Research Article
Genetic and Metabolic Diseases

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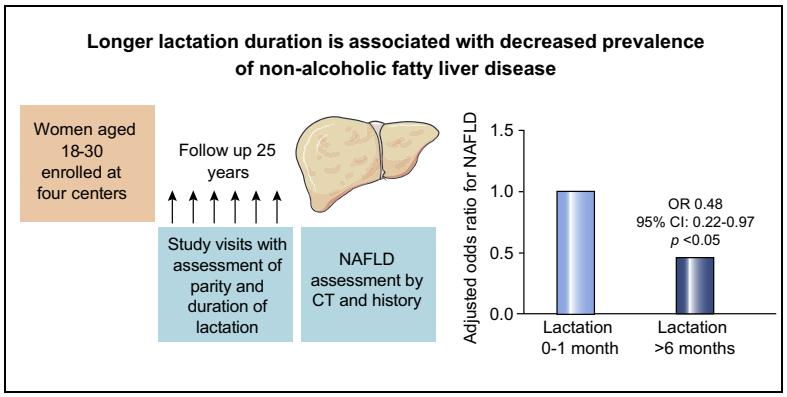
# Longer lactation duration is associated with decreased prevalence of non-alcoholic fatty liver disease in women

## Graphical abstract




## Highlights

- Lactation duration >6 months was protective against NAFLD in mid-life after adjustment for confounders.
- The benefits of increased lactation duration on weight and waist circumference mediated <1/4 of this benefit.
- Longer lactation duration may be an important lifestyle intervention to prevent the NAFLD during mid-life.

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## Lay summary

A longer duration of breastfeeding has been associated with multiple potential health benefits for the mother including reduction in heart disease, diabetes and certain cancers. In this study we found that breastfeeding for longer than 6 months was associated with a lower risk of non-alcoholic fatty liver disease in mid-life.

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# Longer lactation duration is associated with decreased prevalence of non-alcoholic fatty liver disease in women

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See Editorial, pages 13–14

**Background & Aims**: Lactation lowers blood glucose and triglycerides, and increases insulin sensitivity. We hypothesized that a longer duration of lactation would be associated with lower prevalence of non-alcoholic fatty liver disease (NAFLD), which is the leading cause of chronic liver disease in the United States.

**Methods**: Participants from the Coronary Artery Risk Development in Young Adults cohort study who delivered ≥ 1 child post-baseline (Y0: 1985–1986), and underwent CT quantification of hepatic steatosis 25 years following cohort entry (Y25: 2010–2011) were included (n = 844). The duration of lactation was summed for all post-baseline births, and NAFLD at Y25 was assessed by central review of CT images and defined by liver attenuation ≤ 40 Hounsfield Units after exclusion of other causes of hepatic steatosis. Unadjusted and multivariable logistic regression analyses were performed using an *a priori* set of confounding variables; age, race, education, and baseline body mass index.

**Results**: Of 844 women who delivered after baseline (48% black, 52% white, mean age 49 years at Y25 exam), 32% reported lactation duration of 0 to 1 month, 25% reported >1 to 6 months, 43% reported more than 6 months, while 54 (6%) had NAFLD. Longer lactation duration was inversely associated with NAFLD in unadjusted logistic regression. For women who reported >6 months lactation compared to those reporting 0–1 month, the odds ratio for NAFLD was 0.48 (95% CI 0.25–0.94; $p = 0.03$) and the association remained after adjustment for confounders (adjusted odds ratio 0.46; 95% CI 0.22–0.97; $p = 0.04$).

**Conclusions**: A longer duration of lactation, particularly greater than 6 months, is associated with lower odds of NAFLD in mid-life and may represent a modifiable risk factor for NAFLD.

**Lay summary**: A longer duration of breastfeeding has been associated with multiple potential health benefits for the mother including reduction in heart disease, diabetes and certain cancers. In this study we found that breastfeeding for longer than 6 months was associated with a lower risk of non-alcoholic fatty liver disease in mid-life.



