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*Original Research*

# Metabolic Flexibility and Weight Status May Contribute to Inter-Individual Changes in Breastmilk Lipid Content in Response to an Acute Bout of Exercise: Preliminary Findings from a Pilot Study

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ABSTRACT

***International Journal of Exercise Science 13(2): 1756-1769, 2020.*** The purposes of this pilot study were to describe changes in breastmilk lipid content in response to an acute bout of moderate intensity exercise and to explore maternal metabolic health factors, including metabolic flexibility, which may impact this change. A cross-sectional, observational, pilot study design was performed in 14 women between 4 and 6 months postpartum. Whole body fasting lipid oxidation was assessed, a standardized high-fat breakfast was consumed, and lipid oxidation was again measured 120-minutes post-meal. Metabolic flexibility was determined by comparing the change in lipid oxidation before and after the meal. Women completed 30-minutes of moderate intensity treadmill walking 150-minutes post-meal. Breastmilk was expressed and analyzed for lipid content before and after exercise. Overall, there was no significant difference between pre- and post-exercise breastmilk lipid content (pre-exercise 59.4±36.1 g/L vs. post-exercise 52.5±20.7 g/L, p=0.26). However, five (36%) women had an increase in breastmilk lipid content in response to the exercise bout, compared to nine (64%) that had a decrease in breastmilk lipid content suggesting inter-individual variability. The change in breastmilk lipid content from pre- to post-exercise was positively correlated to metabolic flexibility (r=0.595, p=0.03). Additionally, post-exercise lipid content was positively correlated with body mass index (BMI), body composition, and postpartum weight retention. Preliminary findings from this pilot study suggest that metabolic flexibility and maternal weight status may help explain the inter-individual changes in breastmilk lipid content in response to an acute bout of moderate intensity exercise.

KEY WORDS: Postprandial metabolism, lipid oxidation, body fat percentage, postpartum, moderate intensity exercise, physical activity, high-fat meal, maternal metabolism

## INTRODUCTION

The American College of Obstetrics and Gynecology (ACOG) recommends maternal exercise in the postpartum period and exclusive breastfeeding for the first six months of life to maximize health outcomes for the mother and her infant (1, 2). Physical activity during the postpartum period has been shown to improve maternal cardiovascular fitness and lower risk for postpartum depression (10). Additionally, it is well-established that breastfeeding has multiple positive long-term effects on societal, maternal, and infant health, including decreased rates of maternal breast and ovarian cancer, diabetes, heart disease and infant infectious diseases, sudden infant death syndrome and metabolic disease (2). Specifically concerning infant health, adequate lipid content in human breastmilk has been shown to improve retinal and brain development in the newborn (18). The macronutrient content of breastmilk is also crucial for managing infant feeding practices (37) because lipids in breastmilk serve as the main source of energy and essential vitamins for optimizing infant development (22). Therefore, understanding the factors that influence breastmilk lipid content are of great importance. Previous research has demonstrated that milk fat is the most variable macronutrient in breastmilk and that large inter and intra-individual variations in breastmilk lipid content exist (23). Furthermore, evaluating individual variations in breastmilk lipid content in the postprandial state is particularly relevant, given that common eating habits among US adults include the consumption of food throughout a large fraction of the 24 hour day with relatively short fasting periods (15).

In response to a high-fat meal, dietary lipids are converted into glycerol and fatty acids. In non-lactating humans these fatty acids have two primary fates: taken up for storage in adipose tissue or oxidation. Previous work in lactating humans has shown that dietary fatty acids consumed can be taken up by lactating mammary glands (12). These diet-derived fatty acids can be transported into breastmilk a few hours post-meal (12); accounting for about 30% of breastmilk lipids (30). Diet-derived fatty acids can also be oxidized by metabolically active tissues (i.e. skeletal muscle). The extent to which an individual upregulates fatty acid oxidation in response to an increase in available fatty acids is termed metabolic flexibility, which relies on dynamic substrate shifting (28). The potential impact that substrate shifting and metabolic flexibility in response to a high-fat meal has on breastmilk composition remains unknown. It is possible that maternal metabolic flexibility and postprandial lipid metabolism may impact individual responses in breastmilk lipid content in response to exercise.

