Cutaneous perfusion of the human lactating breast: a pilot study with laser Doppler perfusion monitoring

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Abstract

Objective: Knowledge on the hemodynamics of the human lactating breast contributes to our understanding of lactation physiology, as well as the development and management of breastfeeding problems. The objective of this pilot study was to investigate whether laser Doppler perfusion monitoring (LDPM) can be employed to measure physiological changes in mammary cutaneous perfusion during milk extraction. Approach: We evaluated mammary cutaneous perfusion with LDPM in nine lactating women during milk extraction in both the ipsilateral (‘milk extracting’) and contralateral (‘passive’) breast. Fourier domain filtering of the LDPM signal was applied to correct for the influence of the periodic tissue movement caused by the breast pump. Main results: Cutaneous perfusion increased temporarily during 23.7 ± 18.9 s by 18%–74% for all women who sensed their milk ejection reflex (n = 6) in both the ipsilateral and contralateral breast. For those women who did not sense a milk ejection reflex (n = 3), the changes in cutaneous perfusion were less outspoken (maximally 26%). Significance: This pilot study provides new insights into mammary hemodynamics and demonstrates that LDPM is a promising method for the further investigation of physiological changes in mammary cutaneous perfusion during milk ejection. Objective feedback on the occurrence and progression of milk ejection can support lactation research in general, maternal breastfeeding confidence, and may be an early indicator for the development of breastfeeding problems.

1. Introduction

Breastfeeding benefits infants, mothers and societies (Rollins et al 2016, Victora et al 2016). Although mothers worldwide are being advised to exclusively breastfeed their infants for 6 months, only 40% of mothers attains this goal (World Health Organization and UNICEF 2017). The most important reasons for the cessation of breastfeeding in the first two months postpartum are the perception of insufficient milk supply and pain, due to breastfeeding problems (Li et al 2008). More knowledge on the physiology of lactation can contribute to a better understanding of milk synthesis, milk transfer to the infant, as well as the development and management of breastfeeding problems. In turn, this may eventually lead to a better support for mothers to reach their own breastfeeding goals.

One aspect of the physiology of lactation that has not yet been studied in a great amount of detail is the hemodynamics of the breast, despite its importance for milk synthesis. It is estimated that mammary blood flow (MBF) increases by a factor 2.5 during pregnancy to meet with the nutritional demands for milk synthesis (Thoresen and Wesche 1988). During a breastfeed, mammary hemodynamics have been studied with Doppler ultrasound and near-infrared spectroscopy (NIRS) (Janbu et al 1985, Ogawa et al 2008, Tanimoto et al 2011, Geddes et al 2012). The most important findings from these studies are that MBF and milk production are not related (Geddes et al 2012), but that the milk ejection reflex (MER) is associated with a temporary decrease in blood flow and total blood volume inside the breast (Janbu et al 1985, Tanimoto et al 2011). For those studies that simultaneously investigated both breasts, the ipsilateral (‘milk extracting’) and contralateral (‘passive’) breast

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gave similar results in hemodynamic changes (Janbu et al. 1985, Ogawa et al. 2008, Tanimoto et al. 2011). This confirms that the hemodynamics of the breast during a breastfeeding greatly rely on the systemic regulation by hormones, such as oxytocin (Tanimoto et al. 2011).

Despite the accessibility of skin for monitoring purposes, the cutaneous hemodynamics of the human lactating breast have not yet been investigated. Nevertheless, there are multiple clues that also this is an interesting and important aspect to the physiology of lactation. Mammary skin temperature increases during a breastfeeding and changes over the course of lactation, which can be ascribed to changes in cutaneous blood flow (Kimura and Matsuoka 2007). In cows, it has been demonstrated that these changes in mammary skin temperature can serve as an early indicator for mastitis (Polat et al. 2010). When studying cutaneous hemodynamics directly, it was demonstrated in rats that cutaneous perfusion temporarily increases during milk ejection (Eriksson et al. 1996). This opposite effect compared to the decrease in total MBF and volume (Janbu et al. 1985, Tanimoto et al. 2011) can be explained by the fact that the cutaneous microvasculature primarily responds to the vasodilating action of oxytocin, whereas it is hypothesized that the deeper mammary vasculature is mechanically compressed by the expanding milk ducts during the MER (Tanimoto et al. 2011).

