Published in final edited form as:

Am J Obstet Gynecol. 2014 October ; 211(4): 424.e1–424.e7. doi:10.1016/j.ajog.2014.06.004.

Effect of lactation on maternal postpartum cardiac function and adiposity - a murine model

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Abstract

OBJECTIVE—Lactation is associated with reduction in maternal metabolic disease and hypertension later in life; however, findings in humans may be confounded by socioeconomic factors. We sought to determine the independent contribution of lactation on cardiovascular parameters and adiposity in a murine model.

STUDY DESIGN—Following delivery, CD-1 female mice were randomly divided into two groups: lactated (L, nursed pups for 3 weeks, n=10), and nonlactated (NL, pups were removed after birth, n=12). Blood pressure (BP) was assessed prepregnancy and at 1 and 2 months postpartum. Visceral (VAT) and subcutaneous adipose tissue (SAT) determined by computed tomography, and left ventricular ejection fraction (EF), cardiac output (CO) and the E/A ratio determined by micro-ultrasound were evaluated at 1 and 2 months postpartum. Results were analyzed using Student's t-test (significance: $P<0.05$).

RESULTS—We observed a significantly different maternal BP at 2 months postpartum with relatively greater BP in NL (systolic BP: NL 122.2±7.2 vs L 96.8±9.8 mmHg; P=0.04; diastolic BP NL 87.0±6.8 vs L 65.9±6.2 mmHg; P=0.04). VAT was significantly increased in NL mice at 1

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DISCLOSURE: The authors report no conflict of interest.

DISCLAIMER: The views expressed in this presentation are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, or the United States Government.

PREVIOUS PRESENTATION: Presented at the 34th annual meeting of the Society for Maternal-Fetal Medicine, New Orleans, LA, Feb. 3–8, 2014.

CONTACT INFORMATION: reprints will not be available

(22.0±4.1 vs 10.7±1.8 %, P=0.04) and 2 months postpartum (22.9±3.5 vs 11.2±2.2 %, P=0.02), while SAT did not differ between the groups. At 2 months postpartum, EF (51.8±1.5 vs 60.5±3.8%; P=0.04), CO (14.2±1.0 vs 18.0±1.3ml/min; P=0.02) and MV E/A (1.38±0.06 vs 1.82±0.13; P=0.04) were significantly lower in NL mice than L mice.

CONCLUSION—Our data provide evidence that interruption of lactation adversely affects postpartum maternal cardiovascular function and adiposity.

Keywords

adipose; blood pressure; CD-1 mouse; lactation; maternal health

1. INTRODUCTION

The benefits of breastfeeding for the health of the child are well described, including a reduction in infectious disease, childhood obesity, and may improve neurocognitive function.[1–3] Animal models and intensive studies of human milk components have elucidated many of the mechanisms through which breast milk affects infectious morbidity risk; however, the mechanisms underlying associations between lactation and maternal health remain to be determined. Multiple observational studies have linked lactation with reduced *maternal* metabolic disease in later life. Longer duration of lactation is associated with reduced risk of type 2 diabetes,[4–6] hypertension,[4,7,8] breast cancer,[9] hyperlipidemia,[4,10] myocardial infarction,[4,11] cardiovascular disease,[4] and metabolic syndrome.[12,13]

Yet these observational studies have several confounding factors. Mothers that breastfeed differ from mothers who formula feed: they are wealthier, better educated, less likely to smoke, and more likely to engage in other beneficial health behaviors.[14–15] In addition, obesity is associated with decreased breastfeeding initiation and duration, and women who are prone to develop metabolic syndrome may have difficulty with lactogenesis.[16,17] Breastfeeding may therefore be a marker for maternal metabolic health, rather than a mechanism conferring reduced risk of disease. Thus, residual or unmeasured confounding factors, rather than breastfeeding itself, may explain observed associations between breastfeeding and maternal health outcomes. Currently, no data exist from randomized clinical trials in US populations to support or refute a causal association between breastfeeding and cardiovascular disease or adiposity in mothers.

