differed between term and preterm births (Figure 1, Panels B and C), p for interaction =0.003. Among term births, C4-OH was significantly associated with a higher risk of child OWO among OWO mothers, but not among non-OWO mothers (Figure 1, Panel B). As shown in Table 2, the top C4-OH tertile (T3) was associated with 2.22 times (95%CI: 1.44–3.43) the risk of childhood OWO among children born to OWO mothers, whereas the association was not significant among children born to non-OWO mothers (P for interaction = 0.016). When we further estimated the joint association of maternal OWO and C4-OH, having an OWO mother with a top tertile C4-OH level was associated with the highest risk (OR= 3.78, 95%CI: 2.47–5.79), compared with having a non-OWO mother with a low tertile C4-OH level (Table 2). Consistent with this finding, there was a 0.41(se=0.12) unit increase in BMI z-scores among children born to OWO mothers in the top tertile of C4-OH compared to those whose mothers were in the low C4-OH tertile, whereas there was no significant association among children born to non-OWO mothers (p for interaction =0.003) (Table S5). However, the aforementioned associations were not observed among preterm births (Figure 1, Panel C, Table 2 and Table S5).

The role of C4-OH in the inter-generational link of OWO

We further determined that C4-OH was both a mediator and a modifier in the inter-generational link of OWO using a regression-based four-way decomposition mediation analysis of term births. Overall, C4-OH mediated 19% of the inter-generational OWO association (P<0.001), and 26% of the inter-generational OWO link could be explained by the interactions between maternal OWO and C4-OH (P=0.002). In total, 27% of the inter-generational OWO associations were attributable to elevated C4-OH. (Table 3).

Robustness of the study findings: Subgroup and sensitivity analyses

These association patterns did not change when Cesarean section, maternal diabetes status, hypertensive disorders, fetal growth and breastfeeding status were controlled for in the sensitivity analysis, although the association became marginally significant for OWO mothers with T1 C4-OH in the joint effect analysis (Table S6). Since our definition of child OWO accounted for gender and age, adding age to the regression models would lead to over-adjustment. Alternatively, we performed sensitivity analyses in three age groups: 2–5 years, 6–9 years, and 10–18 years, and found that the association patterns persisted across different age groups (Table S7). To avoid the influence of underweight, we evaluated the associations after removing the underweight mothers and obtained similar results (Table S8). To further assess the robustness of our results, we conducted a propensity-score–matched analysis that included 480 mother-child pairs. Consistently, the top tertile of maternal C4-OH, as compared with the low tertile, was associated with an increased risk of childhood OWO (OR, 1.28; 95% CI, 1.06–1.54) and an increased BMI z-score (mean±SD): 0.85±1.22 vs. 0.62±1.13, p=0.039 (Table S9).

In additional analyses, we examined the associations of C4-OH with children's plasma insulin, leptin, and adiponectin/leptin ratio among term births. As shown in Tables S10, C4-OH was positively associated with child's plasma leptin and inversely associated with adiponectin/leptin ratio, though the associations became insignificant after adjusting for confounders. Importantly, top teritle of C4-OH combined with maternal OWO was strongly associated with higher child's leptin and lower adiponectin ratio levels compared to low tertile of C4-OH with non-OWO. Top tertile C4-OH increased child's plasma insulin level, while the finding was not statistically significant.

Discussion

Several key findings were observed in this large, prospective birth cohort study. First, in this high-risk birth cohort, term births and preterm births had a similar rate of OWO across early childhood, school age, and adolescence. Second, we showed that maternal OWO is a significant risk factor for offspring OWO, both in term and preterm births. Most previous studies were conducted in term births only or did not differentiate term vs. preterm births. Third, our study showed that maternal C4-OH levels were significantly and positively associated with both maternal and child OWO risk, and may explain 27% of the link between maternal OWO and the child's OWO as a mediator and modifier.

We found that several acylcarnitines accumulated in OWO mothers, including short-, medium- and long-chain species. These findings fit with previous studies that showed a positive association of plasma acylcarnitine levels with BMI during pregnancy[17] and prepregnancy BMI.[27] Consistently, non-pregnant obese individuals also had higher acylcarnitines than did normal weight individuals.[15] More interestingly, we found that C4-OH was associated with both maternal and child OWO risk. C4-OH is an fatty acid oxidation intermediate derived directly from β-hydroxybutyryl-CoA, the concentration of which reflects the abundance of β-hydroxybutyrate,[28] a ketone that accounts for 70–80% of total ketone bodies.[29] Previous studies have provided evidence that chronically elevated free fatty acids might enhance ketone synthesis and/or expression of ketogenic enzymes in muscles.[28] In addition, human and animal studies consistently showed that C4-OH was associated with insulin resistance.[28, 30] Therefore, maternally elevated C4-OH may be a marker of underlying metabolic alterations in the setting of maternal obesity and insulin resistance, with implications for increased risk of offspring OWO.

