Validity of a 3-Hour Breast Milk Expression Protocol in Estimating Current Maternal Milk Production Capacity and Infant Breast Milk Intake in Exclusively Breastfeeding Dyads

Dayna M. Roznowski,¹ Erin A. Wagner,¹ Sarah W. Riddle,² and Laurie A. Nommsen-Rivers¹

Abstract

Objective: An efficient method for measuring maternal milk production is needed. Our objectives were to: (1) validate a milk production rate (MPR) protocol in exclusively breastfeeding mothers; (2) determine MPR change following 48 hours of increased breast emptying; (3) assess agreement between MPR and infant test-weighing; and (4) characterize MPR in early postpartum exclusively breastfeeding mothers.

Materials and Methods: $N=23$ mothers emptied both breasts hourly over 3 hours (h0, h1, h2, and h3). We estimated steady-state MPR as mean (h2 and h3). Subset A mothers ($n=5$) also completed MPR measurements after 48 hours of increased breast emptying. Subset B mothers ($n=16$) also test-weighed for 48 hours. We used paired t -test to examine within-participant change in hourly milk yield and MPR; and we used Bland-Altman analysis to compare 24-hour milk production (g/24 hours) measured using test-weight versus MPR. Results are reported as mean $\pm$ standard deviation or ($\pm 95\%$ limits of agreement).

Results: Mothers were 54 ± 14 days postpartum. Paired difference in h3–h2 hourly milk yield was not significantly different ($p > 0.05$, 3 ± 10 g/hour). In Subset A ($n=5$), MPR declined from 50 ± 13 to 43 ± 16 g/hour ($p=0.003$) following 48 hours of increased breast emptying. In Study B ($n=16$), mean infant test-weighed intake (TW) was 717 ± 119 g/24 hours, and mean MPR was $1,085 \pm 300$ g/24 hours. Mean difference (MPR–test-weigh) and mean ratio (MPR/test-weigh) significantly increased as MPR increased ($p < 0.05$). For infants with adequate weight gain (>20 g/24 hours, $n=12$), mean MPR = 48 ± 12 g/hour (range, 35–78 g/hour).

Conclusion: MPR is a valid measure of current maternal milk production capacity, but is not accurate for evaluating infant intake in exclusively breastfeeding dyads.

Keywords: human lactation, breastfeeding, insufficient milk production, low milk supply, test-weighing, measurement of breast milk output, milk production rate

Introduction

MULTIPLE PUBLIC HEALTH entities recommend exclusive breastfeeding until 6 months of age due to many benefits for both mother and infant.^{1,2} Unfortunately, mothers in the United States are not meeting breastfeeding recommendations.³ The most consistently cited reasons why mothers stop breastfeeding are maternal perception of an unsatisfied infant and maternal concern of insufficient milk production.⁴ More than three out of four mothers who breastfed <3 months stated they wanted to breastfeed longer.⁵

The extent to which milk supply concerns are based on maternal misperception versus a true deficit in milk production is unknown because health care providers lack a validated, clinically accessible procedure for measuring current milk production and change in milk production in response to an intervention or change in breastfeeding pattern. Clinically, the gold standard method of measuring milk production in lactating women is 2–4 days of 24-hour infant intake test-weighing⁶; however, test-weighing is both cumbersome and time consuming. To better support exclusive breastfeeding in mothers with normal milk production, and to identify

¹College of Allied Health Sciences, University of Cincinnati, Cincinnati, Ohio, USA.

²Department of Rehabilitation, Exercise, and Nutritional Sciences, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA.

mothers with truly insufficient milk supply, there is a need for an efficient, clinically accessible method of estimating maternal milk production.

In 2010, the Hartmann research group, in an article by Lai et al., proposed estimating milk production in breastfeeding mothers using an hourly milk expression protocol.⁷ Lai et al. reported steady-state milk production was reached at hour 2 of hourly breast emptying, and thus proposed averaging hour 2 and hour 3 of hourly breast emptying to estimate mother's milk output. In addition, Lai et al. reported estimated milk output using this approach is not significantly different from 24-hour test-weigh measurement of infant milk intake. However, the participants in the above study were breastfeeding older infants; these results may not be generalizable to the critical time period of early lactation. Lai et al. did not report limits of agreement,⁷ which is the gold standard method for quantifying agreement between two methods measuring the same biological phenomenon.⁸ A subsequent article by the Hartmann research group again evaluated measurement of milk output from an hourly breast expression protocol. In this more recent study, Kent et al. also reported that the difference between milk production as assessed by 24-hour infant intake test-weigh and hour 3 of hourly breast expression was significantly correlated, but, again, did not assess limits of agreement.⁹ Thus, the validity of an hourly breast expression protocol in estimating maternal milk production in early lactation, and its agreement with infant breast milk intake, remain unknown.