Animal models are essential to quantify effects of lactation on later maternal health because lactation duration is assigned by experimental design and is therefore not confounded by other maternal behaviors. The very few studies in rodents have demonstrated that pregnancy without subsequent lactation results in an increase in fat content, lipoprotein lipase activity, larger adipocytes, altered glucose levels, and insulin resistance.[18–20]

Our objective was to determine the independent associations of lactation with subsequent blood pressure, cardiac function and adiposity in an *in vivo* mouse model.. We hypothesized that interruption of lactation would lead to elevated blood pressure, increase in visceral adipose tissue, and diminished cardiac function.

2. MATERIALS AND METHODS

2.1 Animals

The Institutional Animal Care and Use Committee (IACUC) at the University of Texas Medical Branch at Galveston approved the study protocol. Three to four week old CD-1 mice were obtained from Charles Rivers Laboratories (Wilmington, MA). The animals were housed in a temperature and humidity controlled facility with automatically controlled 12:12-hour light and dark cycles. Mice were allowed to consume regular chow and drinking solution ad libitum. Certified personnel and veterinary staff provided regular maintenance and animal care according to IACUC guidelines. The animals were sacrificed by carbon dioxide inhalation according to the IUCUC and American Veterinary Medical Association guidelines.

CD-1 mice have an average lifespan of 2 years. They are ready for breeding at about 5–8 weeks of age. Estrus occurs every 4 to 5 days. Pregnancy lasts about 20–21 days. Mice deliver somewhere between 10 to 16 fetuses. Females could be bred right after delivery or 1 week after weaning pups. Pups are typically weaned by 21 days of age.

2.2 Breeding

Male CD1 mice were placed with individual 6 week old females overnight for breeding. Pregnant female CD1 mice were randomized to lactated (L group, n=10) or nonlactated (NL group, n=12) groups. Litter mate status at the time of randomization of animals to NL versus L was unknown. Numbers of animals were chosen based on our previous studies.[21] In NL group, pups were removed after delivery and the pups in the L group weaned at 21 day of age.

2.3 Maternal data

Since this was an exploratory study, maternal parameters were obtained before pregnancy and in immediate postpartum period – 1 and 2 months post delivery. Because of constricted time frame for obtaining maternal parameters and to avoid stress due to multiple manipulations, the estrus cycle during measurements was not assessed. Maternal weight was recorded at baseline (before pregnancy), 1- and 2-months postpartum. Tail vein blood (100 μL) was collected from all dams after overnight fasting. Blood was centrifuged for 20 minutes at 3000 rpm and serum was collected and stored at −80°C until the time of testing. Glucose was measured with an OneTouch Ultra glucometer (LifeScan Inc, Milipitas, CA) after overnight fasting. Fasting serum LDL, HDL, and triglycerides were determined using a quantitative colorimetric assay (BioAssay Systems, Hayward, CA) according to the manufacturer's instructions and were interpreted by an automated spectrophotometer (Fusion 5.0; Ortho Clinical Diagnostics, Rochester, NY). All samples were run in duplicate.

2.4 Blood Pressure

Blood pressure was determined in vivo using CODA non-invasive system (Kent Scientific, Torrington, CT) before pregnancy and 1 and 2 months postpartum. In vivo volume-pressure recording (VPR) via tail following previously validated methods[22] obtains measurement of systolic and diastolic blood pressure with ability to detect changes in tail blood volume in up

to eight mice simultaneously.[23] To obtain these measures, mice were first acclimated to restraints followed by 20 cycles of blood pressure measurement. Systolic blood pressure (SBP) was determined from the point in which tail blood volume increases. Diastolic blood pressure (DBP) was indicated by the occlusion cuff pressure at which the blood flow into the tail equilibrates. CODA has been validated with telemetry with 99% correlation.[24]

