Original Article

Changes in milk composition associated with pethidine-PCEA usage after Caesarean section

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Abstract

The effect of pethidine as patient-controlled epidural analgesia (PCEA) on specific biochemical components in breast milk in relation to the timing of secretory activation is not well investigated. The aim of this study was to compare biochemical timing of secretory activation between women who had a vaginal (V) or Caesarean birth with pethidine-PCEA (CBP). Several milk samples were collected daily from 36 mothers (17 V, 19 CBP) for the first 265 h post-partum. Protein and lactose concentrations and Na⁺ and K⁺ ion levels were measured. Samples were assigned to three time periods: 0–72, >72–165 and >165–265 h post-partum for statistical analyses. Data were analyzed using linear mixed effect models. In the first 72 h post-partum, the mean difference in lactose concentration was 5 gL⁻¹ higher in group V ($P < 0.05$). From >72–165 h post-partum, protein and Na⁺ concentrations were lower in group V ($P = 0.05$, $P = 0.02$), and K⁺ levels were higher in group V ($P < 0.001$). From >165–265 h post-partum, there were no significant differences between the groups. Biochemically, secretory activation had occurred by 72 h post-partum in both groups. There were greater variations in measured biochemical components observed within group CBP initially. However, by 165 h post-partum, there were no differences in the biochemical components between the groups. This suggests that effects of pethidine-PCEA are diminished by 72 h post-partum and undetected by 165 h.

Keywords: breast milk composition, lactation, breastfeeding, Caesarean, pethidine-PCEA.

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Introduction

Delayed onset of lactation and shorter breastfeeding durations have been reported after delivery by Caesarean birth (Chapman & Pérez-Escamilla 1999; Dewey *et al.* 2003; Scott *et al.* 2007; Prior *et al.* 2012). Given that Caesarean birth (CB) delivery currently accounts for 16% of births worldwide, the impact on lactation may be substantial (WHO 2013). CB-delivery rates have increased substantially from 23.3% to 31.5% in Australia over a 10-year period between 2000 and 2009 (Li *et al.* 2011) so a sizeable number of women appear at risk of a delayed initiation of lactation. Because breastfeeding provides protection against many diseases, both in the short and longer term, and these benefits are often dose dependent, a reduction in breastfeeding duration due to

birth mode may have far reaching health and economic consequences (Ip *et al.* 2007; Eidelman *et al.* 2012).

Secretory activation is the initiation of copious milk production at around 24–72 h post-partum. This process begins with the removal of placenta, initiating a decline in progesterone level that triggers secretory activation (Kulski *et al.* 1977; Pang & Hartmann 2007). The increase in milk production is associated with biochemical changes in milk components, such that there is an increase in lactose, a decrease in protein and sodium (Na⁺) and an increase in potassium (K⁺) concentration (Kulski & Hartmann 1981; Mitoulas *et al.* 2002; Pang & Hartmann 2007). These changes are due to the closure of the tight junctions between the lactocytes (mammary epithelial cells) (Neville 2001). Thus, the absence of or delay in changes to these milk

components reflects a delay in secretory activation (Kulski & Hartmann 1981; Arthur *et al.* 1989; Montagne *et al.* 1999).

Studies investigating the initiation of lactation after birth by CB show conflicting results: either a delay (Chen *et al.* 1998; Chapman & Pérez-Escamilla 1999; Dewey 2001; Dewey *et al.* 2003; Scott *et al.* 2007; Prior *et al.* 2012) or no effect (Kulski *et al.* 1981; Ahluwalia *et al.* 2012). The reasons for the reported delay in secretory activation associated with CB are not clear. Reduced oxytocin levels, remaining placental fragments inhibiting the decline in progesterone levels and extended times to the first breastfeed, have been implicated. Anaesthetics and analgesics vary globally and regionally, yet little research has been carried out on their effect on lactation.