The observed mediating effect of C4-OH may be explained by maternal OWO associated metabolic alterations, including insulin resistance and dyslipidemia, which subsequently result in mitochondrial overload and incomplete fatty acid oxidation.[17, 31] As an intermediate of fatty acid oxidation, elevated C4-OH may be viewed as an indicator of insulin resistance and incomplete fatty acid oxidation,[28, 30] implying an dysfunctional in-utero metabolic environment for the developing fetus. Additional mechanistic studies are needed to better understand maternal OWO and C4-OH interactions on inter-generational link of OWO. Our data on metabolic biomarkers lends further support to the biological plausibility of our findings. These data suggest that part of maternal C4-OH action on child OWO may be via alterations in metabolic programing. Future studies are warranted to replicate these findings and further elucidate the underlying biologic pathways.

We also found an inverse association between maternal plasma C4-OH levels and gestational age among non-OWO mothers, but a positive association among OWO mothers. Inconsistent with our findings, a previous longitudinal study reported a significant decline in short-chain acylcarnitine across the 3rd trimester in normal pregnancies.[32] Nevertheless, as our data showing a relationship between C4-OH and gestational age was cross-sectional, the interpretation of these data will require caution.

Another novel aspect of this study was our ability to study a large sample of term and preterm births and examine the similarities and differences between term and preterm births in the inter-generational link of OWO. In the US, preterm births consist of about 10% of all live births and is significantly higher in Blacks at about 14%.[33] Our study confirmed previous findings that urban, low-income, minority populations, especially, Black and Hispanic, have a higher prevalence of OWO.[34, 35] Alarmingly, almost 40% of these children had OWO by age 5 years. It is also noteworthy that preterm births had a similar rate of OWO as term births across developmental stages (preschool, school age, and adolescence), underscoring the importance of preventing OWO across the entire gestational age spectrum. We also found that maternal prepregnancy OWO was associated with child OWO risk in both term and preterm births, but the association was stronger in term births. Furthermore, we found that C4-OH mediated the inter-generational link of OWO only in term births, not in preterm births, suggesting that the underlying mechanism of inter-generational OWO may be different for term and preterm births. This may be due to several reasons. First, the third trimester is the period of the most rapid fetal weight gain; compared to term births, preterm births had either a missed or shortened 3rd trimester due to premature delivery. As such, preterm births had a shorter duration of exposure to maternal metabolites during the critical 3rd trimester, for which we demonstrated remarkable differences in plasma C4-OH levels between non-OWO and OWO mothers. Second, preterm births had a very different neonatal course, feeding pattern (such as high caloric formula), and clinical interventions (including neonatal intensive care) from term births. As we demonstrated in an early report, extremely rapid weight gain in the first 4 months of life was more common among preterm births, which was associated with a higher risk of OWO in later years.[36] In short, our study findings underscore that preterm births are at the same risk of OWO as term births during childhood and adolescence, but their clinical course and underlying mechanisms for OWO may differ from term births.

Our study has several strengths. This is the first prospective birth cohort study with a large sample size and long duration of follow-up to systematically investigate and quantify the role of maternal plasma acylcarnitines in inter-generational OWO. In particular, our study was conducted in a high-risk US population, which has been disproportionally affected by the obesity epidemic across the life span and generations. Analytically, we have applied advanced statistically methods; carefully considered epidemiological and clinical covariables relevant to OWO; accounted for multiple comparisons; and performed various sensitivity analyses to minimize the impact of uncontrolled confounders.