## Introduction

Non-alcoholic fatty liver disease (NAFLD) is an increasingly common cause of cirrhosis and hepatocellular carcinoma and is on trajectory to become the most frequent indication for liver transplantation in the United States,[1,2] however therapeutic options are limited. While NAFLD is recognized as the hepatic manifestation of metabolic dysfunction, it also portends an increased risk for incident diabetes mellitus (DM) and metabolic syndrome suggesting a complex bidirectional relationship.[3,4]

We recently demonstrated that gestational DM (GDM), a condition of glucose intolerance during pregnancy, is a risk marker for NAFLD in mid-life in women.[5] In addition, other perinatal factors may potentially lower the risk of NAFLD. Lactation lowers maternal blood glucose, lipids and insulin concentrations through non-insulin facilitated cellular uptake of circulating glucose for milk production.[6] Furthermore, in-depth studies of glucose homeostasis in the post-partum period suggest that lactation also improves insulin sensitivity.[7] Lactation may also have favorable metabolic effects that persist post-weaning. A longer duration of lactation has been associated with lower future risk of developing the metabolic syndrome and type 2 diabetes mellitus in women of childbearing age, independent of changes in lifestyle behaviors, body weight, and other risk factors.[8–12] The biological mechanism behind this prolonged benefit is not clear. The association between lactation and increased weight loss is equivocal, however lactation may result in metabolically beneficial changes in blood lipids, regional fat mobilization, and/or reduced insulin secretion and resistance that may prevent beta cell exhaustion.[13,14] The impact of lactation on maternal risk of NAFLD is unknown. Therefore, we sought to evaluate if a longer lactation duration was associated with a lower prevalence of NAFLD in mid-life. Modification of

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the risk for NAFLD in childbearing women represents a novel opportunity to decrease disease burden on a large scale.

## Patients and methods

### Study population

The CARDIA study is a multicenter community-based longitudinal cohort study evaluating cardiovascular disease in young black and white adults 18–30 years of age from 4 US cities (Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA) in 1985–1986. Participants were not selected based on risk factors for metabolic disease and were recruited by random-digit dialing from total communities, census tract information, or from their healthcare plan. The study design has been published previously.[15] In 1985–1986 a total of 5,115 individuals (2,787 women, 50% black) were recruited and had scheduled follow-up at years 2, 5, 7, 10, 15, 20, and 25. The retention rate was 72% of the surviving cohort at year 25. Participants provided written informed consent at each examination, and institutional review boards from each field center (University of Alabama at Birmingham, Birmingham, Alabama; Northwestern University, Chicago, Illinois; University of Minnesota, Minneapolis, Minnesota; and Kaiser Permanente, Oakland, California) approved the study annually. From the 2,787 women enrolled at baseline we selected women who delivered one or more post-baseline children with lactation data available, who underwent computed tomography (CT) quantification of hepatic steatosis 25 years following cohort entry (n = 917). We excluded women with other potential causes of hepatic steatosis including alcohol use >2 drinks/day (n = 38), self-report of HIV or chronic hepatitis (n = 15), and medication use associated with steatosis; amiodarone, methotrexate, valproic acid, tamoxifen, steroids, and diltiazem (n = 20) (Fig. 1).

### Measurements

*Pregnancies and lactation status*

The number of pregnancies and births were assessed by self-report at each exam. Participants reported the number of pregnancies and births, length(s) of gestation, gestational hypertensive disorders, and GDM, as well as how the pregnancy ended (abortion, miscarriage, and live or stillbirths). Births were defined as pregnancies of ≥20 weeks gestation. Total parity was equal to the total number of live births. Women reported lactation duration for each prior birth at the year 7 exam, and for subsequent births at later exams in years 10, 15, 20 and 25. For each birth, women reported whether they had breastfed (yes or no). If they responded "yes", then they selected one of the following duration categories: <6 weeks, 6–11 weeks, 3–6 months, or >6 months. To calculate cumulative duration across births, we assigned the midpoint of each lactation category for each birth: 21 days for <6 weeks, 66 days for 6–11 weeks, 135 days for 3–6 months, and 210 days as the upper limit for >6 months. We summed the number of days to obtain the overall lactation duration for all birth(s) during the study period.

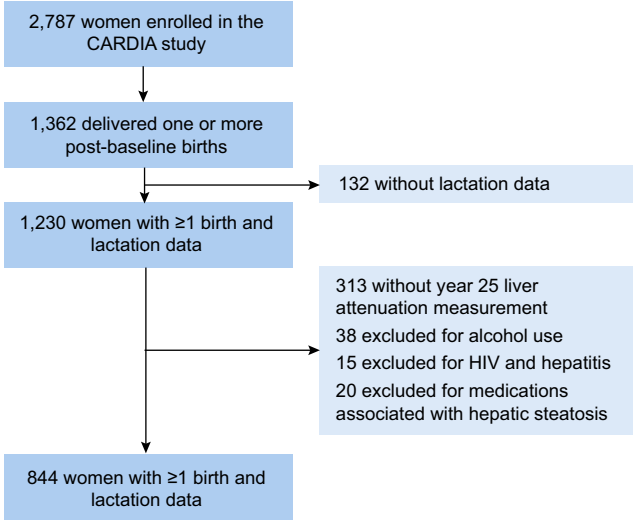


**Fig. 1. Cohort of CARDIA participants meeting inclusion and exclusion criteria.** Alcohol use defined as >14 drinks/week. Medications leading to exclusion; amiodarone, methotrexate, valproic acid, tamoxifen, steroids, or diltiazem.