Overall, the study of the cutaneous hemodynamics of the human lactating breast will contribute to a more complete understanding of the total hemodynamics of the lactating breast, and its physiology in general. Therefore, the objective of this pilot study was to investigate whether laser Doppler perfusion monitoring (LDPM) can be employed to investigate physiological changes in human mammary cutaneous perfusion during milk extraction. LDPM is a widely applied clinical method to noninvasively study skin perfusion, which is a measure of cutaneous blood flow (Klaessens et al. 2003, Lima and Bakker 2005, Janssen et al. 2007). We investigated cutaneous perfusion on nine lactating women in both the ipsilateral and contralateral breast and related our findings to the occurrence of the MER.

2. Methods

2.1. Participants

This pilot study was approved by the Committee on Research Involving Human Subjects (CMO Arnhem-Nijmegen, The Netherlands). The study included mothers across The Netherlands and all data were collected between April 2018 and July 2018. Exclusion criteria were a lactation period of less than 2 weeks postpartum, as well as breastfeeding problems at the time of the experiment: engorgement, mastitis, Raynaud’s syndrome, etc. The experiments were conducted in the participant’s own environment for milk expression, which was either their home, or the designated room for milk extraction at work.

A total of nine healthy women between 3 and 8 months of lactation were included (table 1) and all participants gave written consent prior to the study. The participants maintained their normal milk extraction behavior during the experiment and used their own breast pump with breast shield size matched to breast size. The time since the last feeding varied between participants from 2 to 15 h (table 1). One participant (p8) indicated that she had no experience with sensing her MER during breastfeeding or milk expression. All other participants indicated that they were able to sense their MER during milk extraction as a tingling and/or stinging sensation in the nipple or breast. All participants had a Caucasian skin type, except participant p2, who had an Asian skin type.

2.2. Measurement

The experiments were conducted with a commercial LDPM device: the PeriFlux System 5000 (Perimed, Sweden), operating at a wavelength of 780 nm. Three fiber-optic sensors (type 408) were connected to the device to independently measure the perfusion from three different skin locations at both the ipsilateral, and contralateral breast: ~5 cm above the areola (ipsilateral-high), ~5 cm above the areola (contralateral-high), and ~1 cm above the areola (contralateral-low), see figure 1(a). The illuminated skin area by the fiber-optic sensors was approximately 1 mm². The breasts were fully uncovered during the measurement and care was taken to avoid skin areas with moles and large veins close to the skin surface. All sensors were calibrated prior to the experiment according to the instructions of the manufacturer, which gave a perfusion level of 250 perfusion units (PU) on the motility standard (PF1001) provided by the manufacturer. Due to either a calibration error or inadequate sensor contact, the contralateral-high signal from participant p7 was excluded for further analysis.

2.3. Data collection

All data were collected through the software PeriSoft v2.10 (Perimed, Sweden) with a sampling frequency of 32 Hz. The participants expressed milk from one breast (ipsilateral) using their own electrical breast pump. Prior to the experiment, it was assured that the participant was at ease in a comfortable position, and that the breast shield of the electrical pump was correctly positioned at the breast. Room temperature was between 22.5 °C–25 °C for all experiments.
The LDPM measurement consisted of the following phases: (1) a baseline measurement of 2 min, prior to milk expression, (2) a milk expression stimulation phase with high frequency vacuum changes from the breast pump to evoke the first MER, (3) a milk expression phase with lower frequency vacuum changes from the breast pump to support milk extraction until the participant indicated that the breast was empty, and (4) a post expression measurement of 2–5 min without any interventions after milk expression was completed. Participants were asked to indicate the timing of their MER, as well as the moments to transfer from phase 2 to phase 3, and from phase 3 to phase 4. After the experiment, the expressed milk volume from the ipsilateral breast was registered (table 2).

### 2.4. Management of movement artifacts

Since LDPM is a method to assess the movement of red blood cells, it is also prone to movement artifacts. Therefore, the participant was made aware of the influence of movement artifacts on the LDPM signal, and she was explicitly asked to avoid talking and nodding, and to move as little as possible during the experiment. Any movements by the participant that still occurred during the experiment were marked in time.