Pethidine, also known as meperidine, is used as an epidural analgesic for the alleviation of pain after CB. Pethidine is a lipophilic opioid, with reported half-life of approximately 3–5 h (Tamsen *et al.* 1982; Molloy 2002). It is metabolized in the liver to norpethidine (half-life of 15–40 h) (Armstrong & Bersten 1986) and then excreted via the kidneys. The concentrations of pethidine and norpethidine in mothers who received pethidine-PCEA after CB have been investigated. Both pethidine and norpethidine concentrations in the infant plasma, maternal plasma and breast milk were found to be within recommended safety levels (Al-Tamimi *et al.* 2011). In another study on the same cohort, CBP mothers' perceptions of the fullness of the breast, which is associated with secretory activation, occurred significantly later than that of mothers that had a vaginal birth. In addition, infants in group CBP were found to have higher suck rates at day 3 post-partum providing further evidence supporting the possibility of a delay in secretory activation (Sakalidis *et al.* 2013).

The aim of this study was to determine whether secretory activation was delayed in CB mothers using pethidine-PCEA compared with those that deliver vaginally as determined by the changes in the biochemical components of breast milk.

Methods

Participants

Breast milk samples were obtained from 36 mothers of healthy, term infants recruited at antenatal clinics at King Edward Memorial Hospital, Perth for Women, Perth, Western Australia. All participating mothers provided written informed consent to participate in the study, which was approved by the Human Research and Ethics Committee of King Edward Memorial Hospital for Women and registered with the Australian and New Zealand Clinical Trials Registry. The sample size was based on previous experience of sparse-sampling study design for drug studies in lactating women (Ilett *et al.* 2008; Al-Tamimi *et al.* 2011). Sparse sampling is a widely used methodology, employed when it is not practical to obtain multiple biological samples and is frequently used in drug studies. Therefore, this methodology is used in situations where access to samples is limited. In this study, all available samples were collected when mothers were able to express adequate volume. The participants were all American Society of Anaesthesiologists physical status classification I (healthy) or II (well-controlled medically). Mothers were either scheduled for elective CB at 37 or more weeks of gestation, and had consented to combined spinal-epidural anaesthesia with pethidine PCEA for postoperative analgesia, or had delivered vaginally with no opioid patient-controlled epidural analgesia.

Key messages

- The impact of Caesarean birth with pethidine-PCEA has minimal effect on biochemical components of breast milk indicative of secretory activation.
- There were no significant differences in biochemical components of breast milk between group V and group CBP by 165 h.
- In the early post-partum period, lactose concentration increased more rapidly for group CBP.
- Changes in Na⁺ and K⁺ concentrations were more rapid in group CBP, potentially due to effect of pethidine on ion channels in the lactocytes.

Demographic data collected included maternal age, gestation and infant birth weight. In addition, the duration and dose of pethidine administered and the infant Neurologic and Adaptive Capacity Score were recorded. Neurologic and Adaptive Capacity Score is used to assess the effect of drugs administered to the mother during labour on the central nervous system of the infant (Amiel-Tison *et al.* 1982).

Analgia regimens

Pethidine hydrochloride (Hospira Australia Pty Ltd., Melbourne, Australia) was administered by a mechanical PCEA device (Go Medical Industries Pty Ltd, Subiaco, WA, Australia). Pethidine 5 mg mL^{-1} was available in a 4 mL bolus (20 mg), with a lockout interval of 20 min and no 4 h limit (Banks & Pavy 2001). Mothers commenced PCEA within a few hours of birth and continued for 24–48 h post-operatively as required. Multimodal analgesia is commonly used, and other drugs taken by women in both groups of this study included paracetamol, ibuprofen, diclofenac and tramadol with the exception of oxycodone, which the CBP group took.

Milk sampling

Daily milk samples (approximately 1 mL) were collected from both left and right breasts from 0 to 265 h post-partum by means of either manual breast pump or hand expression into 5 mL polypropylene vials (Disposable Products Pty Ltd, Adelaide, Australia). However, there were days (usually days 3 and 4 post-partum) when some participants provided more than one set of milk samples at different times throughout the day. The number of samples provided by participants depended on the availability of the colostrum/milk, which is consistent with the sparse sampling methodology employed in the study. Samples were immediately frozen and subsequently transported on ice to the laboratory where they were stored at -80°C until analysis.