### Assessment of hepatic steatosis

The CT protocol used a non-contrast abdominal CT scan performed using GE (GE 750HD (64) at Birmingham and GE LightSpeed VCT (64) at Oakland; GE Healthcare, Waukesha, Wisconsin) or Siemens (Sensation 64 at Chicago and Minneapolis Centers; Siemens Medical Solutions, Erlangen, Germany) multidetector CT scanners and has been published previously.[16] Quality control and image analysis were performed at a core reading center (Wake Forest University Health Sciences, Winston-Salem, North Carolina). CT diagnosis of hepatic steatosis was made by measuring liver attenuation (LA) in Hounsfield Units (HU). NAFLD was defined as an LA value ≤40 HU. LA was the average of 9 measurements on 3 CT slices of the right lobe of the liver. A cut-off of LA value ≤40 HU correlates with moderate-to-severe steatosis (>30%) in unenhanced CT scans in multiple studies.[17–19] The characterization of LA in this cohort used a dedicated workflow within the National Institute of Health's Center of Information Technology Medical Image Processing, Analysis, and Visualization application has previously published.[16] The interclass correlation coefficient between different readers on a random sample of 156 participants was 0.975 for LA, indicating high reproducibility of CT measured LA in this cohort.

### Risk factor measurements

Participant demographics, medical history, and alcohol use were obtained through standardized surveys. GDM was defined by self-report among those without overt diabetes before pregnancy based on CARDIA laboratory tests and medical diagnoses. Self-report of GDM has previously been validated in this cohort by review of prenatal glucose tolerance tests for a subsample of 165 women who delivered 200 births and revealed a sensitivity of 100% and specificity of 92%.[20] Education status was dichotomized to greater than high school compared to less than or equal to completion of high school. Medication use was determined through self-report and participants brought medications to study visits for verification. For laboratory parameters utilized in this study, participants were asked to fast for ≥8 h prior to venous blood draws at each visit from baseline to year 25. Diet was assessed by interviewer-administered CARDIA Diet History as described previously.[21,22] Briefly, foods were assigned to 1 of 166 food groups using the food grouping system devised by the University of Minnesota Nutrition Coordinating Center then further collapsed into 46 food groups based on similar

nutrient characteristics and comparability. The *a priori* diet classified the 46 food groups as beneficial, adverse or neutral and the *a priori* diet quality score was the sum of category scores 0–4 for the beneficial items plus scores in reverse order (4–0) for adverse foods. The theoretical maximum score was 132 with higher scores indicating a healthier dietary pattern. This was assessed at baseline. Assessments of physical activity levels were obtained using the interviewer-based CARDIA physical activity history, which covers 13 types of activities and has been published previously.[23] The physical activity score was expressed in exercise units and consisted of a sum of moderate intensity and vigorous intensity scores in exercise units, which are related to caloric expenditure. An increased score was consistent with more exercise. This was assessed at baseline and at year 25. Change in physical activity score was calculated as the difference from baseline to year 25. Change in body mass index (BMI), homeostatic model assessment of insulin resistance (HOMA-IR), and waist circumference were calculated as the difference from the exam before first post-baseline birth to year 25. HOMA-IR was not available at years 2 and 5. Incident DM was defined as the development of overt diabetes based on criteria between year 0 and year 25 as previously described.[20]