Our objectives in this study are to: (1) confirm steady state is reached by hour 2 of the Lai et al. hourly breast expression protocol, and thus externally validate that milk production rate (MPR) can be estimated from the mean of hour 2 and hour 3 milk yield; (2) determine if an increase in MPR is detectable following 48 hours of increased breast emptying; (3) assess agreement between maternal milk production estimated by MPR compared to infant intake test-weighing; and (4) characterize MPR mean and variance in a sample of mothers who are exclusively breastfeeding infants who are achieving appropriate weight gain.

Materials and Methods

Subjects

We recruited exclusively breastfeeding mothers through parent support groups and parenting social media sites during two waves of enrollment: September to December 2017 (Study A) and March to June 2018 (Study B).

Inclusion criteria. Inclusion criteria were as follows: mother resides in Cincinnati, OH metropolitan area, is at least 18 years of age, is 4–10 weeks postpartum at the time of initial study contact, and is exclusively breastfeeding a healthy singleton infant who was born at term (≥ 37 weeks gestation).

Exclusion criteria. Exclusion criteria were as follows: evidence of unresolved insufficient infant weight gain (growth velocity consistently <10 th percentile based on maternal report of infant weight history),¹⁰ infant does not accept expressed milk from a bottle or cup, maternal report of insufficient glandular tissue diagnosis or history of major breast surgery, maternal report of a current diagnosis of type

1 or type 2 diabetes, mothers who are breastfeeding more than one child (e.g., tandem breastfeeding). Eligible and willing mothers were scheduled for enrollment within 10 weeks postpartum, and during a time when they and their infant were without symptoms of infectious illness and still exclusively breastfeeding.

Hourly breast expression protocol

Participants arrived at Cincinnati Children's Hospital Medical Center (CCHMC) Schubert Research Clinic in the morning following an overnight fast. A fasting blood sample was taken, but these results are not related to the current objectives. Mothers were free to breastfeed ad libitum leading up to the hourly breast expression protocol, which involved thorough breast emptying at baseline (h0) and hourly for the subsequent 3 hours (h1, h2, and h3), as described below. At h0, the participant first breastfed her infant if desired, and then she expressed both of her breasts using a hospital-grade breast pump until we observed milk ejection had decreased to infrequent drips (Spectra S2 Plus, Davie, FL). We weighed the infant fully clothed (± 2 g) before and after breastfeeding with no change to clothing to estimate intake. Milk output at h0 was recorded as the sum of breast milk transfer at the h0 breastfeed plus milk expressed. Then, starting 60 minutes after the h0 breast expression was complete, the participant expressed her breasts using pump until milk flow was reduced to infrequent drips (~ 10 minutes) at hourly intervals over the next 3 hours (h1, h2, and h3). We recorded the exact time that each hourly breast expression ended, and we weighed the milk expressed from each breast at each time point on a digital scale (± 1 g). We calculated standardized hourly milk production for h1, h2, and h3 as $([\text{total milk output from both breasts in hourly expression episode}] / [\text{exact number of minutes from end of previous expression to end of current expression}]) \times 60$ minutes, to derive hourly milk production (g/hour). The participant refrained from feeding her infant at the breast for this time period, and instead fed her infant expressed breast milk. During the same research clinic visit, we measured (in duplicate) maternal height and weight and infant naked weight and length; and we interviewed mothers to obtain demographic, perinatal history, and breastfeeding information.

Study A procedures (milk production upregulation through increased breast emptying)

Following the hourly breast expression protocol, we instructed Study A participants to completely empty their breasts after breastfeeding on two occasions on the day of the protocol and three occasions on the following day (spread out over the day). We encouraged participants to refrain from feeding the extra milk expressed to the infant, and to instead breastfeed in response to infant hunger cues, even if it required breastfeeding more frequently than usual. Our rationale for this guidance was to increase the degree of breast emptying during the 48 hours of upregulation, which is thought to stimulate an increase in MPR, as described by Dewey and Lonnerdal, designed to upregulate milk production.¹¹ We provided participants with a hospital-grade breast pump (Spectra S2 Plus). We then repeated the hourly breast expression protocol (as described above, including obtaining naked infant weight) 48 hours after participant's initial visit

to the research clinic. We originally planned to complete Study A with 12 mothers, but decided to terminate Study A after midpoint interim analysis revealed a small, but significant, decrease in infant weight gain velocity during the 48 hours of increased breast emptying.