During the experiment, the researcher operated the electrical breast pump according to the instructions by the participant. Hereto, the participant communicated with the researchers by pointing her index finger on an icon card (4 cm × 4 cm), without moving her arm. On the icon card, the participant could indicate the occurrence of her MER, how the researchers should operate her breast pump (+ increase, − decrease vacuum), as well as a yes/no to questions of the researchers (figure 1(b)). During the second minute of the baseline measurement (experiment phase 1), the participant was asked to move her index finger three times across all icons to allow for checking the absence of movement artifacts from this procedure.

### 2.5. Data analysis

After acquisition of the LDPM perfusion signal as a function of time, the data were analyzed in Matlab. Since the electrical breast pump causes a periodic movement of the breast tissue, these artifacts were filtered out by applying a Notch filter in the Fourier domain around the pump frequency (table 2), which depended on breast pump brand and milk expression phase. If vasomotion was observed in the LDPM signal, an additional Notch
A filter around 0.1 Hz was applied (Krupatkin 2009). Prior to fast Fourier transformation, the individual time traces of experiment phase 2 and 3 were multiplied by a Hamming window. Zero padding of experiment phase 2 was applied to ensure equal sample numbers for both time traces, in order to prevent frequency information loss in phase 2. The remaining high-frequency noise was smoothed with a moving average filter with a time constant of 3 s in the time domain. The data analysis procedure was identical for the ipsilateral and contralateral breast.

Around the timing of the MER, the LDPM signal was investigated in more detail. To facilitate comparison between measurement sites and participants, we investigated the relative changes in the LDPM signal in [%] with respect to the average perfusion signal value over 5 s at approximately 30 s prior to milk ejection. The duration of the MER was defined as the time that the relative change remained above a threshold of 10%, around the maximum perfusion change associated with the MER.

Table 2. Experimental outcomes per participant.

| Participant | Expressed milk volume (ml) | \( f_{\text{pump},2} \) (Hz) | \( f_{\text{pump},3} \) (Hz) | Measurement site | Absolute perfusion level prior to MER1 average ± sd (PU) | Maximum relative perfusion change (%) | Duration of MER (s) |
|-------------|-----------------------------|-----------------------------|-----------------------------|------------------|------------------------------------------------------|-------------------------------------|-------------------|
| p1          | 150                         | 1.8                         | 1.0                         | Ipsi-high        | 68.8 ± 1.9                                           | 37.2                                | 21.7              |
|             |                             |                             |                             | contra-high      | 18.3 ± 0.1                                           | 17.8                                | 2.4               |
|             |                             |                             |                             | Contra-low       | 9.1 ± 0.02                                           | 47.6                                | 27.4              |
| p2          | 120                         | 1.6                         | 0.8                         | Ipsi-high        | 6.8 ± 0.3                                            | 42.5                                | 12.4              |
|             |                             |                             |                             | Contra-high      | 22.8 ± 0.3                                           | 20.6                                | 6.0               |
|             |                             |                             |                             | Contra-low       | 10.5 ± 0.2                                           | 24.4                                | 8.0               |
| p3          | 80                          | (-)2                        | (-)2                        | Ipsi-high        | 16.2 ± 0.1                                           | 47.3                                | 13.6              |
|             |                             |                             |                             | Contra-high      | 7.8 ± 0.2                                            | 39.8                                | 23.9              |
|             |                             |                             |                             | Contra-low       | 2.5 ± 0.1                                            | 49.4                                | 71.0              |
| p4          | 50                          | 1.8                         | 1.0                         | Ipsi-high        | 32.5 ± 1.3                                           | 74.2                                | 13.9              |
|             |                             |                             |                             | Contra-high      | 9.8 ± 0.4                                            | 70.8                                | 24.8              |
|             |                             |                             |                             | Contra-low       | 5.5 ± 0.2                                            | 35.2                                | 10.9              |
| p5          | 160                         | 1.8                         | 1.0                         | Ipsi-high        | 38.7 ± 0.6                                           | 33.0                                | 68.4              |
|             |                             |                             |                             | Contra-high      | 16.1 ± 0.3                                           | 42.0                                | 19.1              |
|             |                             |                             |                             | Contra-low       | 23.8 ± 1.1                                           | 23.3                                | 14.7              |
| p6          | 105                         | 1.8                         | 1.0                         | Ipsi-high        | 50.0 ± 0.7                                           | contact loss                        | contact loss      |
|             |                             |                             |                             | Contra-high      | 2.1 ± 0.02                                           | 64.0                                | 38.3              |
|             |                             |                             |                             | Contra-low       | 9.0 ± 0.01                                           | 30.1                                | 26.3              |
|             |                             |                             |                             |                 | Average ± sd                                        | Average ± sd                        |
|             |                             |                             |                             |                 | 39.4 ± 17.1                                          | 23.7 ± 18.9                         |