### Statistical analyses

A sample size calculation for >6 months lactation as a protective factor for NAFLD was performed with a 2-tailed alpha of 0.05 and beta of 0.2 and revealed that 283 women would be required per lactation group assuming a 4% prevalence of NAFLD associated with >6 months lactation and a 10% prevalence of NAFLD in the reference group (0–1 month lactation). With an approximately even distribution of women through 3 lactation categories, as seen in previously published data from CARDIA, the sample size of 844 is reasonable for this exploratory analysis. Baseline and follow-up differences in participant characteristics were compared by lactation duration in 3 categories; 0 to 1 month, >1 to 6 months, and >6 months with chi-square and ANOVA tests for significance. Logistic regression was used to evaluate the association between lactation duration categories and NAFLD at year 25. Linear trend tests were performed across lactation duration categories. Bivariate models assessed the association between variables chosen *a priori* as possible confounders. These covariates included age, race, education, baseline BMI. Confounding variables were selected for the final multivariable model by backwards elimination with $p$ value <0.05 used as the threshold for covariable inclusion after adjustment for post-baseline parity. Changes in BMI, HOMA-IR, and waist circumference and incident DM were evaluated as mediators of lactation duration and NAFLD at year 25. Mediation effects on the development of NAFLD were calculated as the product of standardized regression coefficients using the binary mediation package. Addition of baseline waist circumference to the final multivariable model was assessed to evaluate for residual confounding not captured by BMI. Addition of physical activity score and dietary assessment variables to the final multivariable model was assessed to evaluate confounding by lifestyle behaviors that may differ by lactation duration category. Effect modification by GDM status was evaluated by adding the cross-product terms to the final model, with $p$ values <0.10 used to identify statistically significant interactions. All $p$ values were 2-sided and statistical significance was defined as a $p$ value <0.05. Analyses were performed using STATA 13.1 (StataCorp, College Station, TX).

## Results

### Study cohort characteristics

Of the 844 women meeting inclusion criteria, 48% were black and 52% were white, with a median (IQR) age of 25 (6) years at baseline. Two-hundred and seventy-six women (32%) reported a lactation duration of 0 to 1 month, 209 (25%) reported >1 to 6 months, 359 (43%) reported >6 months. Fifty-four (6.4%) met the CT definition for NAFLD at year 25. Of the women with NAFLD, 43% breastfed for 0–1 month, 30% for >1 to 6 months and 28% for >6 months. As expected, women with NAFLD had higher BMI, HOMA-IR, triglycerides, waist circumference, more gestational diabetes and lower HDL-cholesterol compared to those without NAFLD (Table S1). Twenty-three (5.6%) black women and 31 (7.1%) white women had NAFLD at year 25. There were significantly more white women with longer lactation duration than black women. At baseline, women with longer lactation duration had lower BMI, HOMA-IR, triglycerides, and waist circumference, and increased physical activity (Table 1). At year 25, women with longer lactation duration had significantly lower BMI, HOMA-IR, triglycerides, total and LDL cholesterol and more post-baseline births and higher educational attainment, but GDM status did not differ by lactation duration during follow-up (Table 2).

**Table 1. Baseline exam (1985–86) characteristics of women with one or more births, by lactation duration category (n = 844).***

| Characteristic | 0 to 1 months (n = 276) | >1 to 6 months (n = 209) | >6 months (n = 359) | *p* value |
|---|---|---|---|---|
| Age, median (IQR) years | 23 (7) | 25 (6) | 24 (7) | 0.68 |
| Race, n (%) | | | | <0.01 |
| Black | 203 (74) | 102 (49) | 103 (29) | |
| White | 73 (26) | 107 (51) | 255 (71) | |
| BMI, median (IQR) kg/m$^2$ | 23.2 (6.9) | 22.5 (5.0) | 21.9 (3.9) | <0.01 |
| HOMA-IR, median (IQR) | 1.5 (0.8) | 1.4 (0.7) | 1.3 (0.7) | <0.01 |
| Total cholesterol, median (IQR) mg/dl | 172 (46) | 175 (42) | 173 (42) | 0.64 |
| LDL, median (IQR) mg/dl | 107 (40) | 109 (39) | 104 (36) | 0.34 |
| HDL, median (IQR) mg/dl | 53 (16) | 56 (17) | 56 (14) | 0.25 |
| Triglycerides, median (IQR) mg/dl | 61 (38) | 55 (28) | 54 (26) | <0.01 |
| Waist circumference, median (IQR) cm | 72 (13) | 70 (10) | 69 (8) | <0.01 |
| Physical activity score, median (IQR) | 226 (303) | 298 (334) | 366 (334) | <0.01 |
| *A priori* dietary quality score, median (IQR) | 59 (14) | 70 (16) | 73 (14) | 0.65 |

Differences in participant characteristics were compared by lactation duration in 3 categories; 0 to 1 month, >1 to 6 months, and >6 months with chi-square and ANOVA tests.
*All variables have <1% missing data, except HOMA-IR, for which n = 725 (86%). BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance.