2.5 Cardiac Function

Echocardiography using Vevo 770 high-resolution system (VisualSonics, Toronto, Ontario, Canada) with 30 MHz transducers was performed at 1 and 2 months postpartum. Animals were anesthetized using 1–2% isoflurane in oxygen delivered by face mask. Abdominal hair was removed with chemical hair removal lotion prior to ultrasound. Animals were placed on a warmed platform for maintaining optimal physiological conditions integrated with electrocardiogram monitoring, heart rate, core temperature, and respiration. M-mode, B-mode, and pulsed Doppler were used with a 30-micrometer resolution and an ability to capture 240 frames per second.

Systolic function was evaluated by left ventricular ejection fraction obtained at the short axis cardiac view using M-mode by obtaining the end diastolic diameter (EDD) and end systolic diameter (ESD).[25–27] Ejection fraction was equal to $(EDD^3 - ESD^3)/EDD^3$. Diastolic function was assessed with mitral valve E/A ratio from the 4-chamber cardiac view where E represents peak velocity and A - the atrial kick during diastole. In early stage diastolic dysfunction, the E peak velocity decreases due to left ventricular stiffness, which results in a reduced E/A ratio.

Cardiac output was determined by measuring the left ventricular outflow diameter from the parasternal long axis view to get the cross-sectional area. Then, the blood flow per unit time through the aorta was used to determine the velocity time integral. The product of these and heart rate resulted in cardiac output (CO=CSA x VTI x HR). Total peripheral resistance (TPR) was calculated by mean arterial pressure divided by cardiac output.

2.6 Visceral and Subcutaneous Adiposity

In-vivo computed tomography with Inveon (Siemens Preclinical Solutions, Knoxville, TN) was used at 1 and 2 months postpartum to assess maternal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT).[28–31] Mice were anesthetized with ketamine (Ketalar, Parke-Davis, Morris Plains, NJ) and xylazine (Gemini, Rugby, Rockville Center, NY) intraperitoneally. Imaging parameters were: voltage 70 kV, current 500 μA, resolution 0.107 mm, exposure time 1000 ms, 520 steps, and 360 degrees of rotation. Scan time was approximately 15 minutes per mouse. Mice were given supplemental oxygen via nose cone during scanning. The transverse views on micro-CT images (1 per animal) at the level of the 5^{th} lumbar vertebra were selected for VAT analysis, and images at the level of the 6^{th} were used for SAT analysis.[30–31] The total body area in cross-section and areas of VAT and SAT were measured using Inveon Research Workplace software. The % VAT and SAT was calculated from the total body area imaged in cross-section.

2.7 Statistical Analysis

For statistical analysis Student *t* or Mann-Whitney *U* tests were performed as appropriate using GraphPad Prism version 6.0c for Mac OSX (GraphPad Software, San Diego, CA, www.graphpad.com). Since this was an exploratory study, comparisons were only performed between the lactated and nonlacted groups at either two (1 and 2 months pospartum) or three (prepgregnancy, 1, and 2, months postpartum) time points. Due to technical problems, such as failed blood pressure, CT or US recordings and death of animals during longitudinal study, numbers of mice in final analyses varied between 5 and 12. A probability value (P-value) of less than or equal to 0.05 was considered statistically significant.

3. RESULTS

3.1 Weights

There was no difference in prepregnancy weight between L and NL mice (Table 1, P=0.39). At 1 month postpartum, NL mice weighed significantly less (Table 1, P=0.004). By 2 months postpartum, this difference in weight resolved (Table 1, P=0.2).

3. 2 Blood Analytes

There was no difference in glucose, HDL, LDL, or triglycerides at 1 or 2 months postpartum (Table 1).