Participants without milk ejection sensation

| Participant | Expressed milk volume (ml) | \( f_{\text{pump},2} \) (Hz) | \( f_{\text{pump},3} \) (Hz) | Measurement site | Absolute perfusion level prior to MER1 average ± sd (PU) | Maximum relative perfusion change (%) | Duration of MER (s) |
|-------------|-----------------------------|-----------------------------|-----------------------------|------------------|------------------------------------------------------|-------------------------------------|-------------------|
| p7          | 70                          | (-)3                        | 0.8                         | Ipsi-high        | 19.6 ± 0.2                                           | 19.4                                | —                 |
|             |                             |                             |                             | Contra-high      | No data                                              | No data                             | —                 |
|             |                             |                             |                             | Contra-low       | 13.2 ± 0.06                                          | 20.3                                | —                 |
| p8          | 65                          | 1.8                         | 1.0                         | Ipsi-high        | 70.1 ± 1.2                                           | 4.7                                 | —                 |
|             |                             |                             |                             | Contra-high      | 17.1 ± 0.5                                           | 8.6                                 | —                 |
|             |                             |                             |                             | Contra-low       | 5.3 ± 0.1                                            | 7.1                                 | —                 |
| p9          | 60                          | 1.8                         | 0.8                         | Ipsi-high        | 34.0 ± 0.7                                           | 3.8                                 | —                 |
|             |                             |                             |                             | Contra-high      | 10.2 ± 0.2                                           | 25.7                                | —                 |
|             |                             |                             |                             | Contra-low       | 4.7 ± 0.1                                            | 23.9                                | —                 |
|             |                             |                             |                             |                 | Average ± sd                                        | Average ± sd                        |
|             |                             |                             |                             |                 | 14.2 ± 8.4                                           | —                                   | —                 |

1 At relative time \( t = 0 \) s (average perfusion value over 5 s).
2 No pump frequency detected in LDPM signal.
3 Participant switched directly from phase 1 to phase 3.

\( f_{\text{pump},2} \): pump frequency in experimental phase 2, \( f_{\text{pump},3} \): pump frequency in experimental phase 3, sd: standard deviation.
3. Results

Figure 2(a) shows an example raw data set for the complete LDPM measurement on participant p1 during all phases of the experiment and for all measurement sites (ipsilateral-high, contralateral-high and contralateral-low). As the pump frequencies are most dominant in the ipsilateral-high signal, the Fourier transformed spectra of this signal is depicted in figures 2(b)–(e) for all experimental phases. The pump frequencies of phase 2 (1.8 Hz) and phase 3 (0.96 Hz) are well defined, as well as their higher harmonics (figures 2(c) and (d)). A relatively constant heart rate during the baseline measurement in phase 1 is reflected by the peak at 1.2 Hz, or 72 beats per minute (figure 2(b)). The pump frequencies that were retrieved from the LDPM signal are listed in table 2 for all participants. Differences can be explained by differences in pump type. No pump frequencies were detected in the LDPM signal from participant p3. Following our data analysis procedure, we obtained the filtered and smoothed data sets, which are depicted in figure 3 for participant p1. This dataset was deliberately selected as an example, to show the influence of movement artifacts (indicated by ‘m’) on the LDPM signal.

3.1. Perfusion changes during all experimental phases

The following changes in the perfusion signal were observed for participant p1 (figure 3), and are analogous to the data of the other participants.