**Table 2. Characteristics of women at year 25 by lactation duration (n = 844).***

| Characteristics | 0 to 1 months (n = 276) | >1 to 6 months (n = 209) | >6 months (n = 359) | *p* value |
|---|---|---|---|---|
| Education (high school or less), n (%) | 95 (34) | 31 (15) | 27 (8) | <0.01 |
| BMI, median (IQR) kg/m$^2$ | 31.6 (11.5) | 28.3 (11.3) | 27.1 (8.4) | 0.04 |
| HOMA-IR, median (IQR) | 2.3 (2.4) | 1.9 (2.2) | 1.6 (1.6) | <0.01 |
| Total cholesterol, median (IQR) mg/dl | 188 (46) | 193 (49) | 192 (43) | 0.04 |
| LDL-C, median (IQR) mg/dl | 108 (44) | 112 (43) | 109 (40) | 0.01 |
| HDL-C, median (IQR) mg/dl | 57 (19) | 62 (22) | 63 (23) | 0.07 |
| Triglycerides, median (IQR) mg/dl | 89 (58) | 81 (48) | 78 (50) | <0.01 |
| Waist circumference, median (IQR) cm | 93 (23) | 88 (24) | 84 (20) | 0.08 |
| Physical activity score, median (IQR) | 150 (289) | 259 (349) | 268 (328) | 0.79 |
| Number of post-baseline births, mean (SD) | 1.7 (0.9) | 1.5 (0.7) | 2.1 (0.9) | <0.01 |
| Gestational diabetes, n (%) | 31 (11) | 26 (12) | 47 (13) | 0.76 |
| NAFLD, n (%) | 23 (8.3) | 16 (7.7) | 15 (4.2) | 0.07 |

Differences in participant characteristics were compared by lactation duration in 3 categories; 0 to 1 month, >1 to 6 months, and >6 months with chi-square and ANOVA tests.
*All variables ≤ 1% missing data. BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; NAFLD, non-alcoholic fatty liver disease.

**Table 3. Association between characteristics of parous women and NAFLD at 25 years: unadjusted and multivariable adjusted odds ratios (95% CI).**

| Characteristic | Unadjusted OR (95% CI) | *p* value | Multivariable* OR (95% CI) | *p* value |
|---|---|---|---|---|
| Age (per 5 year) | 1.38 (0.94–2.02) | 0.10 | | |
| White race (*vs.* Black) | 1.29 (0.74–2.25) | 0.37 | 2.36 (1.24–4.50) | 0.01 |
| Total post-baseline parity (per birth)† | 0.66 (0.46–0.97) | 0.03 | 0.75 (0.51–1.11) | 0.15 |
| BMI (per 3 kg/m$^2$ increase) | 1.25 (1.10–1.43) | <0.01 | 1.30 (1.13–1.50) | <0.01 |
| Education >high school *vs.* ≤high school | 0.76 (0.39–1.49) | 0.43 | | |
| Lactation duration (0–1-month reference) | | | | |
| >1 to 6 months | 0.91 (0.47–1.78) | 0.79 | 0.84 (0.42–1.70) | 0.64 |
| >6 months | 0.48 (0.25–0.94) | 0.03 | 0.46 (0.22–0.97) | 0.04 |

*Multivariable model determined by backward stepwise elimination with race, BMI and lactation duration in final model.
† Forced into final model to adjust lactation duration for number of post-baseline births.
BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio.

### Lactation duration and prevalent NAFLD at year-25

NAFLD prevalence decreased with increasing lactation duration; 8.3% for 0 to 1 month, 7.7% for >1 to 6 months and 4.2% for >6 months. In unadjusted logistic regression models, the odds ratio (OR) of NAFLD at year 25 was lower, 0.48 (95% CI 0.25–0.94, $p = 0.03$) for those with >6 months lactation duration compared to 0 to 1 month (Table 3). A test for linear trend was statistically significant, $p = 0.03$. Any breastfeeding when compared to none did not have a statistically significant protective association against NAFLD at year 25 (OR 0.90; 95% CI 0.48–1.69; $p = 0.74$) consistent with a threshold effect for duration >6 months. In addition, lactation duration of >1 to 6 months was not associated with significantly lower odds of NAFLD (OR 0.91; 95% CI 0.47–1.78; $p = 0.79$).