Study B procedures (agreement between hourly breast expression and infant intake test-weigh)

We conducted a home visit 2–7 days before the research clinic visit to instruct the participant on proper test-weighing technique. We provided a digital infant scale (± 2 g; Tanita BD-815U, Arlington Heights, IL) and employed the “Teach-Back” method to ensure participant understanding of test-weighing.¹² The participant then completed 48 hours of at-home infant intake test-weighing, which included weighing the infant in the exact same clothing before and after every feeding at the breast and weighing all milk expressed for 48 consecutive hours. We reviewed the completed test-weigh record for each participant and assessed it for accuracy and biological plausibility. We considered odd-numbered entries as inaccurate (because the study scales only record even numbers in the weight range of the study infants), and we considered pairs of entries resulting in >200 g gain or <2 g loss in milk transfer from a single breast as suspect. In these situations, we asked the participant to repeat or extend the test-weigh time period. We then calculated infant intake and exact time interval of the intake record based on total intake from the start time of their first breastfeed during the test-weigh to the start time of the breastfeed closest to 48 hours later (the latter was used to calculate exact test-weigh interval, but was not included in total milk intake), and standardized to intake/24 hours. To calculate total test-weigh milk production (g/24 hours), expressed breast milk was added to infant intake test-weigh result before standardizing to 24 hours. We obtained infant naked weight before starting the test weigh procedure and assessed rate of weight gain between this time point and the time of the MPR procedure, 2–7 days later.

Ethics

We followed procedures in accordance with the ethical standards of the University of Cincinnati Institutional Review Board on human research participation. Study participants provided written, informed consent before study protocol initiation. Whenever we identified infants with weight gain <20 g/24 hours,¹⁰ the study pediatrician followed up with the mother. This follow-up always included sending written documentation to the infant’s pediatrician and, as appropriate, a recommendation to breastfeed more frequently or to make an appointment with Cincinnati Children’s Hospital Center for Breastfeeding Medicine.

Statistics

We summarized participants’ descriptive characteristics as mean $\pm$ standard deviation (SD) for continuous variables and as frequency (%) for categorical variables. We used paired *t*-test to determine if the amount of milk expressed at h0, h1, and h2 was significantly different from that expressed at h3 to confirm steady state was reached by hour 2 of hourly breast

expression. Upon confirmation of the latter, we then estimated MPR as mean (h2 and h3).

For Study A participants, we used data from their initial research clinic visit in this paired comparison of hourly milk yield. In the Study A subset, we also used paired *t*-test to determine if there was a significant increase in breast-emptying episodes, and if there was a significant change in hourly milk output and MPR, following 48 hours of increased breast emptying.

In the Study B subset, we used paired *t*-test to determine if the average difference between infant intake test-weigh and MPR measurements of 24-hour milk production was significantly different from zero. In an a priori analysis, we determined that a sample size of 16 paired measurements enabled us to detect 0.75 SD difference between measurements as significantly different from 0, assuming 80% power and $p < 0.05$ significance. We then used the Bland-Altman approach to assess agreement, bias, and uniformity between paired measurements of milk output using infant intake test-weigh and MPR.⁸ Finally, we summarized MPR mean and range for Study B mother-infant dyads with adequate infant weight gain, defined as gain of 20 g/24 hours or more, to provide an estimate of MPR representative of successful exclusive breastfeeding. We a priori defined adequate infant weight gain in accordance with World Health Organization Child Growth Standards, which show about 90% of male and female infants gaining at least 20 g/24 hours during the second month of life.¹⁰

Results

A total of 41 participants were screened for study participation, 24 were determined eligible and enrolled, 1 dropped out before MPR measurements, and 23 participants completed the primary hourly breast expression protocol. Five of these participants completed Study A (milk production up-regulation through increased breast emptying), and 16 completed Study B (agreement between hourly breast expression and infant intake test-weigh measurements of milk production). One participant initiated, but did not complete Study A, and one participant initiated, but did not complete Study B. Demographic and perinatal history, and maternal and infant status at the time of the research clinic visit, are summarized in Table 1.

Objective 1

Milk yield sum from both breasts at h0 and h1 was significantly more than milk yield at h3 (paired *t*-test $p < 0.0001$ and $p = 0.004$, respectively); but milk yield at h2 was not significantly different from milk yield at h3 (paired *t* test, $p = 0.20$). A summary of hourly breast expression results is shown in Table 2, first results column. This confirms that steady state is reached by h2 and, thus, it is valid to average milk yield at h2 and h3 in estimating MPR.