Biochemical analysis

Protein

The protein content of defatted milk samples was determined using the Bradford protein assay using a

commercial protein reagent (Bio-Rad Laboratories, Richmond, California). Human milk protein standards were prepared by determining the concentration of an aliquot of mature breast milk (Atwood & Hartmann 1992). Protein assays (Mitoulas *et al.* 2002) were adapted and carried out by JANUS workstation (PerkinElmer, Inc, Waltham, US) and measured on EnSpire (PerkinElmer, Inc., Waltham, US). The recovery of a known amount of protein added to milk samples was 99.7% (standard deviation (SD) 3.3, $n = 5$). The detection limit of this assay was 0.048 g L^{-1} , and the inter-assay coefficient of variation (CV) was 18.5% ($n = 28$).

Lactose

The lactose content of defatted milk samples was analyzed by an enzymatic spectrophotometric method (Arthur *et al.* 1989; Mitoulas *et al.* 2002), which was adapted for use on JANUS (PerkinElmer, Inc., Waltham, US) workstation and absorbance measured with EnSpire (PerkinElmer, Inc., Waltham, US). The recovery of a known amount of lactose added to milk samples was 100.3% (SD 3.3%, $n = 8$). The detection limit (3X SD low concentration standard) of this assay was 2.11 g L^{-1} , and the inter-assay CV was 5.9% ($n = 23$).

Fat

The fat content of milk samples was determined by the creatocrit method (Fleet & Linzell 1964) using the Creatocrit Plus device (Medela, Inc., McHenry, Illinois). This method has been demonstrated to have a strong correlation with results obtained by the spectroscopic esterified fatty acid assay (Mitoulas *et al.* 2002; Czank *et al.* 2009).

Na^{+} and K^{+}

The Na^{+} and K^{+} concentrations of whole milk samples were determined by Na^{+} and K^{+} ion selective electrodes [HORIBA LAQUAtwin B-722 (Na^{+}); B-731 (K^{+}), HORIBA Ltd, Kyoto, Japan]. The principle of the ion selective electrode was demonstrated by Goulet *et al.* (2012) on sweat samples (Goulet *et al.* 2012). The recovery of a known amount of Na^{+} added to milk samples was 86.2% (SD 8.7, $n = 10$) and for K^{+} 86.5% (SD 10,

$n = 10$). The detection limit of these assays was 0.0561 mM and 0.0142 mM for Na^+ and K^+ , respectively, with inter-assay CVs of 0.92% ($n = 5$) for Na^+ and 0.78% ($n = 5$) for K^+ . Na^+/K^+ ratio was categorized in accordance with the scale used by Filteau *et al.* (1999a,1999b; Allen *et al.* 1991) A milk Na^+/K^+ ratio >1.0 indicated pre-secretory activation; <1.0 and >0.6 was indicative of 'transitional' milk and <0.6 was associated with 'mature' milk. The declining Na^+/K^+ ratio signified closure of tight junctions and increased milk synthesis as secretory activation progressed (Neville *et al.* 1991).

Statistical analysis

Statistical analysis was performed using R Statistical Software programme, version 3.1.1 (The R Core Team 2015). The packages nlme (Pinheiro *et al.* 2009) and lattice (Sarkar 2008) were used for linear mixed effect models and graphical plots, respectively. P -values less than 0.05 were considered statistically significant.

Macronutrient and ion concentrations were compared between groups V and CBP using linear mixed effects models with random intercepts, grouped by participants. Mode of delivery was the fixed effect of interest; time post-partum was included as a covariate; the possibility of different time courses by mode of delivery was tested with an interaction term (time post-partum $\times$ mode of delivery).

Random effects of left and right breasts within participant were considered not significant. Analyses were run separately for data for 0–72, >72–165 and >165–265 h post-partum. Linear-mixed effect models was also used to analyze participant characteristics, with mode of delivery as fixed effect and with random intercepts grouped

by participants (Table 1). The effects of pethidine-PCEA dose and duration in relation to the time of first breastfeed in CBP were compared separately using linear mixed effect models with random intercepts, grouped by participants.