Higher total post-baseline parity was associated with less NAFLD per additional birth (OR 0.66; 95% CI 0.46–0.97; $p = 0.03$). Higher baseline BMI was associated with higher odds of NAFLD (OR 1.45; 95% CI 1.17–1.81; $p < 0.01$). White race was associated with a trend towards higher odds of NAFLD (OR 1.29; 95% CI 0.74–2.25; $p = 0.37$), which was not statistically significant. White race was also associated with more education (89% *vs.* 74% >high school, $p < 0.01$), lower baseline BMI (mean ± SD; 22 ± 3 *vs.* 25 ± 6 kg/m$^2$; $p < 0.01$), older age (mean ± SD; 50 ± 3 *vs.* 48 ± 4 years; $p = <0.01$) and longer duration of breastfeeding (mean ± SD; 262 ± 227 *vs.* 118 ± 162 days; $p < 0.01$).

### Multivariable analysis of lactation duration and NAFLD at year 25

In the final fully adjusted multivariable model, lactation duration >6 months *vs.* 0 to 1 month lactation was associated with lower odds of NAFLD (aOR 0.46; 95% CI 0.22–0.97; $p = 0.04$) adjusted for baseline BMI, race and post-baseline parity. In addition, the test of trend across lactation categories remained statistically significant $p = 0.04$. Race and baseline BMI were significantly associated with NAFLD at year 25 in the final multivariable model and remained in the adjusted model after backward stepwise elimination (Table 3).

Addition of baseline waist circumference to the fully adjusted multivariable model to assess potential confounding from higher abdominal fat level at baseline had minimal impact on the lactation and NAFLD association (OR for >6 months lactation duration compared to 0 to 1 month was 0.48; 95% CI 0.23–1.01; $p = 0.06$) (Table 4), and the test for trend across lactation categories was slightly above the threshold for statistical significance, $p = 0.06$. Addition of baseline physical activity score and diet score to the fully adjusted multivariable model to assess for potential lifestyle confounders by lactation group had little effect on the association between increased lactation duration and lower odds of NAFLD (OR 0.50; 95% CI 0.23–1.09; $p = 0.09$), with $p = 0.08$ for the test for linear trend across lactation categories.

Mean change in BMI and waist circumference were inversely related to lactation duration ([BMI −0.87 kg/m$^2$; 95% CI −1.81 to 0.08; $p = 0.07$] and [waist circumference −1.86 cm; 95% CI −3.90 to 0.17; $p = 0.07$] for >6 months lactation duration compared to 0–1 month in adjusted linear regression). Inclusion of change in BMI from pre-conception visit to follow-up in the fully adjusted multivariable model slightly attenuated the association between >6 months lactation and NAFLD at year 25 (OR 0.51; 95% CI 0.23–1.11; $p = 0.09$). Higher change in BMI was associated with NAFLD (OR per 3 kg/m$^2$ increase was 1.77;

**Table 4. Multivariable models for NAFLD for >6 months lactation compared to 0–1 months of lactation.**

| Model | OR NAFLD | 95% CI | *p* value* |
|---|---|---|---|
| Lactation >6 months, unadjusted | 0.48 | (0.25–0.94) | 0.03 |
| Model 1: Lactation duration categories + Baseline BMI + race + post baseline parity | 0.46 | (0.22–0.97) | 0.04 |
| Model 2: Model 1 + Baseline waist circumference | 0.48 | (0.23–1.01) | 0.06 |
| Model 3: Model 1 + Baseline diet & physical activity | 0.50 | (0.23–1.09) | 0.09 |
| Model 4: Model 3 + Baseline HOMA-IR | 0.45 | (0.21–0.98) | 0.04 |
| Model 5: Model 1 + Change in BMI | 0.51 | (0.23–1.11) | 0.09 |
| Model 6: Model 1 + Change in HOMA-IR† | 0.44 | (0.20–0.97) | 0.04 |
| Model 7: Model 1 + Change in waist circumference | 0.51 | (0.23–1.14) | 0.10 |
| Model 8: Model 1 + Change in physical activity | 0.46 | (0.22–0.97) | 0.04 |

*Calculated by logistic regression.
† Sample size limited to 716 (85%), due to missing data.
BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; NAFLD, non-alcoholic fatty liver disease; OR, odds ratio.

95% CI 1.53–2.04; $p < 0.01$). Binary mediation analysis suggests that changes in BMI may account for 20% of the association between lactation and NAFLD. Addition of change in waist circumferences slightly attenuated the association between breastfeeding duration >6 months and NAFLD (OR 0.51; 95% CI 0.23–1.14; $p = 0.10$) and lactation category, with $p = 0.07$ for test of trend across lactation categories. Higher change in waist circumference was associated with NAFLD (OR per cm increase was 1.11; 95% CI 1.08–1.14; $p < 0.01$). Binary mediation analysis suggests that changes in waist circumference may account for 23% of the association of lactation with NAFLD.