Objective 2

Table 3 summarizes results for participants in Study A. The number of breast-emptying episodes significantly increased over Study A participants’ 48 hours of increased breast emptying, compared to the 24 hours before their initial research clinic visit (increase of 6 ± 0 per day, counting each

TABLE 1. CHARACTERISTICS OF PARTICIPANTS BY MILK PRODUCTION RATE STUDY GROUP

<i>Variable description</i>	<i>Study A,^a n=5 x ± SD or n (%)</i>	<i>Study B,^b n=16 x ± SD or n (%)</i>	<i>All with MPR,^c n=23 x ± SD or n (%)</i>
Demographics and perinatal history			
Maternal age, years	28 ± 5	31 ± 4	31 ± 5
Maternal race/ethnicity			
Asian	0 (0)	1 (6)	1 (4)
White	5 (100)	15 (94)	21 (91)
Black/African American	0 (0)	0 (0)	1 (4)
Maternal Hispanic ethnicity			
Yes	1 (20)	0 (0)	1 (4)
No	4 (80)	16 (100)	22 (96)
Maternal education			
High school diploma or less	0 (0)	0 (0)	0 (0)
Vocational/technical/some college	0 (0)	1 (6)	3 (13)
Bachelor's degree	1 (20)	6 (38)	7 (30)
Post-Bachelor's degree	4 (80)	9 (56)	13 (57)
Public health insurance			
Yes	0 (0)	2 (12)	2 (9)
No	5 (100)	14 (88)	21 (91)
Primiparous			
Yes	3 (60)	11 (69)	15 (65)
No	2 (40)	5 (31)	8 (35)
Delivery mode			
Vaginal	4 (80)	13 (81)	18 (78)
Planned cesarean	1 (20)	1 (6)	3 (13)
Unplanned cesarean	0 (0)	2 (13)	2 (9)
Infant sex			
Male	3 (60)	6 (37)	9 (39)
Female	2 (40)	10 (63)	14 (61)
Infant gestational age, weeks	40 ± 1	40 ± 1	40 ± 1
Infant birth weight, g	3,311 ± 377	3,552 ± 424	3,455 ± 419
Status at time of research clinic visit			
Infant age, days	50 ± 5	56 ± 13	54 ± 14
Infant weight-for-age, percentile ^d	49 ± 28	53 ± 27	51 ± 26
Exclusive breastfeeding since birth			
Yes	3 (60)	16 (100)	21 (91)
No	2 (40)	0 (0)	2 (9)
Ankyloglossia concern			
Yes	2 (40)	4 (25)	7 (30)
No	2 (40)	5 (31)	8 (35)
Unknown/not evaluated	1 (20)	7 (44)	8 (35)
If yes, lingual frenotomy completed			
Yes	2 (100)	2 (50)	5 (71)
No	0 (0)	2 (50)	2 (29)
Maternal body mass index, kg/m ²	26 ± 6	27 ± 7	27 ± 7
Maternal body mass index category			
Obese (≥30.0 kg/m ²)	2 (40)	4 (25)	7 (30)
Non-obese (<30.0 kg/m ²)	3 (60)	12 (75)	16 (70)
Resumption of menstrual cycle			
Yes	0 (0)	0 (0)	0 (0)
No	5 (100)	16 (100)	23 (100)
Hormonal contraception use			
Yes	2 (40)	1 (6)	3 (13)
No	3 (60)	15 (94)	20 (87)

All infants were exclusively breastfeeding at the time of study participation.

^aStudy A: paired comparison of MPR before and after increased breast-emptying intervention.

^bStudy B: paired comparison of MPR as measured by an hourly breast-emptying protocol, and infant intake as measured by test weighing.

^cAll enrolled participants who completed MPR measurements at the research clinic, includes one Study A participant and one Study B participant who did not complete the non-MPR measurements.

^dBased on World Health Organization growth velocity tables.

MPR, milk production rate; SD, standard deviation.