Results

Participants

Seventeen participants delivered vaginally, and 19 delivered by Caesarean birth. Participant characteristics are shown in Table 1. The first breastfeed was significantly later for CBP group infants ($P = 0.002$; Table 1). Neither overall pethidine-PCEA dose nor duration was associated with the time to first breastfeed for the CBP mothers ($P = 0.766$; $P = 0.418$).

Milk composition

The concentrations of the macronutrients and micronutrients measured in the breast milk are shown in Table 2. Fat content is within the normal range. Regression curves for the results discussed later are shown in Fig. 1.

0–72 h post-partum

Macronutrient content was analyzed in 108 milk samples (13 V, $n = 49$; 14 CBP, $n = 59$) and ion concentrations in 31 (5 V, $n = 20$; 4 CBP, $n = 11$). Fewer milk samples were available for the micronutrient analyses because of the small volumes collected during this time period. Protein concentration ($P = 0.06$) was not significantly different between the groups and decreased over time. Lactose concentration increased over time with group CBP

Table 1. Participants' characteristics by mode of delivery

Characteristics	Vaginal delivery	Caesarean birth with pethidine-PCEA	P -values
Maternal age (year)	31.53 ± 5.06 ($n = 17$)	31.16 ± 4.75 ($n = 19$)	0.820
Gestational age (week)	40.07 ± 0.79 ($n = 17$)	38.94 ± 0.96 ($n = 19$)	0.0005
Parity	2.00 (1.00–3.00)* ($n = 17$)	2.00 (1.50–3.00)* ($n = 19$)	0.970
NACS	35.12 ± 2.20 ($n = 17$)	33.53 ± 2.99 ($n = 19$)	0.081
Time of first breastfeed (min)	30.50 (24.25–72.50)* ($n = 16$)	112.00 (79.50–150.00)* ($n = 19$)	0.002
Pethidine-PCEA duration (h)		38.66 ± 12.96 ($n = 19$)	
Total pethidine-PCEA dosage (mg)		610.79 ± 281.83 ($n = 19$)	

NACS is neonatal adaptive capacity score (0–40); Data are $\bar{x} \pm \text{SD}$ unless specified; P -values <0.05 indicates significant differences between vaginal delivery and Caesarean birth with pethidine-patient-controlled epidural analgesia (PCEA); *Median (IQR).

Table 2. Mean changes in concentration of milk components within period of analysis

Time post-partum	Mode of delivery	Protein (g L ⁻¹)	Lactose (g L ⁻¹)	Fat (g L ⁻¹)	Sodium (mM)	Potassium (mM)
0–72 h	V	24.8 ± 11.8 (<i>n</i> = 49)	48.4 ± 8.9 (<i>n</i> = 49)	37.8 ± 14.7 (<i>n</i> = 49)	16.9 ± 5.7 (<i>n</i> = 20)	17.2 ± 1.7 (<i>n</i> = 20)
	CBP	32.0 ± 21.9 (<i>n</i> = 59)	43.4 ± 13.2 (<i>n</i> = 59)	34.8 ± 17.6 (<i>n</i> = 59)	14.9 ± 2.7 (<i>n</i> = 11)	15.2 ± 2.1 (<i>n</i> = 11)
	<i>P</i> -value	0.061	0.005	0.069	0.095	0.787
>72–165 h	V	20.3 ± 3.2 (<i>n</i> = 155)	56.0 ± 6.1 (<i>n</i> = 155)	45.6 ± 15.7 (<i>n</i> = 155)	10.5 ± 2.6 (<i>n</i> = 98)	18.2 ± 2.4 (<i>n</i> = 98)
	CBP	21.0 ± 4.2 (<i>n</i> = 151)	55.1 ± 7.9 (<i>n</i> = 151)	42.1 ± 16.6 (<i>n</i> = 151)	13.9 ± 4.6 (<i>n</i> = 98)	16.1 ± 1.9 (<i>n</i> = 98)
	<i>P</i> -value	0.046	0.352	0.944	0.022	0.0001
>165–252 h	V	18.9 ± 2.7 (<i>n</i> = 119)	58.9 ± 5.8 (<i>n</i> = 119)	47.7 ± 17.3 (<i>n</i> = 119)	9.0 ± 3.4 (<i>n</i> = 79)	16.0 ± 2.5 (<i>n</i> = 79)
	CBP	18.3 ± 3.0 (<i>n</i> = 108)	59.9 ± 8.8 (<i>n</i> = 108)	46.0 ± 16.5 (<i>n</i> = 108)	10.6 ± 4.7 (<i>n</i> = 91)	15.1 ± 1.4 (<i>n</i> = 91)
	<i>P</i> -value	0.549	0.641	0.129	0.151	0.972