The limitations of our study should also be acknowledged. We profiled acylcarnitines in plasma samples taken at 1–3 days after delivery. It is uncertain to what extent this one-time measurement can reflect *in utero* exposure. Previous studies have suggested that plasma lipid

levels return to prepregnancy levels within a year postpartum in humans,[37] and within in a week in monkeys.[38] Although we did not measure fetal acylcarnitine concentrations, a previous study suggested a strong correlation between maternal and cord blood acylcarnitines.[39] Lastly, maternal acylcarnitine concentrations were measured in random plasma samples. As we discussed in our previous report,[18] the timing of blood sampling occurred randomly (any time during the clinical hours), which may have introduced background noise and thus biased our results towards the null. Finally, because this study was conducted in a high-risk U.S. urban, low-income population, caution is needed when generalizing its findings to other populations with different characteristics.

In conclusion, in this urban low-income birth cohort, nearly 40% of the children had OWO by age 5 in both term births and preterm births. C4-OH levels were significantly and positively associated with child OWO among term births, but not in preterm births. Our findings provide evidence of an inter-generational link of OWO and reveal potentially different pathways for OWO between term and preterm births. These findings raise the possibility that a child's risk of OWO may be traced back to their metabolic environment *in utero*. Given the limited success of current obesity prevention programs in childhood, findings from our study underscore the importance of interventions starting at the earliest possible developmental stage (preconception, in-utero and infancy), which may in turn, impact their child's life-long metabolic health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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A.
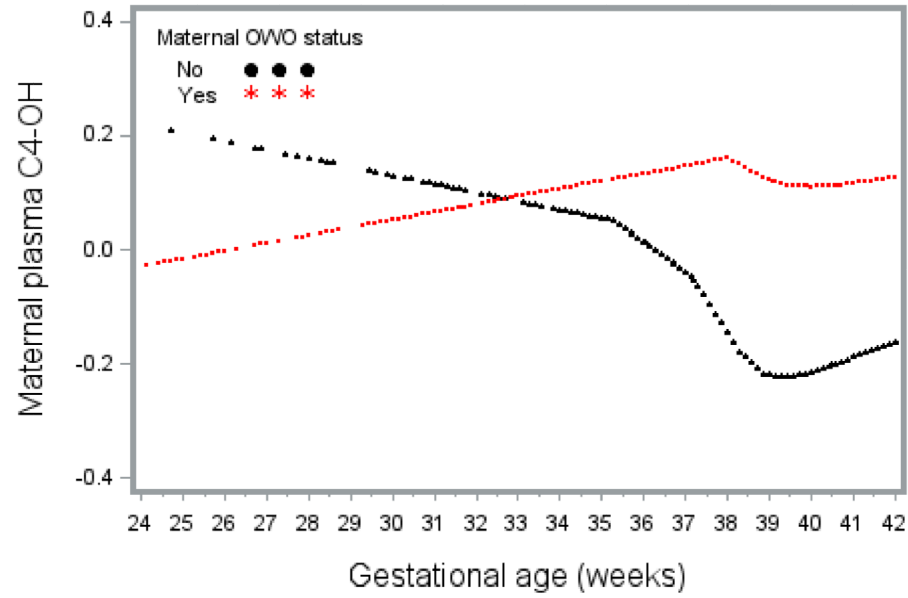
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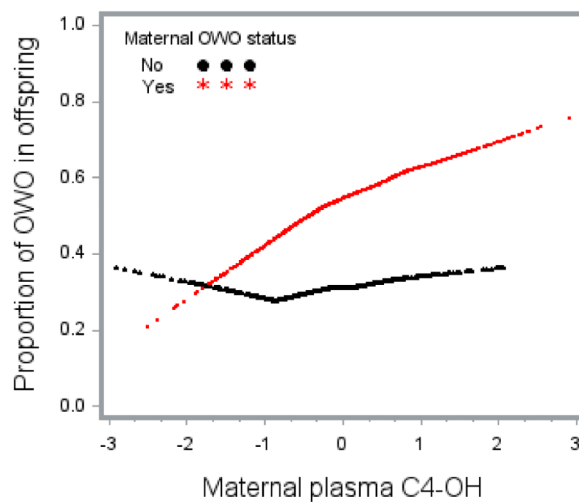
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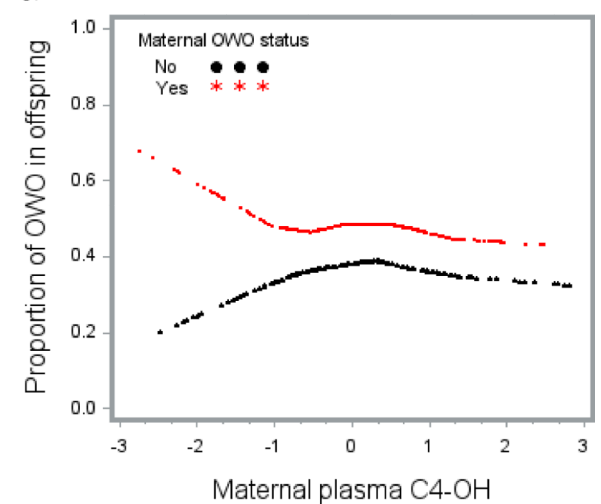
Figure 1.
Smooth plot of the relationship between gestational age and maternal plasma C4-OH carnitine levels stratified by maternal prepregnancy BMI categories (Panel A), the differential relationships between maternal plasma C4-OH carnitine and the child's risk of overweight or obesity (OWO) stratified by maternal prepregnancy OWO status among term births (Panel B), and among preterm births (panel C).