- Phase 1 (baseline): the perfusion remains constant for both the ipsilateral and contralateral breast. The movement of the participant’s finger on the icon card does not induce any movement artifacts.
- Phase 2 (milk expression stimulation): the perfusion signal increases for both the ipsilateral and contralateral breast. For this participant, this is primarily caused by a movement artifact (m). In the absence of movement artifacts, the ipsilateral breast also shows an increased perfusion signal directly after the start of phase 2—as was observed for the other participants. This can be explained by the mechanical action of the breast pump on the mammary tissue, which can induce both an ‘actual’ increase in perfusion due to a physiological response by the breast, as well as an ‘artificial’ increase in perfusion due to incomplete removal of the tissue motion artifacts induced by the breast pump.
- MER: when the MER is sensed by the participant, the perfusion signal increases temporally in both the ipsilateral and contralateral breast.
- Phase 3 (milk expression): for the ipsilateral breast, the perfusion signal remains relatively constant around a value that is higher than the baseline value before milk expression. Temporal increases in the perfusion signal can be observed, but these are generally slower and smaller in amplitude than the response to the MER. For the contralateral breast, the perfusion signal remains constant at baseline value. Again, movement artifacts (m) can cause a temporal increase in the perfusion signal.
- Phase 4 (post expression): the perfusion declines to the baseline value before milk expression for the ipsilateral breast. For the contralateral breast, the perfusion signal remains constant at baseline value.

The perfusion values (expressed in PU as standardized by the instrument’s calibration) vary between the three different sensors on the contralateral and ipsilateral breast, due to differences in baseline skin perfusion.
3.2. Perfusion changes in the presence of milk ejection sensation

Omitting movement artifacts, the most prominent changes in the perfusion signal can be observed around the moment that the participant senses her MER. Hence, this event was investigated in more detail. Figure 4 presents the perfusion change in [%] as a function of relative time for all participants. To facilitate comparison, relative time is displayed on the horizontal axis during 100 s, around the occurrence of the MER (figures 4(a)–(f)). The absolute perfusion in (PU) at relative time \( t = 0 \) s is listed in table 2.

In general, we can observe an increase in perfusion for all three measurement sites of 18%–74% during, or briefly after the moment that the participant indicated to sense her MER (figures 4(a)–(f), table 2). Only for participant p2, the most prominent increase in perfusion after the sensation of milk ejection is delayed with approximately 20 s. For participant p6, the drift in the ipsilateral perfusion signal can be explained by a loss of adequate contact between the LDPM sensor and the skin. For participant p1, p2 and p3, the timing of the perfusion changes is similar for all three measurement sites. However, for participant p4, p5 and p6, the ipsilateral perfusion signal changes asynchronously with respect to the contralateral perfusion signals. On average, the perfusion signal remains above the 10% threshold during 23.7 \( \pm \) 18.9 s (table 2).

3.3. Perfusion changes in the absence of milk ejection sensation

For those mothers who did not sense their MER, the change in perfusion is displayed for the first 100 s after the transfer from phase 1 to phase 2 (figures 4(g)–(i)), except for participant p7 (figure 4(g)), who transferred directly from phase 1 to phase 3. Although these participants did not sense a MER, milk flow did commence during the time period displayed in figures 4(g)–(i). In general, the perfusion signal does not change as dramatically (largest increase 26%) as for the participants who did sense their MER (table 2). For the contralateral measurement sites in participants p7 and p9, we can observe a temporal increase in the perfusion signal, which for participant p9 is partly caused by a movement artifact.

4. Discussion

In this pilot study, we used LDPM to learn more about the perfusion in the skin of the lactating breast, in particular its response to the MER. When the participants sensed their MER, the perfusion signal increased substantially in both the ipsilateral and contralateral breast. Omitting the drifting ipsilateral signal from participant p6, this increase in perfusion amounted to 18%–74% (figures 4(a)–(f)). Our findings are similar to those from the cutaneous perfusion study on lactating rats, where a 30%–50% increase in perfusion was observed after the intravenous administration of oxytocin (Eriksson et al 1996). Also, the duration of the temporary increase in perfusion during milk ejection (23.7 \( \pm \) 18.9 s) is comparable to the time it takes for the milk ducts to reach their maximum diameter when dilating during milk ejection (26.5 \( \pm \) 11.2 s) (Ramsay et al 2005b). It is therefore plausible that the measured increase in perfusion is caused by the MER, rather than a general autonomic response. The observed increase in skin perfusion may either be due to the vasodilating action of oxytocin, or the mechanical pressure of the dilating milk ducts that forces blood into the microvasculature of the skin. The vasodilating action of oxytocin may be verified in future studies that measure skin perfusion at other body
locations, whereas the influence of dilating milk ducts can be investigated by combining LDPM with ultrasound imaging of milk duct dynamics (Ramsay et al 2004, 2005b). To relate skin perfusion to the hemodynamics inside the deeper lying breast tissue, LDPM can be combined with Doppler ultrasound and NIRS (Tanimoto et al 2011, Geddes et al 2012). The combination of LDPM with ultrasound imaging will further objectify the validation of the occurrence and timing of the MER. It may also explain the asynchronous behavior between the ipsilateral and contralateral measurement sites for some participants (Gardner et al 2015), and it will allow to study the response of the perfusion signal to any other MERs occurring after the first reflex (Ramsay et al 2004).