V, vaginal delivery; CBP, Caesarean birth with pethidine-patient-controlled epidural analgesia (PCEA); *n*, number of samples used in analysis. Values are presented as $\bar{x} \pm \text{SD}$.

Fig. 1. Post-partum changes in milk components. Scatterplots of regression lines showing time course post-partum (hours) in respect to milk components. Order of boxes in each row (from left): protein, lactose, sodium (Na⁺), potassium (K⁺). (A) 0–72 h; (B) >72–165 h; (C) >165–252 h. Caesarean birth with pethidine-PCEA (CBP) = ■; Vaginal delivery (V) = ●. Regression lines CBP = —; V = —. No regression lines are drawn in Na⁺ and K⁺ in row A.

increasing significantly more rapidly than group V ($P=0.005$). This difference remained when data were restricted to 30–72 h (3 V, $n=45$; 4 CBP, $n=51$; $P=0.018$). When one individual in group CBP with suspected delayed secretory activation was removed

from the 0–72 h lactose analysis ($P=0.017$), the difference remained significant, although after further restriction of the data to 30–72 h, the significant interaction ceased ($P=0.12$). Na⁺ concentrations decreased, and K⁺ increased with no difference

between groups (Na^+ $P=0.10$; K^+ $P=0.79$). The mean Na^+/K^+ ratio of the participants during this time period was 1.01 ± 0.40 for group V and 1.01 ± 0.32 for group CBP.

>72–165 h post-partum

At 72.01–165 h, 306 samples were analyzed for macronutrient content (16 V, $n=155$; 17 CBP, $n=151$) (Fig. 1). Protein concentration decreased slightly with a significant difference between the groups ($P=0.047$). However, this significance was due to the single CBP sample (Fig. 1), which was from the same individual reported earlier with suspected delayed secretory activation. If this sample is removed, significance does not persist ($P=0.12$). Lactose levels continued to increase with no difference between groups ($P=0.35$). Fat levels continued to increase with no difference between the groups ($P=0.94$). One hundred ninety-six samples were analyzed for ion concentrations (11 V, $n=98$; 14 CBP, $n=98$). Na^+ concentrations continued to decrease in both groups but were slightly more pronounced in group CBP ($P=0.022$), with an increased mean difference of 0.0458 mM. There was a significant association between the increasing K^+ levels and the interaction between groups over hours post-partum ($P < 0.001$); the K^+ levels in group CBP remained constant, while group V showed a trend to reduced concentration, with a higher mean difference of 0.0409 mM. The mean Na^+/K^+ ratio of the participants during this time period was 0.59 ± 0.20 for group V and 0.88 ± 0.34 for group CBP.

>165–265 h post-partum

At >165–265 h, 227 samples were analyzed for macronutrient content (15 V, $n=119$; 15 CBP, $n=108$), while 170 samples were used for ion concentration analysis (12 V, $n=79$; 13 CBP, $n=91$) (Fig. 1). In both groups, all macronutrient and ion content showed no significant interaction ($P > 0.10$). Fat levels remained constant, and protein and lactose concentrations continued to minimally decrease and increase, respectively, over this time period. Similarly, both Na^+ and K^+ concentrations decreased marginally. The mean Na^+/K^+ ratio of the participants during this time period was 0.60 ± 0.32 for group V and 0.71 ± 0.34 for group CBP.