TABLE 2. MILK PRODUCTION RATE RESULTS

	Study B		
	All with MPR, n = 23 x ± SD	All completers, n = 16 x ± SD	Adequate gain subset, ^a n = 12 x ± SD
Hour 0, yield from complete breast emptying			
Left breast, g	89 ± 36	86 ± 39	92 ± 42
Right breast, g	96 ± 38	95 ± 44	101 ± 46
Total, g	185 ± 55	181 ± 59	193 ± 64
Hour 1, yield from complete breast emptying			
Left breast, g/hour	27 ± 13	28 ± 10	28 ± 10
Right breast, g/hour	33 ± 18	33 ± 21	34 ± 24
Total, g/hour	60 ± 26	61 ± 26	62 ± 30
Hour 2, yield from complete breast emptying			
Left breast, g/hour	21 ± 8	22 ± 7	23 ± 7
Right breast, g/hour	26 ± 10	25 ± 11	27 ± 12
Total, g/hour	47 ± 13	47 ± 14	50 ± 14
Hour 3, yield from complete breast emptying			
Left breast, g/hour	20 ± 8	20 ± 7	22 ± 8
Right breast, g/hour	24 ± 10	23 ± 10	25 ± 10
Total, g/hour	44 ± 13	43 ± 13	47 ± 12
Paired difference from hour 3 total yield			
Hours 0–3, g	141 ± 50*	138 ± 56*	147 ± 62*
Hours 1–3, g	16 ± 23*	18 ± 26*	15 ± 29*
Hours 2–3, g	3 ± 10	4 ± 10	3 ± 11
MPR ^b			
MPR, g/hour ^b	46 ± 12	45 ± 13	48 ± 12
MPR, extrapolated to 24 hours (MPR × 24)	1,092 ± 297	1,085 ± 300	1,164 ± 283
Paired difference in estimate of 24-hour milk output, (MPR × 24) – (Test Weigh), g	n/a	368 ± 234	406 ± 249
Ratio, (MPR × 24)/(Test weigh)	n/a	1.5 ± 0.2	1.5 ± 0.3

All infants were exclusively breastfeeding at the time of study participation.

^aAdequate infant weight gain during study participation defined as >20 g/day.

^bEstimated steady-state maternal MPR, g/hour, calculated as: (total hour 2 yield, g/hour + total hour 3 yield, g/hour)/2, during a morning protocol of hourly breast emptying.

*Paired *t* test *p*-value <0.05.

MPR, milk production rate; SD, standard deviation.

side separately, $p=0.0004$). Average ($\pm$ SD) MPR (mean of h2, h3) was significantly lower at the follow-up hourly breast expression measurements compared to the initial clinic visit (decline of 7.3 ± 4.5 g/hour, $p=0.003$), and compared to infant weight gain history in the 2–3 weeks before the initial clinic visit, the rate of infant weight gain significantly declined during the 48 hours of increased breast emptying (decline of 26.9 ± 9.3 g/day, $p=0.02$). Due to the negative, although small, impact on infant weight gain velocity, we decided to terminate Study A midway through completion. All mothers reported resumption of normal feeding patterns following the study.

Objectives 3 and 4

Table 4 summarizes test-weight results for participants in Study B. For all participants who completed Study B test-weight measurements, average ($\pm$ SD) breast milk intake was $717 \text{ g} \pm 119 \text{ g/24 hours}$. However, we identified infants with weight gain velocity below 20 g/24 hours. Average ($\pm$ SD) weight gain was $33 \pm 11 \text{ g/24 hours}$ for infants with adequate weight gain ($n=12$) and $13 \pm 3 \text{ g/24 hours}$ for infants with inadequate weight gain ($n=4$). Among infants with

adequate weight gain, average breast milk intake was $758 \text{ g} \pm 71 \text{ g/24 hours}$ and ranged from a minimum of 699 g/24 hours to a maximum of 915 g/24 hours. Among infants with inadequate weight gain, average breast milk intake was $594 \pm 160 \text{ g/24 hours}$ and ranged from a minimum of 405 g/24 hours to a maximum of 789 g/24 hours.

Table 2, in the Study B columns, summarizes agreement between MPR and infant intake test-weigh. Among the entire sample with MPR data, average ($\pm$ SD) MPR was $46 \pm 12 \text{ g/hour}$, which extrapolates to $1,092 \pm 297 \text{ g/24 hours}$ (MPR g/hour $\times$ 24). Milk output as estimated by MPR was on average 1.5-fold greater than infant intake test-weigh and ranged from 118% to 206% of the infant intake test-weigh measurement. For the Study B subset with adequate infant weight gain velocity, average MPR was $48 \pm 12 \text{ g/hour}$ (range, 35–78 g/hour), which extrapolates to $1,164 \pm 283 \text{ g/24 hours}$. For the Study B subset with inadequate infant weight gain velocity, average MPR was $35 \pm 10 \text{ g/hour}$ (range, 28–50 g/hour), which extrapolates to $848 \pm 237 \text{ g/24 hours}$.