3.3 Blood Pressure

There was no difference in SBP or DBP at baseline between the two groups (Figure 1A and B, P=0.65 and P=0.39, respectively). At 1 month postpartum, a trend was seen for higher SBP and DBP in NL group (Figure 1,A and B, P=0.53 and P=0.51, respectively). At 2 months postpartum, the SBP and DBP became significantly different with a relatively greater BP in NL mice (Figure 1, A and B, P=0.04 for both).

3.4 Cardiac Function

Cardiac output, while the same at 1 month, was also lower among the nonlactated group at 2 months postpartum (Figure 4, P=0.27 and P=0.02, respectively). There was no statistically significant difference in heart rate between lactated and nonlactated mice (370±12 vs 348±14 bpm, P=0.29). Total peripheral resistance, while the same at 1 month postpartum, was different and relatively higher in the nonlactated mice at 2 months postpartum (Figure 1C, P=0.95 and P=0.05, respectively).

Systolic function, as evaluated with ejection fraction, was similar between groups at 1 month postpartum, but, while it remained the same in the lactated group, it was lower in the nonlactated group at 2 months postpartum (Figure 2B, P=0.85 and P=0.04, respectively). Mitral valve E/A ratio, similarly, was no different at 1 month postpartum and significantly lower at 2 months postpartum in the nonlactated group, indicative of diastolic dysfunction (Figure 2C, P=0.48 and P=0.04, respectively).

3.5 Visceral and Subcutaneous Adiposity

Subcutaneous adipose tissue determined via micro-CT was not different at 1 or 2 months postpartum (Figure 3A, P=0.44 and P=0.92 respectively) between the groups. However, the percent of visceral adipose tissue was significantly higher at 1 and 2 months postpartum in the nonlactated group (Figure 3B, P=0.04 and P=0.02, respectively).

4. COMMENT

In an animal model, we found that interruption of lactation adversely affected maternal cardiovascular function and increased visceral adipose tissue in the immediate postpartum period. In this study we demonstrate that mice that did not lactate had relatively greater systolic and diastolic blood pressure and concomitant total peripheral resistance at two months postpartum when compared to lactated animals. Cardiac function was preserved after pregnancy and lactation while a diminished systolic and diastolic function was noted in mice that did not lactate. The increase in mean arterial pressure and reduction in cardiac output are both indicative of higher total peripheral resistance in the mice that did not lactate. Moreover, a relatively greater accumulation of visceral adipose tissue was found in postpartum nonlactated mice. Thus, our data suggest a direct harmful effect of interrupting lactation on immediate postpartum maternal cardiovascular function and adiposity.

Several limitations of our study need to be noted. First, this is an animal model involving lactating dams with a multiple pups rather than a single infant as in humans. The greater metabolic load in this animal model likely intensifies differences between lactated and nonlactated animals compared to humans. Secondly, our model used complete separation from pups in the nonlactated condition, and thus did not evaluate the effect of separation from the effects of suckling on maternal physiology. Studies among breastfeeding women have shown that lactation immediately before a stressor presentation reduces HPA axis activation more than holding an infant without breastfeeding, suggesting that the act of lactation impacts stress reactivity independent of physical contact with the infant.[32] Thus, differences seen between two animal groups in our model could be confounded by the stress of separation in maternal physiology. Thirdly, the follow up only during immediate postpartum period in our study limits extrapolation of results to long-term maternal effect.. Fourth, although several parameters were similar at 1 month postpartum, relatively greater or lesser values in the non lactated group were noted only at 2 months postpartum. We propose that differences become evident due to delayed effect of lactation on cardiovascular function and adiposity. Further investigations are needed, to address limitations of the current study.

It had been proposed that lactation plays a central role in mobilizing accumulated fat stores and “resetting” maternal metabolism.[33–35] The “reset hypothesis” links lactation with a protective effect on maternal health.[33] During pregnancy, dramatic changes occur in a woman’s metabolism as she accommodates the demands of “metabolizing for two.”[36] These well-described increases in visceral fat, insulin production, insulin resistance, and circulating lipid levels both support the developing fetus and allow accumulation of energy stores for lactation.[37–38] However, if no lactation occurs, maternal metabolism does not return to prepregnancy state and results in a negative impact on the mother’s health. Our study is a

first step towards providing evidence for the “reset hypothesis”. Further studies are needed to support or refute this theory.