Addition of changes in HOMA-IR did not significantly affect the association between lactation duration and NAFLD nor was lactation duration category associated with changes in HOMA-IR, $p = 0.98$. Adding change in physical activity to the fully adjusted model did not significantly affect the association between breastfeeding duration >6 months and NAFLD (OR 0.46; 95% CI 0.22–0.97; $p = 0.04$). We also adjusted for incident DM during the 25-year follow-up in the multivariable model, however it had minimal impact on the association between lactation duration >6 months and NAFLD (OR 0.47; 95% CI 0.22–1.00; $p = 0.05$).

Additionally, interaction terms for lactation duration >6 months and GDM status were not statistically significant ($p = 0.67$). As race was associated with multiple socioeconomic and metabolic factors we performed sensitivity analysis evaluating the association between lactation duration >6 months and NAFLD specifically for white (OR 0.34; 95% CI 0.14–0.85; $p = 0.02$) and black women (OR 0.48; 95% CI 0.13–1.74; $p = 0.26$). Finally, among women with any lactation duration, we evaluated the effect of a 90 day increase in lactation duration as a continuous variable in the fully adjusted model (n = 639) and found a trend towards decreased NAFLD with increasing lactation duration that was not statistically significant (OR 0.87; 95% CI 0.71–1.08; $p = 0.21$).

## Discussion

Our findings provide evidence that longer duration of lactation, particularly >6 months compared to never or very short lactation (<1 month), is associated with approximately half the odds of prevalent NAFLD in parous women in mid-life. This association was minimally affected by other risk factors for NAFLD such as race and baseline pre-pregnancy BMI and HOMA-IR. This is the first study to evaluate the relationship between lactation duration and NAFLD and to show that lactation may be an important lifestyle intervention that can be targeted to parous women to prevent the development of NAFLD during mid-life. In mediation analysis, changes in body size and waist circumference accounted for less than one-fourth of the inverse association of lactation and NAFLD, suggesting any potential benefit of lactation may go beyond improvement in weight.

Long-term benefits of lactation on maternal metabolism are well described, including lower risk of developing the metabolic syndrome more than a decade later,[8] as well as atherosclerosis,[24] cardiovascular disease and type 2 diabetes during mid to later life.[25,26] The physiologic stress of normal pregnancy is characterized by elevations in circulating triglycerides and greater insulin resistance that reach a peak by mid-gestation. The benefits of lactation may counteract the adverse effects of pregnancy by increasing the maternal basal metabolic rate, mobilizing fat stores, increasing insulin sensitivity and persistent favorable effects on blood lipids, particularly lowering of maternal circulating triglycerides and lack of decline in HDL-C, a strong risk factor for type 2 diabetes in women.[27] Previous studies of CARDIA women have demonstrated lasting effects of lactation on maternal metabolic factors, which may be responsible for the beneficial impact of lactation on NAFLD.

In our study, the only baseline confounding risk factors associated with NAFLD on multivariable analysis were race and BMI. Both factors demonstrated strong associations with lactation duration and NAFLD. Black race is protective against NAFLD and may be related to genetic factors including patatin-like phospholipase domain containing protein 3 (*PNPLA3*) genotype.[28] The association between race and NAFLD is complex and affected by significant socioeconomic differences including less education, physical activity and poorer diet quality in the black women in this study. Furthermore, metabolic differences including higher BMI, waist circumference and HOMA-IR but lower fasting triglycerides in the black women may confound the relationship with NAFLD. We performed sensitivity analysis restricted to white women and the protective association between lactation duration >6 months and NAFLD persisted. In sensitivity analysis restricted to black women the direction and magnitude of the association were similar but no longer reached statistical significance. This may be related to an attenuated association in black women, or to a lack of precision resulting from the smaller sample size in this sensitivity analysis. Overall and abdominal obesity have been inversely associated with lactation duration and are also associated with NAFLD.[29,30] We evaluated change in BMI, HOMA-IR, and waist circumference as possible mediators of the impact of lactation duration on NAFLD. Addition of change in BMI or waist circumference to the adjusted model resulted in mild attenuation of the association between lactation duration and NAFLD, and lactation duration showed only borderline associations with these