Discussion

Secretory activation can be susceptible to physical, psychological or pharmacological disruption, and this can result in a suboptimal breastfeeding experience for mothers and infants. In this study, we found that although there were differences in the initial nutrient levels and the rate of change in concentrations over the first 7 days post-partum, there was no significant difference between mothers who delivered vaginally (group V) and mothers who delivered by CB with pethidine as post-operation patient-controlled epidural analgesia (group CBP) by day 11. While there were small differences in the biochemical components of the milk, the Na^+/K^+ ratio of 1.01 is indicative that secretory activation had occurred in all women by 72 h (Allen *et al.* 1991; Neville *et al.* 1991; Filteau *et al.* 1999a,1999b).

A delay in secretory activation has been shown to be associated with increased neonatal weight loss, suboptimal milk production, formula supplementation and decreased duration of breastfeeding (Chapman & Pérez-Escamilla 1999). Methods of assessing secretory activation include measurement of biochemical components of milk that indicate closure of tight junctions between the lactocytes and the mothers' perception of fullness of the breast, although this clinical sign tends to be noticed later than the biochemical changes (Arthur *et al.* 1989). At secretory activation, it would be expected that protein and Na^+ concentrations decrease and lactose and K^+ increase (Kulski *et al.* 1977; Kulski & Hartmann 1981; Mitoulas *et al.* 2002; Pang & Hartmann 2007) and these trends were observed after both vaginal and Caesarean birth (Gunther *et al.* 1965; Anderson *et al.* 1981; Kulski *et al.* 1981; Hibberd *et al.* 1982; Lemons *et al.* 1982; Neville *et al.* 1991; Mastroeni *et al.* 2006). Further, all study values were within the normal limits reported by others (Gunther *et al.* 1965; Anderson *et al.* 1981; Kulski *et al.* 1981; Hibberd *et al.* 1982; Lemons *et al.* 1982; Neville *et al.* 1991; Mastroeni *et al.* 2006).

Typically, biochemical changes of secretory activation are expected to occur by day 2–3 post-partum (Kulski & Hartmann 1981; Arthur *et al.* 1989; Neville *et al.* 2006; Pang & Hartmann 2007). Protein levels are high, and lactose levels are low in the immediate post-natal period as the primary functions of colostrum are immunologic and trophic rather than nutritive (Ballard

& Morrow 2013). Closure of the tight junctions at secretory activation are associated with reductions in the Na^+/K^+ ratio, decreased protein and increased lactose concentrations. In the current study, protein and lactose concentrations indicated that secretory activation had occurred in both groups by 72 h post-partum (Table 2; Fig. 1). While all levels were within a normal range, there was a significant difference in the rate of change in lactose concentration, being more rapid for group CBP (Fig. 1). This might either be due to complete removal of the placenta at birth resulting in a more rapid decline in progesterone triggering secretory activation immediately after birth or due to the timing of sampling during this period.

Fat is an important nutritional component of breast milk contributing to over 50% of the energy of milk, but it is not considered to be a marker for secretory activation. However, as with the other biochemical components examined in this study, fat was within the reported range for normal initiation of lactation (Mitoulas *et al.* 2002; Kent *et al.* 2006; Khan *et al.* 2013a, 2013b). Further, we found no differences by birth mode, and rate of change in fat content was consistent in both groups despite fat content being the most variable component in breast milk (Mitoulas *et al.* 2002; Kent *et al.* 2006; Khan *et al.* 2013a, 2013b).

The >72–165 h post-partum period is important in the establishment of lactation and has been linked to subsequent milk production (Hill *et al.* 2005). Changes in lactose levels were not significantly different between the two groups during this period; however, protein concentrations were significantly different, probably because of a single sample from group CBP.

The rate of decrease in Na^+ levels in group CBP was significantly faster than in group V (Fig. 1). This difference in ion concentration could potentially be attributed to the use of pethidine-PCEA. An *in vitro* experiment found that pethidine had the ability to affect the action potential on Na^+ and K^+ ion channels in cells (Wagner *et al.* 1999; Brau *et al.* 2000). Therefore, it is possible that the ion channels in the lactocytes and surrounding blood vessels in the breasts of mothers that delivered by CB in this study may have been affected when pethidine was used as epidural analgesia after labour. The group CBP used pethidine-PCEA for 38.7 h on average (Table 1), and considering the drug half-life is 3–5 h in adults, the effect of pethidine use may not be apparent early in the establishment of lactation