During moderate intensity exercise there is a continuous mobilization of fatty acids from adipose tissue that exceeds total fat oxidation, resulting in excess free fatty acids (14, 19). In lactating humans, these fatty acids could be taken up by the mammary glands. In fact, it is estimated that 60% of breastmilk lipids are derived from the mobilization of fatty acids from adipose tissue (30). Therefore, it could be logical to expect, an increase in breastmilk lipid content post-exercise among women with adequate body fat stores, particularly in a fed state. Only two studies have investigated the impact that 30 minutes of moderate intensity exercise has on breastmilk lipid content (5, 9). In one study, there was a non-significant trend for an increase in select fatty acid concentrations in breastmilk after exercise (5). However, another study reported

no overall change in breastmilk lipid content post-exercise (9). These studies did not report food intake prior to the exercise bout. It might be possible that exercise, after high-fat meal consumption, could have an additive effect resulting in increased breastmilk lipid content.

To date, no study has investigated how an acute bout of moderate intensity exercise may impact breastmilk lipid content after eating a high-fat meal. Evidence suggests that fatty acids are rapidly transferred into breastmilk after eating a high-fat meal (12). It is possible that lactating women, in the postprandial state, will preferentially deliver the excess fatty acids mobilized during exercise to the mammary glands, resulting in an increase in breastmilk lipid content. Therefore, the purpose of this pilot study is to describe the change in postprandial breastmilk lipid content in response to an acute bout of moderate intensity exercise. A secondary purpose of the study is to report potential maternal metabolic health factors (i.e. metabolic flexibility, postprandial lipid metabolism, weight status, dietary intake) that are associated with post-exercise breastmilk lipid content. Because many of these factors (diet, exercise, metabolic health, weight status) are modifiable, the implications from understanding their connection/s to breastmilk composition are important.

## METHODS

*Participants*

Participants were recruited from a previously studied cohort of pregnant women during their third trimester (34). An additional inclusion criterion was exclusively breastfeeding their infant between 4 and 6 months postpartum. Additional exclusion criteria were: current use of illegal drugs and tobacco products, current usage of medications known to alter metabolism (corticosteroids, anti-psychotics), history of gestational diabetes and/or pre-pregnancy diabetes, and any medical contraindication to exercise. Informed consent was obtained for all participants prior to study participation. All aspects of this project were approved by the Western Kentucky University IRB (#17-412) prior to the start of the study and authors adhered to the guidelines for the ethical conduct of research set forth by Navalta, et al (29).

*Protocol*

The 24 hours prior to the study visit, participants were instructed to avoid caffeine and alcohol. Participants were provided with written instructions for consuming a standardized meal the night before the study visit, which consisted of approximately 50% carbohydrate, 30% fat, and 20% protein, based on body weight. They were instructed to consume the meal between 6pm and 9pm the evening before the study visit and then encouraged to drink water before and during the study visit. On the day of the exercise study visit, participants reported to the lab at approximately 8:00am after a 10-hour fast. Baseline assessments were taken upon arrival and included assessed the participant's weight, height, vitals (including resting heart rate), and body composition. Body fat percentage was determined using seven site skinfold anthropometry and entered into standardized equations that accounted for age and gender (20). To assess dietary intake, participants completed the National Institutes of Health's validated Dietary History Questionnaire II (NIH DHQII) (33).

Resting metabolic rate and respiratory exchange ratio were measured using the TrueOne Canopy Option and TrueOne Metabolic Cart (TrueOne 2400, Parvomedics, Sandy, UT). Lipid oxidation rates were calculated by measurement of oxygen consumption and carbon dioxide production as previously described (13). Then participants consumed a standard 1000-kcal high-fat blended beverage from Smoothie King© that was prepared specifically for the study. The blended beverage contained almond milk, banana, apple, peanut butter, super grains, cocoa, vegan protein, and almond. This high-fat meal was similar in fat content to previous studies (17, 21, 34). The blended beverage was 1062 total kilocalories, of which 594 were from fat (55.93%), 312 from carbohydrates (29.38%), and 156 from protein (14.69%). Participants hand expressed approximately 1ml of breastmilk into a microcentrifuge tube approximately 90 minutes after the high-fat meal. Breastmilk was subsequently analyzed for caloric density and lipid content using the Creamatocrit Plus Breast Milk Analyzer (EKF Diagnostics, Inc. USA) using the manufacturer's protocol (26). Approximately 30 minutes later, resting metabolic rate and respiratory exchange ratio were obtained a second time (~120 minutes post high-fat load).