Agreement in estimating breast milk production from infant intake test-weigh intake versus maternal MPR is shown in Figure 1a and b. Bias is represented by the mean difference between MPR and infant intake test-weigh measurements of

TABLE 3. STUDY A: BASELINE AND POSTINTERVENTION MEASUREMENTS (N=5)

	Baseline <i>x</i> ± <i>SD</i>	Intervention <i>x</i> ± <i>SD</i>	<i>p</i> ^a
Hour 0 breast expression, g	196 ± 54	167 ± 34	
Hour 1 breast expression, g	74 ± 35	71 ± 21	
Hour 2 breast expression, g	51 ± 11	46 ± 19	
Hour 3 breast expression, g	49 ± 17	40 ± 14	
Breast-emptying frequency ^b	15 ± 2	21 ± 2	0.0004
MPR, g/hour ^c	50 ± 13	43 ± 16	0.003
Infant rate of weight gain, g/day ^d	43 ± 14	17 ± 15	0.02

All infants were exclusively breastfeeding at the time of study participation.

^aPaired *t* test *p*-value.

^bSum of all breastfeeding and breast-emptying episodes in left breast + all episodes in right breast, standardized to 24 hours; baseline=episodes in the 24 hours before the baseline MPR measurements; intervention=episodes during the intervention, standardized to 24 hours.

^cEstimated steady-state maternal MPR, g/hour, calculated as: ([total hour 2 yield, g/hour] + [total hour 3 yield, g/hour])/2, during a morning protocol of hourly breast emptying.

^dBaseline=weight at time of baseline MPR measurements – weight at most recent pediatric visit; Intervention=weight at time of intervention MPR measurements – weight at time of Baseline MPR measurements.

MPR, milk production rate; SD, standard deviation.

milk output. Our results reveal a positive bias, indicating that MPR overestimates measurement of maternal milk production compared to infant intake test-weighing by an average of 368 ± 234 g/24 hours. Individual overestimation ranged from a minimum of 114 g/24 hours to a maximum of 968 g/24 hours. Variability in agreement between MPR and infant intake test-weigh measurements of milk output is represented by the 95% limits of agreement, which are drawn at ±1.96 SDs from mean bias and ranged from –90 to +826 g/24 hours.

Homogeneity of bias across MPR values would be represented by even distribution of individual data points above and below the mean bias line consistently across the range of MPR values. The results shown in Figure 1a indicate that the bias was not homogenous across MPR values. For mothers with lower MPR values, there was less bias in milk production as measured by MPR versus infant intake test-weigh, and increasingly greater bias between these two measurements as MPR increased. The correlation between the MPR and bias was significant ($r=0.93$; $p<0.0001$), indicating that as MPR increased, bias increased.

Figure 1b examines homogeneity in bias across MPR values when bias is expressed as a ratio of MPR to infant intake test-weigh. Even after expressing bias as the ratio of MPR/infant intake test-weigh, the correlation between MPR and bias was significant ($r=0.72$; $p=0.002$).

We also assessed agreement between test-weigh milk production (i.e., infant intake recorded during test-weighing plus all milk expressed during the test-weigh interval) and MPR. The mean bias between MPR and test-weigh milk production revealed a similar positive bias, indicating that MPR overestimated maternal milk output compared to test-weigh by an average (±SD) of 319 ± 173 g/24 hours (range, 115–727 g/24 hours). For mothers with lower MPR values, there was less bias in milk output measured by MPR versus

TABLE 4. STUDY B: TEST WEIGH DATA

	All completers, <i>n</i> = 16 <i>x</i> ± <i>SD</i>	Adequate gain subset, ^a <i>n</i> = 12 <i>x</i> ± <i>SD</i>
Infant gain during study participation, g/day	28 ± 13	33 ± 11
Duration of study participation, days	8 ± 4	8 ± 5
Infant breast milk intake		
Total, g/24 hours	717 ± 119	758 ± 71
Total, g/kg body weight/24 hours	150 ± 30	153 ± 22
Left breast, g/24 hours	323 ± 95	347 ± 78
Right breast, g/24 hours	347 ± 112	382 ± 88
Peak intake from a single feed on left breast, g	93 ± 39	103 ± 39
Peak intake from a single feed on right breast, g	98 ± 35	104 ± 37
Longest gap between feedings, hours	6 ± 2	6 ± 2
Maternal production		
Total, g/24 hours	766 ± 188	822 ± 172
Left breast, emptying episodes/24 hours	7 ± 2	7 ± 2
Right breast, emptying episodes/24 hours	7 ± 2	7 ± 2
Total emptying episodes/24 hours	14 ± 4	14 ± 4
Longest gap in left breast-emptying episodes, hours	7 ± 2	7 ± 2
Longest gap in right breast-emptying episodes, hours	7 ± 1	7 ± 2

All infants were exclusively breastfeeding at the time of study participation.