BMI, body mass index; OWO, overweight or obesity. Non-OWO was defined as BMI<25kg/m^2; OWO was defined as BMI 25kg/m^2.

Table 1.

Characteristics of mothers-child pairs in total sample and stratified by term vs. preterm births.

	Total sample	Term births	Preterm births	P value
Maternal characteristic				
N	1402	1043	359	
Maternal age (years)	28.5±6.6	28.2±6.6	29.4±6.6	0.003
Race/ethnicity				0.816
Black	980(69.9)	730(70.0)	250(69.6)	
Hispanic	288(20.5)	211(20.2)	77(21.5)	
Other	134(9.6)	102(9.8)	32(8.9)	
Education				0.569
High school and less	920(65.6)	680(65.2)	240(66.9)	
College and above	482(34.4)	363(34.8)	119(33.2)	
Smoking during pregnancy				<0.001
Never	1174(83.7)	901(86.4)	273(76.1)	
Ever	103(7.4)	71(6.8)	32(8.9)	
Continuous	125(8.9)	71(6.8)	54(15.0)	
Prepregnancy overweight or obesity				0.062
No	649(46.3)	498(47.8)	151(42.1)	
Yes	753(53.7)	545(52.2)	208(57.9)	
Diabetes status				<0.001
Nondiabetes	1227(87.5)	931(89.3)	296(82.4)	
Gestational diabetes	107(7.6)	74(7.1)	33(9.2)	
Preexisting diabetes	68(4.9)	38(3.6)	30(8.4)	
Hypertensive disorders	206(14.7)	100(9.6)	106(29.5)	<0.001
Cesarean section	504(36.0)	341(32.7)	163(45.4)	<0.001
Plasma C4-OH	0.00±1.00	−0.03±0.98	0.09±1.06	0.042
Child's characteristics				
Child's age (years)[a]	9.2 (6.3–11.9)	9.2(6.3–11.7)	9.1(6.3–12.2)	0.555
Gender				0.379
Boy	710(50.6)	521(50.0)	189(52.7)	
Girl	692(49.4)	522(50.0)	170(47.3)	
Birthweight (g)	2979±790	3274±520	2120±815	<0.001
Gestational age (weeks)	37.9±3.3	39.4±1.2	33.4±3.5	<0.001
Large for gestational age	156(11.1)	111(10.6)	45(12.5)	0.325
Breastfeeding				0.042
Formula exclusively	346(24.7)	241(23.1)	105(29.3)	
Breastfeeding exclusively	104(7.4)	83(8.0)	21(5.8)	
Both	952(67.9)	719(68.9)	233(64.9)	
BMI z-score at last well-child visit	0.77±1.22	0.78±1.18	0.74±1.32	0.646
OWO at age 2–18 years	608(43.4)	453(43.4)	155(43.2)	0.933
OWO at age 2–5 years[b]	532(39.3)	397(39.3)	135(39.4)	0.976

	Total sample	Term births	Preterm births	P value
OWO at age 6–9 years[c]	497(46.7)	369(46.4)	128(47.6)	0.740
OWO at age 10–18 years[d]	287(47.7)	212(47.6)	75(47.8)	0.978

Data are presented as mean±SD, no.(%).

[a]Data are presented as median (IQR).

[b]N=1354

[c]N=1064

[d]N=602.

C4-OH, β-hydroxybutyryl-carnitine; BMI, body mass index; OWO, overweight or obesity.

Table 2.

The individual and joint associations of maternal prepregnancy overweight or obesity (OWO) and plasma C4-OH at delivery on child's OWO during childhood (age range: 2–18 years).