Participants were assessed for their readiness for physical activity using the PAR-Q and then they completed an acute bout of moderate intensity exercise approximately 150 minutes after the high-fat meal. Before the exercise session began the participant's age-predicted maximal heart rate (220-age) was calculated. Using the age-predicted maximum heart rate and resting heart rate (assessed at baseline) a heart rate range of 40-60% heart rate reserve (HRR) was calculated. The 30-minute bout was performed on a treadmill and participants were instructed to maintain intensity between 40-60% of HRR, which is classified as "moderate intensity" by the American College of Sports Medicine (25). Heart rate was monitored continuously using a Polar Heart Rate Monitor and participants reported perceived exertion on the Rating of Perceived Exertion (RPE) Borg 6-20 Scale (6) every 5 minutes during the exercise bout. During the exercise bout, participants self-selected an appropriate belt speed and incline, which allowed them to maintain their heart rate within the given range (40-60% HRR). If their heart rate fell outside of this range, they were instructed to increase or decrease the intensity accordingly. This exercise mode/intensity/self-selected method was chosen to best represent a typical exercise session for a woman during the postpartum period (7). Oxygen consumption and carbon dioxide production were assessed at three different 2-5-minute time segments at minutes 5, 15, and 25 during the exercise bout using the Parvo Metabolic Analyzer and exercise version of the software. This protocol for assessing oxygen consumption and carbon dioxide production is consistent with previous work among pregnant women (35) and chosen to reduce participant burden. Participants were also asked to report their rate of perceived exertion during the exercise bout as well. An additional 1ml of breastmilk was hand expressed by the participant in a private location 15 minutes post-exercise, and analyzed for caloric density and lipid content.

Prior to leaving the study visit, participants were given an Actigraph GT9X Link Accelerometer (ActiGraph LLC, Pensacola, FL) to wear for a week in order to assess their physical activity levels as previously described (36). The accelerometer device uses established cut points to determine percentage of wear time, in this case 7 days for 24 hours per day, for each category of activity (i.e. sedentary time, light physical activity, moderate physical activity).

*Statistical Analysis*
Normality of the distribution for each variable was tested using Kolmogorov-Smirnov tests. The significance level was set at 0.05. Paired t-tests were used to assess differences in breastmilk lipid content before and after exercise. Pearson product-moment correlation coefficients for normally distributed variables or Spearman's rank-order correlation coefficient for non-normally distributed variables were used to assess the degree of the relationship between variables. Partial correlations were used to adjust for potential confounders including infant age, gestational weight gain, postpartum weight retention, total maternal caloric intake, maternal dietary fat intake, maternal physical activity (sedentary time and moderate physical activity), and acute exercise intensity (average %HRR) assessed during the study visit. All data analyses were conducted using IBM SPSS Statistics, Version 25 (Armonk, New York) on raw or log-transformed data. Because this was a cross-sectional, observational, pilot study where participants were recruited from a previously studied cohort of pregnant women during their third trimester (34), no formal power analysis was performed. Additionally, other data on this topic is very limited making it hard to establish a reasonable effect size.

To assess changes in breastmilk lipid content in response to exercise, two different calculations were performed. Fold change was assessed by dividing post-exercise lipid content by pre-exercise lipid content. Percent change was assessed using the following equation: ((post-exercise breastmilk lipid content - pre-exercise breastmilk lipid content) / pre-exercise breastmilk lipid content) * 100.

Metabolic flexibility (in response to the high fat meal) was determined by assessing fold change in lipid oxidation in response to the high-fat meal. Metabolic flexibility was calculated using the following equation, where a value greater than 1.0 represents an increase in lipid oxidation in response to the high-fat meal: post-prandial lipid oxidation / baseline (fasting) lipid oxidation. A larger the value represents a more "metabolically flexible" individual.