^aAdequate infant weight gain during study participation defined as >20 g/day.

SD, standard deviation.

test-weigh of milk production, and increasingly greater bias between these two measurements as MPR increased ($r=0.81$; $p=0.0001$), and thus bias was not homogeneous when milk expressed during the test weigh was considered. However, when bias was expressed as a ratio of MPR to test-weigh milk production, the correlation between MPR and bias approached homogeneity ($r=0.45$; $p=0.08$).

Discussion

Objective 1

Mean difference between milk volumes expressed at hour 2 and hour 3 of the hourly breast expression protocol was not significantly different from each other, supporting the work of Lai et al., who also found that steady-state milk production was achieved by hour 2.⁷ Although steady-state milk production was achieved by h2 of the pumping protocol, the SD of this mean difference, ±10 g, is large enough to warrant averaging milk output from h2 and h3 because obtaining two measurements of steady-state milk yield will provide a more precise estimate of MPR compared to using h2 alone.

FIG. 1. (Top) and (bottom): Bland-Altman plot of agreement in estimating 24-hour breast milk output from infant test-weighing of intake versus milk production rate, $n = 16$. Infant test-weighing of intake per 24 hours is calculated from 2 days of continuous test weighing of the infant before and after every feeding. Milk production rate per 24 hours is estimated from the hourly breast-emptying protocol as follows: $([\text{hour } 2 + \text{hour } 3]/2) \times 24$ hours. The top figure shows the difference between these two measurements of milk output, and the bottom figure shows the ratio. Mean $\pm$ SD of individual differences, 368 ± 234 ; mean $\pm$ SD of individual ratios, 1.5 ± 0.3 . Black data points = daily infant weight gain within normal limits; gray data points = infant weight gain < 20 g/24 hours; solid line = mean; dashed lines = 95% limits of agreement (95% confidence interval in limits of agreement estimate, ± 0.191 [SD] 2). SD, standard deviation.

Mothers began the protocol with varying levels of breast fullness. However, correlation in milk yield between h0 and MPR (i.e., mean h2 and h3) was not significant ($r = 0.22$, $p = 0.32$), demonstrating that MPR is not biased by degree of breast fullness at the start of the protocol.

Objective 2

Contrary to our Study A hypothesis, we observed a significant decrease in MPR at the follow-up hourly breast expression measurements compared to the initial clinic visit. We observed a very strong within-mother correlation from the initial clinic visit to the follow-up hourly breast expression measurements, indicating reliability in the repeatability of the measurement ($r = 0.95$; $p = 0.014$). Our results contrast with Daly et al., who examined the relationship between frequency of milk removal and subsequent short-term response in MPR in lactating mothers and concluded that if the breast is emptied to a greater degree than at the previous breastfeed, the rate of milk synthesis will increase.¹³ In an intervention by Dewey and Lonnerdal, lactating mothers expressed extra milk several times daily for 14 days, with a mean increase in milk production of 124 ± 98 g/24 hours.¹¹ Our intervention examined MPR response to increased breast emptying on a medium-term basis—longer than the feed-to-feed response examined by Daly, but shorter term than the

14-day response of Dewey. Collectively, the studies to date point to the need for more research to understand the factors that stimulate increase in MPR and the duration it takes to detect a change.

Objective 3

Our results reveal that MPR greatly overestimates measurement of infant breast milk intake compared to infant test-weighing and lead us to conclude that MPR cannot be used to estimate infant intake.

Both Lai et al.⁷ and Kent et al.⁹ report a significant correlation between maternal milk production and infant intake test-weighing, but correlation alone does not imply one measure can be substituted for the other.⁸ We also observed a significant within-subject correlation between MPR and infant intake test-weigh ($r = 0.69$, $p = 0.003$). However, our use of the Bland-Altman approach revealed a general lack of agreement, indicating that one measurement cannot be substituted for the other. Of note, infants included in the Lai et al.'s⁷ and Kent et al.'s⁹ studies were much older at the time of the hourly MPR measurements. It is possible that MPR is more tightly tied to infant intake later in lactation.