changes. This suggests a possible mechanism by which lactation duration may affect NAFLD prevalence. Further study of the impact of lactation on regional body fat distribution are required. Because BMI does not adequately capture regional fat distribution, we added waist circumference, an indirect measure of abdominal fat mass, to our adjusted model. However, this had minimal impact on the lactation duration and NAFLD association. Change in weight and body composition may account for some of the effect of lactation on decreased NAFLD, however, longitudinal studies have not demonstrated a clear association between lactation and beneficial changes in body composition.[6] Furthermore, weight change only minimally mediated the association between lactation duration and a decreased risk of incident DM in a separate analysis of the CARDIA cohort.[12,25] Future studies should evaluate weight independent effects of lactation on lipid metabolism and their impact on NAFLD.

While our study describes longer lactation duration as a novel, modifiable factor associated with a decreased risk of NAFLD, we acknowledge certain limitations. The lack of liver tests including aminotransferases and measures of synthetic function is a limitation of our study, however, laboratory testing is neither sensitive nor specific for the diagnosis of NAFLD.[31] We excluded secondary causes of hepatic steatosis (*e.g.* alcohol, medications, and concomitant viral hepatitis) through detailed surveys but could not take into account other potential disease modifiers including coffee and fructose intake, antibiotic use and the gut-liver axis or other secondary causes of fatty liver disease. Our study quantitated duration of lactation by self-report at five time-points prospectively over a 25-year period. However, we did not have data on exact duration of lactation or lactation intensity. Assessment for NAFLD was only performed at the year 25 follow-up visit and limited our ability to assess for incident NAFLD, however, morbidity and mortality in NAFLD is most likely to occur at older ages, making prevalent NAFLD in mid-life a clinically relevant outcome. NAFLD was assessed by LA on non-contrast CT scan. Liver biopsy, the gold standard for diagnosis of NAFLD, is invasive and impractical to scale for a screening study of this size with a low prevalence of NAFLD. We chose our LA cut-off which showed excellent specificity but lower sensitivity for the detection of NAFLD with liver histology as the reference.[19] Maintaining high specificity reduces the impact of measurement bias in our study,[32] however, it also limits our ability to detect lesser degrees of pathologic steatosis between 5% and 30%. Our cohort also did not include Hispanic women or data on genetic polymorphisms associated with NAFLD prevalence and severity including *PNPLA3* and *TM6SF2*. The combination of a highly specific but less sensitive cut-off points in LA, lack of inclusion of Hispanic women and CT assessment at a median age of 49 when many women remained pre-menopausal likely contributed to the low prevalence of NAFLD in our study. Unfortunately, a minority of women with NAFLD with advanced fibrosis may have lesser degrees of steatosis and could be misclassified. Future studies should consider alternate non-invasive methods including liver stiffness measurements or clinical prediction rules to assess the association between lactation duration and NAFLD severity. Finally, women who lactated for longer than 6 months may have healthier lifestyles, however, we evaluated self-reported diet and physical activity, which did not materially change findings. In addition, despite adjustment for age, race, obesity and education the strong protective association between lactation duration and NAFLD remained. Nevertheless, residual confounding cannot be ruled out.

In conclusion, our study shows that increased duration of lactation, particularly greater than 6 months, is inversely associated with the odds of NAFLD in middle-aged women after adjusting for socio-demographics and key confounding factors. This work adds to the growing list of potential long-term maternal health benefits of lactation including decreased risk of breast and ovarian cancer, metabolic syndrome and cardiovascular disease. Recently, Ayonrinde and colleagues demonstrated that longer lactation duration may decrease the risk of NAFLD in offspring[33] and here we demonstrate a maternal benefit that we hypothesize may be related to lasting effects on body fat distribution, circulating lipids and insulin sensitivity. Promotion of breastfeeding among parous women during the perinatal period may represent a unique opportunity to decrease the prevalence of NAFLD.

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## Conflicts of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

## Authors' contributions

Veeral H. Ajmera: Study concept and design, Analysis and interpretation of data, Drafting of Manuscript, Critical Revision. Norah A. Terrault: Study concept and design, interpretation of data, Critical Revision. Lisa B. VanWagner: Interpretation of data, Critical Revision. Monika Sarkar: Interpretation of data, Critical Revision. Cora E. Lewis: Collection and interpretation of data, Critical Revision. John J. Carr: Collection and interpretation of data, Critical Revision. Erica P. Gunderson: Study concept and design, Analysis and interpretation of data, Critical Revision.

## Supplementary data

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