## RESULTS

All demographic characteristics and lifestyle behaviors, including physical activity, sedentary time, and dietary intake, are presented in Table 1. All participants were highly educated and white. During the exercise bout, participants maintained an average of 46.7±10.9% HRR and reported an average RPE of 11.9±2.0.

*Breastmilk lipid content in response to an acute bout of moderate intensity exercise.*
There was no difference between pre- versus post-exercise breastmilk lipid content (pre-exercise 59.4±36.1 g/L vs. post-exercise 52.5±20.7 g/L, p=0.26) (Figure 1) or caloric density (pre-exercise 906.8±339.3 kcal/L vs. post-exercise 841.8±194.4 kcal/L, p=0.26).

**Table 1.** Demographic characteristics and lifestyle behaviors.

| Participants, N=14 | |
|---|---|
| Parity, *N=13* | |
| *Nulliparous* | 8 (57%) |
| *Multiparous* | 5 (36%) |
| Education | |
| *High School Graduate* | 1 (7%) |
| *College Graduate* | 7 (50%) |
| *Post-Graduate Degree* | 6 (43%) |
| Age (y) | 30.7±4.0 |
| Months Postpartum | 5.8±0.5 |
| Systolic Blood Pressure (mmHg) | 120.8±11.5 |
| Diastolic Blood Pressure (mmHg) | 77.7±9.1 |
| Resting Heart Rate (bpm) | 70.0±10.0 |
| Weight Status Indicators | |
| *BMI ($kg/m^2$)* | 24.1±3.8 |
| *Body Fat %* | 25.1±5.8 |
| *Postpartum Weight Retention (kg)* | 1.7±6.0 |
| *Pre-Pregnancy BMI ($kg/m^2$)* | 23.1±2.7 |
| *Gestational Weight Gain (kg)* | 15.0±5.7 |
| Physical Activity | |
| *Sedentary Time (%)* | 51.9±9.1 |
| *Moderate Physical Activity (%)* | 14.6±3.9 |
| Dietary Intake, *N=11* | |
| *Total Kilocalories (kcal/day)* | 1867.3±958.6 |
| *Total Fat (g)* | 76.1±35.8 |
| *Monounsaturated Fat (g)* | 29.6±13.2 |
| *Saturated Fat (g)* | 25.0±15.0 |
| *Total Carbohydrates (g)* | 221.3±120.0 |
| *Total Protein (g)* | 82.3±44.8 |
| Baseline (fasting) Metabolism | |
| *Resting Metabolic Rate (kcal/day)* | 1,474.6±244.9 |
| *Lipid Oxidation (g/min)* | 0.06±0.02 |
| Postprandial Metabolism | |
| *Resting Metabolic Rate (kcal/day)* | 1,786.8±355.5 |
| *Lipid Oxidation (g/min)* | 0.08±0.02 |
| Metabolic Flexibility | |
| *Fold Change in Lipid Oxidation* | 1.34±0.30 |

However, when the change in breastmilk lipid content in response to exercise was assessed for each individual, a wide variety of responses were noted (Figure 2). Overall the mean percent change in breastmilk lipid content was -3.8±29.6% in response to the acute bout of exercise. However, five of the 14 (36%) women had an increase in breastmilk lipid content post-exercise (Figure 2).

