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 ≥ 85th percentile for age and sex. The primary exposures were maternal prepregnancy OWO defined as BMI ≥ 25 kg/m² 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 the highest risk of OWO: OR = 3.78 (95%CI: 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 USA, 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 a growing recognition that childhood obesity may originate 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 intrauterine 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 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 a written informed consent.

As illustrated in the flowchart (Fig. 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 eight children who had missing body mass index (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/m²) and OWO (≥25 kg/m²). 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 were abstracted from the electronic medical record (EMR). Maternal diabetes status was classified as non-diabetic, gestational diabetes, and preexisting 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 (≥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 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 US national reference data [21]. OWO was defined as BMI ≥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 was 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 25 kg/m² (Fig. 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 (INTref), mediated interaction (INTmed), 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 INTref (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 INTmed 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 INTmed 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 to 18 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, and 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 (Fig. 1, 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 (Fig. 1a).

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 (Fig. 1b, 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 (Fig. 1b). 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 = 0.035). 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 = 0.016) (Table S5). However, the aforementioned associations were not observed among preterm births (Fig. 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
### Table 1 Characteristics of mother–child pairs in total sample and stratified by term vs. preterm births

| Maternal characteristic                          | Total sample | Term births | Preterm births | P value |
|--------------------------------------------------|--------------|-------------|---------------|---------|
| 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 (33.1)    |         |
| 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 age (years)                              | 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                             | 532 (39.3)   | 397 (39.3)  | 135 (39.4)    | 0.976   |
| OWO at age 6–9 years                             | 497 (46.7)   | 369 (46.4)  | 128 (47.6)    | 0.740   |
| OWO at age 10–18 years                           | 287 (47.7)   | 212 (47.6)  | 75 (47.8)     | 0.978   |

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

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

aData are presented as median (IQR)

bN = 1354
cN = 1064
dN = 602
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 Table 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 tertile of C4-OH combined with maternal OWO was strongly associated with higher child’s leptin and lower adiponectin/leptin 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.

Fig. 1 Smoothing plot of the relationship between gestational age and maternal plasma C4-OH levels stratified by maternal prepregnancy BMI categories (a), the differential relationships between maternal plasma C4-OH and the child’s risk of overweight or obesity (OWO) stratified by term vs. preterm births (Panels b, c). BMI, body mass index; OWO, overweight or obesity. Non-OWO was defined as BMI < 25 kg/m²; OWO was defined as BMI ≥ 25 kg/m².
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 versus 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, nonpregnant obese individuals had higher acylcarnitines than normal weight individuals [15]. More interestingly, we found that C4-OH was associated with both maternal and child OWO risk. C4-OH is a 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].

### 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 OWO | Term births (n = 1043) | Preterm births (n = 359) |
|--------------|------------------------|-------------------------|
|              | 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 | 19  | 40 (40.4)  | 1.33 | 0.57–3.10 |
|              | T3 | 143         | 49 (34.3)  | 1.37 | 0.85–2.21 | 17  | 36 (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 |
| Jointa       | 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 | 19  | 40 (40.4)  | 1.50 | 0.66–3.40 |
|              | No | T3 | 143         | 49 (34.3)  | 1.26 | 0.79–2.02 | 17  | 36 (36.2)  | 1.22 | 0.53–2.79 |
|              | Yes| T1  | 152        | 62 (40.8)  | 1.67 | 1.06–2.62 | 30  | 48 (48.4)  | 2.07 | 0.97–4.43 |
|              | Yes| T2  | 188        | 109 (58.0) | 3.26 | 2.12–5.02 | 39  | 34 (35.4)  | 2.52 | 1.20–5.29 |
|              | Yes| T3  | 205        | 127 (62.0) | 3.78 | 2.47–5.79 | 32  | 32 (43.8)  | 1.73 | 0.81–3.71 |
| P for interaction of maternal OWO and C4-OH |    |        | 0.035 | 0.544 |

Non-OWO was defined as body mass index (BMI) < 25 kg/m²; OWO was defined as BMI ≥ 25 kg/m².

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

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.

OWO: overweight or obese, T tertile

Where the common reference group is non-OWO mothers with C4-OH in the low tertile.
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 lend 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. Consistent with our findings, a previous longitudinal study reported a significant decline in short-chain acylcarnitine across the third 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 USA, 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 third trimester due to premature delivery. As such, preterm births had a shorter duration of exposure to maternal metabolites during the critical third 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].

**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 (INTeff)  | 0.15 (0.11)   | 0.182   | 0.08 (0.05)               | 0.135   |
| Mediated interaction (INTmed)   | 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 attributed to Interaction | 0.26 (0.08) | 0.002 |
| Overall proportion eliminated   | 0.27 (0.08)   | 0.001   |                          |         |

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 INTeff (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 INTmed 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 (= INTeff + INTmed + PIE). Direct effect = CDE + INTeff, indirect effect = INTmed + PIE. The model was adjusted for maternal age, race, smoking, education, and parity.

CDE controlled direct effect, INTeff reference interaction, INTmed mediated interaction, PIE pure indirect effect.
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 disproportionately affected by the obesity epidemic across the life span and generations. Analytically, we have applied advanced statistically methods; carefully considered epidemiological and clinical covariates 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 pre-pregnancy 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 toward the null. Finally, because this study was conducted in a high-risk US 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 (pre-conception, in-utero and infancy), which may in turn, impact their child’s life-long metabolic health.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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Inter-generational link of obesity in term and preterm births: role of maternal plasma acylcarnitines

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