We observed significant correlations between MPR and bias, indicating that mothers with higher milk production have a greater reserve in milk supply above and beyond infant

intake. This finding challenges the long-held belief that infant demand is the main driver of maternal milk supply.¹⁴ It is important to note that regular expression of “surplus milk” (i.e., milk volumes that regularly exceed the volume of expressed breast milk fed to the infant) is an additional source of variation to consider when comparing infant test-weigh intake to MPR. The extent to which mothers regularly express surplus milk may further widen the bias between 24-hour test-weigh measurements of infant intake and MPR. In our study, expression of surplus milk was quite small (64 g/24 hours), and even when taken into consideration, there was still substantial bias between 24-hour milk production and MPR. These results point to the need for further research to identify additional factors that drive maternal milk production.

Objective 4

In the subset with adequate infant weight gain velocity, MPR averaged 48 ± 12 g/hour (range, 35–78 g/hour), which extrapolates to $1,164 \pm 283$ g/24 hours. Infant intake by test-weighing in this subset was very similar to values reported for exclusively breastfeeding infants by Butte and King¹⁵ and Dewey et al.¹⁶

Among the four infants identified with inadequate weight gain velocity, two of their mothers were identified with inadequate milk production, with MPRs of 28 and 31 g/hour. Their infants were consuming nearly all available milk (MPRs were 124% and 118% of test-weigh intake, respectively), but this was not enough to support adequate weight gain. As mothers were not followed from the beginning of lactation, we are not able to ascertain if infrequent or insufficient breast emptying eventually led to low MPR, or conversely, if low MPR is due to a primary physiologic condition. For a third infant, their breast milk intake was inadequate (405 g/24 hours), yet the mother’s MPR indicated the infant was only consuming 53% of available milk. The fourth infant presented with erratic weight gain patterns from week to week, despite adequate infant milk transfer during the test-weigh period (789 g/24 hours) and maternal milk production (50 g/hour). For example, during the interval before the hourly breast expression protocol initiation, infant weight gain velocity was >50th percentile, but was <10th percentile the previous interval according to maternal report.

It is notable that 25% of study infants exhibited an inadequate rate of weight gain, given that all recruited study participants reported exclusive breastfeeding was going well at the time of screening. A limitation of our study is the short time frame of 2–7 days within which we assessed infant weight gain, which may have led to misclassification of some infants. Nonetheless, our unexpected results call attention to the need for continued breastfeeding evaluation and support beyond the newborn period, especially during the duration of exclusive breastfeeding. Since this study did not intend to include infants with insufficient weight gain and, therefore, only four infants were identified with weight gain <20 g/24 hours, our preliminary data on the healthy range for MPR may not be generalizable to a larger population. We recommend further research in a larger group of exclusively breastfeeding women and babies to set recommendations for monitoring of adequate infant weight gain, breast milk intake, and maternal MPR.

Conclusion

Our goal was to validate a protocol for assessing and managing the breastfeeding dyad with insufficient milk production concerns. Although MPR and infant intake test-weigh methods are related, they are measuring different entities, and therefore their use depends on what is being investigated. Validation of MPR is most useful when the aim is to determine mother’s current milk production capacity—for example, in evaluating if poor infant weight gain is due to insufficient maternal milk supply. On the other hand, test weighing is most useful for determining infant milk transfer during breastfeeding in the context of adequate maternal supply. Through the measurement of both MPR and infant intake test-weighing, it is possible to differentiate poor infant weight gain that is the result of inadequate infant milk transfer versus current insufficient milk production. Our results suggest an MPR ≥ 35 g/hour is consistent with successful exclusive breastfeeding in early lactation. Future research should aim to field test both clinical and research applications of hourly breast expression and assess the potential for mother’s self-assessment of current milk production capacity by utilizing the MPR protocol.

Authors’ Contributions

L.A.N.-R., E.A.W., D.M.R., and S.W.R. designed the research study; E.A.W. and D.M.R. conducted the research; L.A.N.-R., D.M.R., and E.A.W. analyzed the data; D.M.R. wrote the first draft of the article; and L.A.N.-R., E.A.W., and S.W.R. made substantive contributions to the final draft of the article; L.A.N.-R. had primary responsibility for final content. All authors read and approved the final article.

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Address correspondence to:
 Laurie A. Nommsen-Rivers, PhD
 Department of Rehabilitation, Exercise,
 and Nutritional Sciences
 College of Allied Health Sciences
 University of Cincinnati
 3225 Eden Avenue
 Cincinnati, OH 45267-0394
 USA

E-mail: laurie.rivers@uc.edu