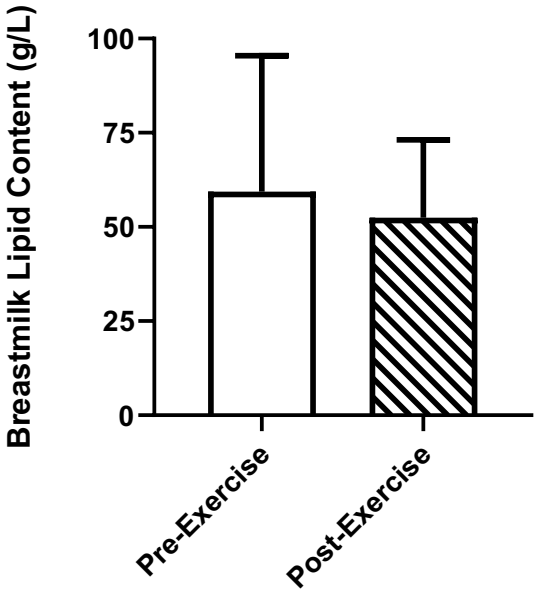
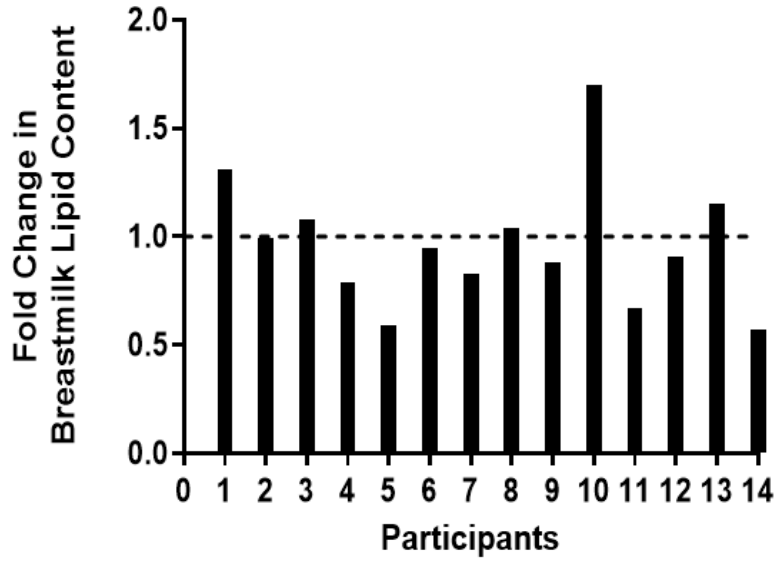



**Figure 1.** Breastmilk lipid content in response to an acute bout of exercise. There was no significant difference between breastmilk lipid content collected pre-exercise and post exercise (pre-exercise 59.4±36.1 g/L vs. post-exercise 52.5±20.7 g/L, p=0.26).

A.




B.

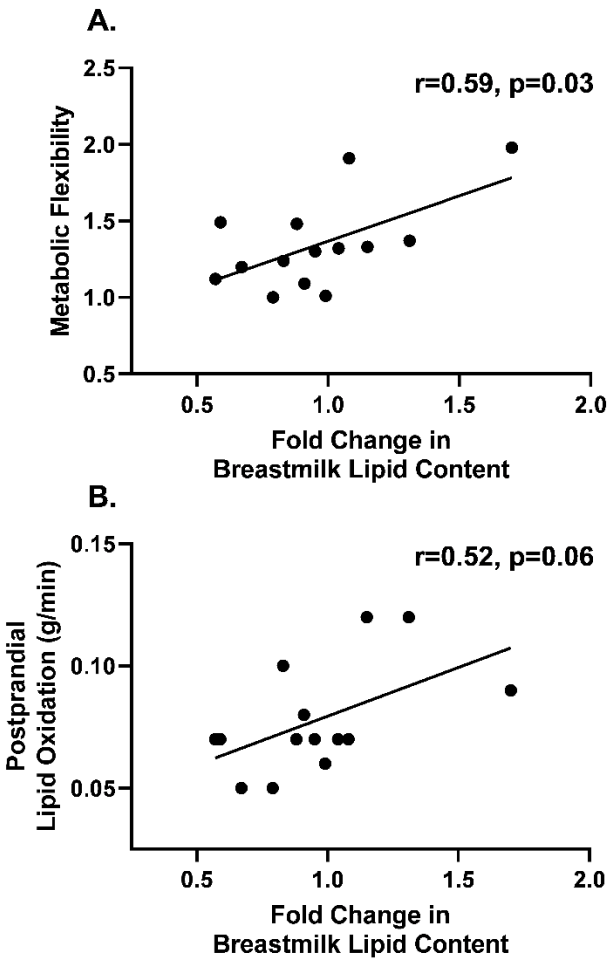
| Participants | % Change |
|---|---|
| 1 | 31.1 |
| 2 | -0.8 |
| 3 | 8.2 |
| 4 | -21.3 |
| 5 | -40.7 |
| 6 | -4.9 |
| 7 | -16.7 |
| 8 | 3.5 |
| 9 | -11.7 |
| 10 | 69.5 |
| 11 | -32.6 |
| 12 | -8.6 |
| 13 | 14.6 |
| 14 | -43.0 |