An unexpected finding may be that the perfusion signal did not change as dramatically for those participants who did not sense their MER, despite the fact that their milk flow also became established during milk extraction and thus, milk ejection must have occurred (figures 4(g)–(i)). Also here, combining LDPM with ultrasound imaging will support the verification of the presence and timing of a MER. Our findings suggest that a relation may exist between the ‘tingling’ sensation of milk ejection and mammary skin perfusion. Literature on this tingling sensation related to the MER is scarce, and remains limited to observations, rather than an explanation for this phenomenon (Waller 1943, Newton and Newton 1948). Therefore, the relation between this sensation, mammary skin perfusion and milk duct dilation will be an intriguing topic for future research.

4.1. Limitations
As explained above, future studies with LDPM on mammary skin perfusion will benefit from including ultrasound imaging as a validation method. Due to the delayed, and more gradual response of skin temperature to changes in skin perfusion, temperature measurements are unlikely to be helpful in validating the occurrence of the MER. However, these may be of interest in future studies to validate the slower perfusion changes during the entire breastfeed (Kimura and Matsuoka 2007).

It should be noted that also the technique LDPM has several limitations. Although our data analysis procedure filters out the pump frequency, pump-induced tissue motion at other frequencies is likely to be the cause of the increase in the absolute value of the LDPM signal in the ipsilateral breast during experimental phases 2 and 3 (figure 2(a)). The conclusions that we draw from this study are therefore all based on the relative changes of the LDPM signal. As can be observed in our data, movement artifacts have a similar effect on the LDPM signal as the MER. In its current implementation, LDPM is therefore unlikely to be useful during live breastfeeding. However, several technological improvements can be investigated, such as the combination of LDPM with specialized
motion sensors. Automated registration of any breast motion will also support the verification of the absence of movement artifacts due to deformation of the areola during milk ejection (Waller 1943, Geddes 2009). For this study, we consider the influence of areola deformation low, as the LDPM signal closest to the areola (contralateral-low) did not show perfusion changes that were substantially higher than for the other two measurement sites.

Another known limitation of LDPM is its decreased signal to noise ratio on highly pigmented skin, because of the attenuation of light by melanin in the epidermal layer (Abbot et al 1996). Eight participants in this study had a Caucasian skin type, and one participant had an Asian skin type. Therefore, future research should also include mothers with a higher degree of skin pigmentation. The penetration depth of LDPM is limited to approximately 0.5–1 mm, which limits LDPM perfusion measurements to the skin only. Care should be taken when applying LDPM at the base of the nipple, as milk ducts can be located close to the skin surface (≥0.7 mm) at this location (Ramsay et al 2005a). When the LDPM photons interact with flowing milk, this will also induce an increase in the perfusion signal.

4.2. Significance

If the current limitations are handled properly, LDPM can potentially become a valuable tool in obtaining a more complete understanding of the hemodynamics of the human lactating breast. The skin is better accessible for monitoring purposes than the breast tissue that it covers, and LDPM requires less complicated equipment and operating skills than, for example, ultrasound imaging. For the practice of breastfeeding research and support, objective feedback on the occurrence of milk ejection can support maternal confidence, and lactation research in general (Geddes 2009). An interesting topic for future research will be if breast skin perfusion can be an early indicator for the development of breastfeeding problems, such as mastitis and Raynaud’s syndrome (Barrett et al 2013).

5. Conclusion

In this pilot study, we investigated mammary skin perfusion with LDPM. For those women who sensed their MER, we measured a temporary increase in mammary skin perfusion in both the ipsilateral and contralateral breast. Despite its sensitivity to movement artifacts, we can conclude that LDPM is a promising method for the further investigation of mammary skin perfusion.

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