Although there is an initial increase in weight in the mice that lactated at 1 month postpartum, we did not find difference in weight at 2 months postpartum. This was likely an initial effect of lactation as it had only been 1 week since the animals had weaned. The association between lactation and postpartum weight loss varies among human studies.[1,39–44] After birth, lactation utilizes the fat stores and maternal metabolism returns to baseline. However, if lactation does not occur, the metabolic changes of pregnancy persist leading to cardiometabolic disease later in life. Visceral adiposity was decreased in the lactated group both at 1 and 2 months postpartum. Thus, the increased visceral adipose tissue among nonlactated animals in our model due to lactation may increase risk for metabolic disease later in life.[33] Visceral adipose tissue has been demonstrated to correlate strongly with metabolic syndrome.[45–47] In the absence of the caloric demands of breastfeeding, our results suggest that visceral adipose tissue accumulated during pregnancy persists, with adverse affects on long-term maternal health.[37, 48, 49]

Although we found no difference in HDL at 2 months postpartum, there appears to be a trend with higher HDL among the group that lactated. Pregnancy is a state of relative hyperlipidemia, and cholesterol and triglyceride levels fall postpartum. Compared with their nonlactating peers, breastfeeding women have lower insulin, glucose, and triglyceride along with higher HDL levels.[11, 50–54] In a small human study, breastfeeding was associated with favorable changes in HDL that persisted after weaning.[11]

The echocardiographic changes seen in our model would unlikely lead to overt disease; however, these changes could contribute to future cardiovascular disease. Mothers who do not breastfeed have been shown to have an increase in subclinical heart disease compared with those who lactated, including an increase in aortic calcification and coronary artery calcification.[55]

The results of this study suggest an effect of lactation on immediate postpartum maternal cardiac function and adiposity. Further study is required to demonstrate a long-term effect of lactation on maternal health. Mechanisms involved in this protective effect could lead to novel preventive strategies to decrease cardiovascular disease in women. In addition, enabling more women to achieve their breastfeeding goals may improve health across two generations[56].

Acknowledgments

FINANCIAL SUPPORT:

Dr. Egle Bytautiene, is supported by a research career development award (K12HD052023: Building Interdisciplinary Research Careers in Women’s Health Program-BIRCWH) from the National Institute of Allergy and Infectious Diseases (NIAID), the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), and the Office of the Director (OD), National Institutes of Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIAID, NICHD, OD, or the National Institutes of Health.

This study was conducted with the support of the Institute for Translational Sciences at the University of Texas Medical Branch, supported in part by a Clinical and Translational Science Award (UL1RR029876) from the National Center for Research Resources, National Institutes of Health

We would like to express gratitude to Phyllis Gamble and Esther Tamayo for their assistance with breeding, blood pressure assessment, weight, and blood draws. We thank Talar Kechichian, BS, MS for her expertise and assistance with HDL/LDL and triglycerides and Jingna Wei, MD, for her help with computed tomography.

We would also like to recognize Maged Costantine, MD for his help with the development of the manuscript and Massoud Motamedi, PhD for the unlimited access to the Bioengineering Center Facilities and equipment. All acknowledged are University of Texas Medical Branch employees at Galveston, Texas. Funding was received from the National Institute of Health NIH UL1RR029876 AND K12HD052023 (BIRCWH) grants.