**Figure 2.** Changes in breastmilk lipid content in response to an acute bout of exercise.

A. Fold changes in breastmilk lipid content in response to an acute bout of exercise for each individual participant. Fold change was calculated by dividing post-exercise lipid content by pre-exercise lipid content. No change is a value of 1, which is represented by the dashed line, with values >1.0 indicative of an increase in breastmilk content in response to the exercise.

B. Percent changes in breastmilk lipid content in response to an acute bout of exercise for each individual participant. Percent change was esimated using the following equation: ((post-exercise breastmilk lipid content - pre-exercise breastmilk lipid content) / pre-exercise breastmilk lipid content) * 100.

The change in breastmilk lipid content in response to exercise was positively correlated to metabolic flexibility (i.e. the upregulation of lipid oxidation in response to the high-fat meal) (r=0.59, p=0.03) (Figure 3A). When adjusting for maternal body fat percentage and the number of months postpartum (which have both been associated with breastmilk lipid content (30)), the positive relationship between metabolic flexibility and the change in breastmilk lipid content in response to exercise remained significant (r=0.63, p=0.03; r=0.70, p=0.01 respectively). Additionally, this relationship remained significant after controlling for gestational weight gain, postpartum weight retention, total caloric intake, dietary fat intake, baseline maternal physical activity (sedentary time and moderate physical activity), and acute exercise intensity (average %HRR)(data not shown). There was a trend for the change in breastmilk lipid content in response to exercise to be positively correlated to postprandial lipid oxidation (r=0.52, p=0.06) (Figure 3B).




A. The change in breastmilk lipid content in response to exercise was positively correlated to metabolic flexibility (i.e. the upregulation of lipid oxidation in response to the high-fat meal) (r=0.59, p=0.03).

B. There was a trend for the change in breastmilk lipid content in response to exercise to be positively correlated to postprandial lipid oxidation (r=0.52, p=0.06).

**Figure 3.** Fold change in breastmilk lipid content in response to an acute bout of exercise correlated with metabolic flexibility and postprandial lipid oxidation.

In an effort to better understand factors that may be associated with breastmilk lipid content post-exercise, additional analyses were performed. Post-exercise breastmilk lipid content (g/L)

was not correlated with metabolic flexibility or postprandial lipid oxidation (r=0.22, p=0.46; r=0.48, p=0.08 respectively). However, there was a positive correlation between post-exercise breastmilk lipid content (g/L) and BMI (r=0.57, p=0.04), body fat percentage (r=0.69, p=0.01), and postpartum weight retention (0.61, p=0.03), but not between post-exercise breastmilk lipid content (g/L) and pre-pregnancy BMI (r=0.23, p=0.46) or gestational weight gain (r=0.40, p=0.17). There were no significant correlations between any of the weight status indicators (i.e. BMI, body fat percentage, postpartum weight retention, pre-pregnancy BMI, gestational weight gain) and pre-exercise breastmilk content (data not shown).

## DISCUSSION

The primary purpose of this pilot study was to describe the change in postprandial breastmilk lipid content in response to an acute bout of moderate intensity exercise that was undertaken approximately 2.5 hours after a high-fat meal. The main finding from this pilot study was that there was no significant decrease in postprandial breastmilk lipid content in response to an acute bout of moderate intensity exercise post high-fat meal consumption (Figure 1); however, inter-individual responses in terms of postprandial breastmilk lipid content in response to exercise are evident (Figure 2). This is clinically relevant information as fear of impacting breastfeeding is a barrier to exercise for many women during the postpartum period (3, 31). Additionally, it is also clinically important for women to recognize individual differences in response to exercise; thus, some women's milk composition (and/or supply) may be altered more than others.