Maternal		Term births (n=1043)				Preterm births (n=359)			
OWO	C4-OH tertile	n	Case,n(%)	OR	95%CI	n	Case,n(%)	OR	95%CI
Individual									
No		498	155(31.1)	1.00		151	54(35.8)	1.00	
Yes		545	298(54.7)	2.59	1.99–3.37	208	101(48.6)	1.73	1.11–2.70
	T1	347	118(34.0)	1.00		119	48(40.3)	1.00	
	T2	348	159(45.7)	1.58	1.16–2.16	120	58(48.3)	1.38	0.82–2.32
	T3	348	176(50.6)	1.94	1.42–2.65	120	49(40.8)	0.99	0.58–1.69
	P for trend				<0.001				0.714
Stratified									
No	T1	195	56(28.7)	1.00		57	18(31.6)	1.00	
	T2	160	50(31.3)	1.15	0.72–1.83	47	19(40.4)	1.33	0.57–3.10
	T3	143	49(34.3)	1.37	0.85–2.21	47	17(36.2)	1.11	0.47–2.61
Yes	T1	152	62(40.8)	1.00		62	30(48.4)	1.00	
	T2	188	109(58.0)	2.01	1.30–3.12	73	39(53.4)	1.32	0.66–2.66
	T3	205	127(62.0)	2.22	1.44–3.43	73	32(43.8)	0.94	0.46–1.92
Joint [*]									
No	T1	195	56(28.7)	1.00		57	18(31.6)	1.00	
No	T2	160	50(31.3)	1.09	0.69–1.73	47	19(40.4)	1.50	0.66–3.40
No	T3	143	49(34.3)	1.26	0.79–2.02	47	17(36.2)	1.22	0.53–2.79
Yes	T1	152	62(40.8)	1.67	1.06–2.62	62	30(48.4)	2.07	0.97–4.43
Yes	T2	188	109(58.0)	3.26	2.12–5.02	73	39(53.4)	2.52	1.20–5.29
Yes	T3	205	127(62.0)	3.78	2.47–5.79	73	32(43.8)	1.73	0.81–3.71
P for Interaction of maternal OWO and C4-OH					0.035				0.544

OWO, overweight or obese. T, tertile. Non-OWO was defined as body mass index (BMI)<25kg/m^2; OWO was defined as BMI 25kg/m^2.

The regression models were adjusted for maternal age, race, smoking, education, and parity.

[*] Where the common reference group is non-OWO mothers with C4-OH in the low tertile.

The interaction of maternal prepregnancy OWO (Yes vs. No) and C4-OH (middle and top tertiles vs. low tertile) on child's odds of OWO was tested by adding a multiplicative term in the models.

Table 3.

Four-way decomposition analysis of the mediation and effect modification of maternal C4-OH on inter-generational overweight or obesity (OWO) among term births.

Effects	Estimate(se)	P value	Proportion attributed (se)	P value
Total effect	1.91(0.43)	<0.001	-	
Controlled direct effect (CDE)	1.40(0.33)	<0.001	0.73(0.08)	<0.001
Reference interaction (INT$_{ref}$)	0.15(0.11)	0.182	0.08(0.05)	0.135
Mediated interaction (INT$_{med}$)	0.34(0.13)	0.011	0.18(0.05)	<0.001
Pure indirect effect (PIE)	0.02(0.04)	0.545	0.01(0.02)	0.555
Overall Proportion Mediated (Total indirect effect)			0.19(0.05)	<0.001
Overall Proportion Attributable to Interaction			0.26(0.08)	0.002
Overall Proportion Eliminated			0.27(0.08)	0.001

CDE, Controlled direct effect; INT$_{ref}$, Reference interaction; INT$_{med}$, Mediated interaction; PIE; Pure indirect effect.

The CDE (the component due to neither mediation nor interaction) is the effect of the exposure on the outcome in the absence of the mediator. The INT$_{ref}$ (the component due to interaction but not mediation) is the interactive effect when the mediator is left to the level it would take in the absence of exposure. The INT$_{med}$ is the component due both to mediation and interaction, and the PIE is the component due to mediation alone, not interaction. Proportion eliminated is the effect due to interaction or mediation or both (=INT$_{ref}$+INT$_{med}$+PIE). Direct effect=CDE+ INT$_{ref}$; indirect effect= INT$_{med}$+PIE. The model was adjusted for maternal age, race, smoking, education, and parity.