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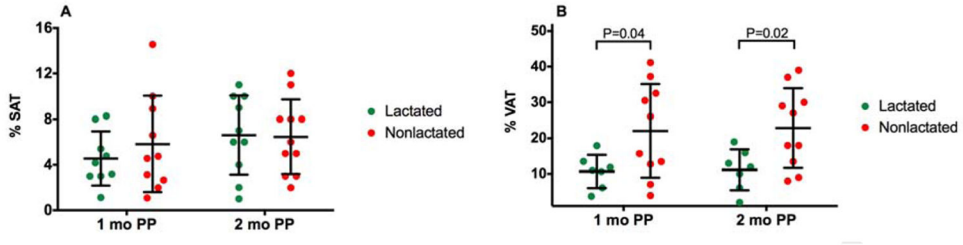

Figure 1. Blood Pressure

Systolic blood pressure (A, SBP), diastolic blood pressure (B, DBP) and total peripheral resistance (C, TPR) in dams that lactated and did not lactate. Data are expressed as dot plot with a line at the mean and whiskers showing standard deviation.

PP, postpartum

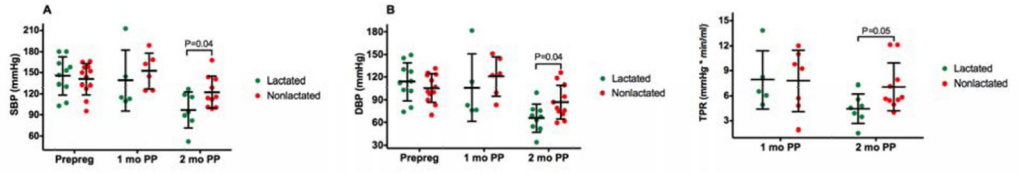

Figure 2. Ejection Fraction

Cardiac output (A), feft ventricular ejection fraction (B) and left ventricular E/A ratio at mitral valve (C) of dams that lactated and did not lactate. Data are expressed as dot plot with a line at the mean and whiskers showing standard deviation. PP, postpartum

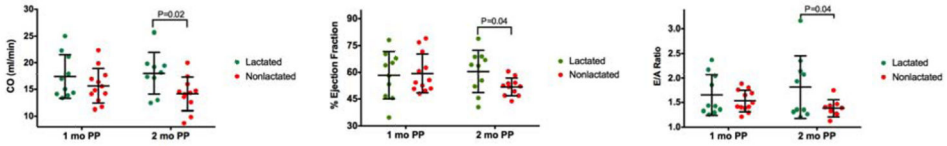

Figure 3. Subcutaneous and Visceral Adipose Tissue

A. % subcutaneous adipose tissue and B, % visceral adipose tissue of dams at 1 and 2 months postpartum that lactated and did not lactate. Data are expressed as dot plot with a line at the mean and whiskers showing standard deviation. PP, postpartum; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue

Table 1

Characteristics of dams

Characteristic	Lactated (n=10)	Nonlactated (n=12)	P value
Maternal weight, g			
Baseline	18.9 ± 1.3	17.6 ± 0.7	0.39
1 month postpartum	37.8 ± 1.3	31.8 ± 1.3	**0.004**
2 months postpartum	31.5 ± 1.3	29.4 ± 0.9	0.20
Glucose, mg/dL			
1 month postpartum	104.5 ± 3.8	97.1 ± 3.8	0.19
2 months postpartum	103.5 ± 2.6	99.3 ± 3.3	0.34
HDL, mmol/L			
1 month postpartum	123.1 ± 8.8	111.9 ± 9.5	0.40
2 months postpartum	127.5 ± 11.4	110.6 ± 6.4	0.20
LDL, mmol/L			
1 month postpartum	33.9 ± 2.8	30.6 ± 1.9	0.35
2 months postpartum	21.5 ± 2.0	22.9 ± 2.0	0.64
Triglycerides, mmol/L			
1 month postpartum	1.12 ± 0.15	1.09 ± 0.12	0.86
2 months postpartum	1.13 ± 0.16	0.97 ± 0.1	0.41

Glucose and lipids are fasting serum values. Data expressed as mean ± standard deviation.