while the tight junctions are still open, allowing free exchange of required Na^+ and K^+ . This explains why we only found a difference in ion levels in group CBP after tight junction closure, during the >72–165 h post-partum period. Furthermore, the mean Na^+/K^+ ratio of the participants during this time period showed that group CBP were producing transitional milk (0.88 ± 0.34) compared with group V (0.59 ± 0.20), whose mothers were producing established milk (Neville *et al.* 1991; Filteau *et al.* 1999a, 1999b; Lemay *et al.* 2013). Neville *et al.* found a nonlinear relationship between protein, Na^+ concentrations and milk volume, with rapid changes initially slowing as milk production increases. This nonlinearity supports the theory that decreases in protein and Na^+ are not solely due to the dilutional effect of increased milk production (Neville *et al.* 1991). The differences in protein and Na^+ concentrations between the groups during this period could be explained by higher values and slower rates of change initially in group CBP, but these differences are not present by day 7 post-partum. By >165–265 h post-partum, both the concentrations of the biochemical components in the milk and lack of difference in the rates of change of the components between the groups suggest that the majority of the participants had progressed past secretory activation.

Previous studies have shown that first breastfeed is delayed among mothers that delivered by CB, and this has a negative effect on the establishment of feeding (Vestermarck *et al.* 1991; Evans *et al.* 2003; Prior *et al.* 2012; Zanardo *et al.* 2012). A delay in sucking stimulus and removal of milk from the breast is thought to exacerbate lower levels of oxytocin and prolactin associated with CB, negatively impacting on both milk ejection and production (Nissen *et al.* 1996). This study also found a significantly longer time to the first breastfeed in group CBP, yet this group did not experience a substantial delay in initiation of lactation, possibly because of good breastfeeding care in the early post-partum period.

Infants play an important role in the establishment of the milk supply and must be able to remove milk effectively to facilitate normal growth and development. Sakalidis *et al.* found that infants delivered via CB had faster suck rates and more tongue movement during sucking but found no difference in milk intake compared with mothers delivering vaginally (Sakalidis *et al.* 2013). The neurobehavioral scores of

infants exposed to pethidine are lower than those exposed to morphine (Wittels *et al.* 1990; Wittels *et al.* 1997), however infants exposed to pethidine in this study did not exhibit signs of abnormal or suboptimal breastfeeding behaviour (Al-Tamimi *et al.* 2011; Sakalidis *et al.* 2013). This may be because patients have lower opioid requirements with epidural administration compared with systemic, such that maternal plasma concentrations of pethidine and norpethidine are less and infant exposure via milk restricted (Paech *et al.* 1994).

The limitations of this study are that this analysis relates only to the composite effect of Caesarean birth with pethidine-PCEA and that the exposure of opioid during both modes of delivery and other types of PCEA were not evaluated. However, the impact of CB with pethidine-PCEA on lactation appears to be short term. Further investigation with a greater number of participants may be useful to confirm these results. Data on maternal milk production would enable discussion of the significance of the results in a clinical context. An understanding of biochemical and physiological consequences of analgesia use post-partum coupled with informed breastfeeding support programmes promoting best practice for lactating mothers would result in improved lactation outcomes for mothers and infants.

Conclusion

In this study, the protein and lactose concentrations and Na^+/K^+ ratio showed that both groups of participants had achieved secretory activation by 72 h post-partum, after which secretory activation is considered to be delayed. There were greater initial variations in measured biochemical components observed within the group having CB with pethidine-PCEA. However, by 165 h post-partum, there were no significant concentration differences between the groups in the biochemical components of milk. The effect of pethidine-PCEA appeared to be diminished after 72 h post-partum.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Contributions

Conception and design of study were done by YA, MJP, PEH. WJT, CTL and ARH analyzed and interpreted the data. Initial drafting of the manuscript was done by WJT. WJT and HG revised manuscript with important intellectual content. All co-authors participated in manuscript preparation and critically reviewed all sections of the text for important intellectual content. Final approval of the version to be published was given by MJP, PEH and DTG.

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