Consistent with our findings, a previous report by Carey, et al. (1997) also noted no overall significant change in breastmilk lipid content at 0, 30, 60, and 90 minutes after exercising (at 75% of VO2max for 30 minutes). Another report by Bopp, et al. (2005) noted the fatty acid content of breast milk 10 and 60 minutes after exercising (at 75% of predicted maximum heart rate for 30 minutes) was not significantly different compared to pre-exercise breastmilk lipid content, although they reported a trend for an increase in the content of specific fatty acids in the breastmilk post-exercise. Neither group reported the individual participant responses in terms of breastmilk lipid content pre- vs post-exercise.

A novel finding from this study was that the change in breastmilk lipid content from pre- to post-exercise was positively correlated to metabolic flexibility in response to a high-fat meal (Figure 3). It has become increasingly clear that *how* an individual responds to a meal (termed metabolic flexibility) and postprandial metabolic profiles are critical factors in overall metabolic health in non-gravid populations (16, 28). Our results suggest that maternal metabolic flexibility and, to some extent, postprandial maternal metabolism could contribute to dynamic inter-individual variations in breastmilk lipid content. This could in turn impact infant feeding and health and potentially have implications for longer-term maternal metabolic health.

In general, the term metabolic flexibility describes the ability of an individual to adjust substrate metabolism according to nutrient availability (28). In response to a high fat meal, metabolically flexible individuals will upregulate lipid metabolism. This involves dynamic substrate shifting in response to signals that are primarily regulated by nutrient availability and sensitivity,

intracellular signaling molecules, and hormones (28). It is interesting to speculate that the most metabolically flexible women had a more dynamic shift of fatty acids into their breastmilk post-exercise or that perhaps they had an enhanced ability to spare breastmilk lipids during exercise, however this was not directly measured. Other studies investigating endurance trained athletes with enhanced metabolic flexibility have reported that these individuals can increase fatty acid oxidation in response to lipid overload and have a tendency to preserve glycogen stores within muscle by decreasing glucose oxidation during exercise (16). The enhanced metabolic flexibility evident in the endurance trained athlete was associated with higher mitochondrial capacity and, likely, enhanced substrate shifting. Perhaps more metabolically flexible lactating women have an increased capacity to spare lipids in breastmilk in response to exercise due to an enhanced lipid mobilization from other depots and favorable substrate shifting during exercise. To date, no studies have evaluated the specific postprandial flux of fatty acids during exercise in lactating women.

The secondary purpose of this pilot study was to report potential maternal metabolic health factors (i.e. maternal metabolic flexibility, postprandial metabolism, maternal weight status, dietary intake) that are associated with post-exercise breastmilk lipid content. We found a significant positive correlation between post-exercise, postprandial breastmilk lipid content (g/L) and BMI, body fat %, and postpartum weight retention, but not between post-exercise breastmilk lipid content (g/L) and pre-pregnancy BMI or gestational weight gain. These results are consistent with those of Bopp et al. who reported a significant correlation between body fat % and concentrations of fatty acids in breastmilk post-exercise (5). It should be noted the current study found no significant correlations between maternal weight status and fatty acid concentrations in breastmilk samples collected pre-exercise, which is also consistent with Bopp et al. Perhaps the additional metabolic stress of exercise was necessary to "uncover" the relationship between maternal weight status and breastmilk lipid content among relatively lean women. Like the study conducted by Bopp et al. the women in the current study were relatively lean and healthy, having a mean BMI of 24.1±3.8 kg/m$^2$.

Other studies have previously reported indicators of maternal weight status are positively associated with breastmilk lipid content, however the majority of previous work has tested the impact of varying weight status classifications (i.e. lean vs obese) in the fasting state and not in response to exercise. Of these, one study found that overweight mothers had significantly lower milk fat content than normal weight mothers (32). In contrast, multiple other studies have found a positive correlation between maternal body mass index and milk fat content in breastmilk (8, 24). For example, Bzikowska-Jura, et al. (2018) found a positive correlation of lactating women's BMI and milkfat content at 1 month postpartum as well as women's weight status and milk fat content at 6 months postpartum. Another study by Marin, et al. (2005) found that total breastmilk lipid content is higher among obese women, however the fatty acid composition pattern differs according to weight status (i.e. lean vs obese). More work in this area needs to be done in order to ascertain optimal fatty acid concentrations in breastmilk.

To our knowledge this is the first study to collect breastmilk samples from women that consumed a standardized meal the evening before and then collected postprandial samples after

a high-fat meal. However, we did not collect a fasting, pre- high-fat meal breastmilk sample, which is a limitation of this study. Previous work has characterized the impact of acute lipid intake on breastmilk lipid content (12), so we did not collect a fasting breastmilk sample to compare to the post high-fat meal breastmilk sample. Although it would have been interesting to see if the more metabolically flexible women had a greater shift of fatty acids into their breastmilk in response to the high-fat meal. It would be interesting to determine how this profile of enhanced maternal metabolic substrate shifting and/or substrate shifting into and out of breastmilk may impact long term maternal weight status and infant weight status. Additionally, we did not control for the exercise history of participants, which could potentially impact substrate shifting into and out of breastmilk and/or substrate use during exercise.

An additional limitation of the present study was the inability to account for all time-related factors and maternal characteristics that could potentially influence breastmilk lipid composition. There are many inherent difficulties in assessing human breastmilk composition. Human breastmilk composition is greatly influenced by time (i.e. the length of time between feedings), feeding frequencies, and feeding volume (which impacts the extent to which breasts are emptied) (11). Additionally, we did not account for all maternal characteristics that are known to influence breastmilk composition, such as maternal genetics, energy balance, environmental exposures, sleep patterns, and breast storage capacity (4, 11, 27). However, there were several strengths in the present study related to controlling some aspects of maternal dietary intake, including the standardized meal consumed the night prior, the overnight fast, and a consistent/same high-fat meal consumed immediately prior to the exercise bout.

The gold standard for assessing lipid content in breastmilk involves collecting fore- and hind-milk samples from the complete emptying of both breasts over a 24-hour period, which is a demanding sampling protocol (27). The fore-milk samples collected and analyzed in the study are therefore not an accurate estimate of total (combined fore- and hind-) breastmilk lipid content. Our findings are therefore only generalizable to other studies reporting the lipid content of fore-milk samples. The decision not to require the complete emptying of the breast was ultimately made considering our ability to recruit exclusively breastfeeding women for participation, from within another larger study, as we thought this requirement would make certain women less eager to participate. We also did not provide extensive instructions for manual expression, which could have impacted breastmilk lipid content in our collected sample. We did, however, control for the diurnal variation in lipid content, as all collection procedures were performed at a consistent time of day (in the morning) after a standardized meal the evening before and a standardized breakfast the day of the study visit.

This study also did not include a control group, that instead of completing the exercise bout, simply rested. Because of this, it is impossible to determine the extent that postprandial lipid shifting into and/or out of breastmilk might occur during resting conditions relative to the exercise condition. For example, the changes observed could have been more dependent on the quality of the participant's diet and/or on the composition of the high-fat meal. While this limitation does not undermine the main finding of the study, that there are varied individual responses in terms of postprandial breastmilk lipid content that are related to other maternal

metabolic health factors, it does leave room for questioning the specific impact that exercise has on postprandial breastmilk lipid content. It is possible the changes we observed in breastmilk lipid content in response to exercise would be no different than the potential changes in breastmilk lipid content in response to a resting period, which is consistent with the findings of others (5).

In summary, there was no overall decrease in breastmilk lipid content in response to the acute bout of exercise that was undertaken approximately 2.5 hours after a high-fat meal, however, there were a wide variety of responses in breastmilk lipid content when participants were evaluated individually. Further, the change in breastmilk lipid content in response to exercise is positively correlated to metabolic flexibility, where a greater increase in breastmilk lipid content from pre- to post- exercise is associated with increased metabolic flexibility in response to a high-fat meal. This study also found that post-exercise lipid content was positively correlated with several markers of maternal weight status (i.e. BMI, body fat %, postpartum weight retention). Taken together, preliminary findings from this pilot study suggests that breastmilk lipid content is highly variable from person-to-person and that maternal metabolic flexibility and weight status help explain this variability. Clinicians should be aware that a variety of maternal health factors (e.g. metabolic profile, weight status, diet, physical activities) will impact breastmilk composition to varying degrees among postpartum women.

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