

Symposium: Human Lactogenesis II: Mechanisms, Determinants and Consequences

Physiology and Endocrine Changes Underlying Human Lactogenesis II^{1,2}

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ABSTRACT Lactogenesis stage II, the onset of copious milk secretion, takes place during the first 4 d postpartum in women and involves a carefully programmed set of changes in milk composition and volume. The evidence is summarized that progesterone withdrawal at parturition provides the trigger for lactogenesis in the presence of high plasma concentrations of prolactin and adequate plasma concentrations of cortisol. Although the process is generally robust, delayed lactogenesis does occur with stressful deliveries and in poorly controlled diabetes. Failure of early removal of colostrum from the breast is associated with high milk sodium and poor prognosis for successful lactation in many women. We speculate that this problem may result from accumulation of a substance in the mammary alveolus that inhibits lactogenesis, even in the face of appropriate hormonal changes after parturition. *J. Nutr.* 131: 3005S–3008S, 2001.

KEY WORDS: • *lactogenesis* • *mammary development* • *milk composition*

The breast is one of the few organs that undergoes most of its development postnatally. At birth the breast is represented by a nipple, a few small ductal elements and an underlying fat pad. Only with the onset of puberty and the secretion of estrogen does the gland initiate a complex developmental process. Ducts grow out into the fat pad guided by an interesting structure, the terminal end bud (1), which guides the elongation, branching and spacing of the ducts. In women, with the onset of menses, and in those rodents in which there is a significant luteal phase, alveolar structures begin to sprout from the sides of the duct stimulated by progesterone and probably prolactin. The mature breast resembles a flowering tree in springtime with lobular alveolar complexes, called terminal duct lobular units (TDLU)⁴ by pathologists, sprouting regularly from the major ducts. The breast reaches a stage of quiescence marked by some waxing and waning of the TDLU driven by the hormonal changes of the menstrual cycle (2–5).

The next stage of development begins in pregnancy. As the levels of progesterone, prolactin and placental lactogen rise, the TDLU undergo a remarkable expansion so that each lobule comes to resemble a large bunch of grapes. During mid-pregnancy, secretory differentiation begins with a rise in

mRNA for many milk proteins and enzymes important to milk formation. Fat droplets begin to increase in size in the mammary cells, becoming a major cell component at the end of pregnancy. This switch to secretory differentiation is called stage I lactogenesis (6,7). The gland remains quiescent but poised to initiate copious milk secretion around parturition. This period of quiescence depends on the presence of high levels of circulating progesterone; when this hormone falls around the time of birth, stage II lactogenesis or the onset of copious milk secretion ensues. As long as prolactin secretion is maintained and milk is removed from the gland, the mature function of the breast, milk secretion, is maintained. After weaning, the TDLU involute with the apoptosis of a large proportion of the alveolar cells and a remodeling of the gland so that it returns to the mature quiescent state (8).

This brief outline of a complex series of hormonally regulated developmental events shows that mammary development is governed by a series of switches. Most of these developmental switches are instigated by external hormonal influences, but lactogenesis stage I may be simply the consequence of the inherent program of alveolar development and involution is most often initiated by a failure of milk removal. In this short article we discuss the developmental switch represented by lactogenesis stage II. The changes in milk composition and volume attendant on this switch in women are outlined first, followed by a discussion of the role of hormones in its initiation. Finally, the scanty findings concerning the role of milk removal and a hypothesis for a role of an inhibitor of lactogenesis are presented as a guide to future research.

Stage II lactogenesis in women

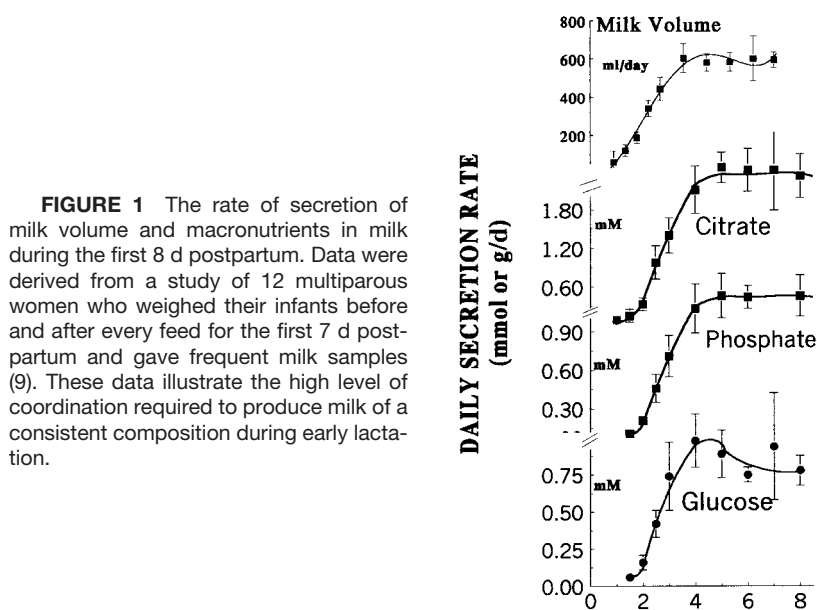
The top left panel in **Figure 1** shows the time course of the increase in milk volume that occurs during the 1st wk post-

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⁴ Abbreviation used: TDLU, terminal duct lobular units.

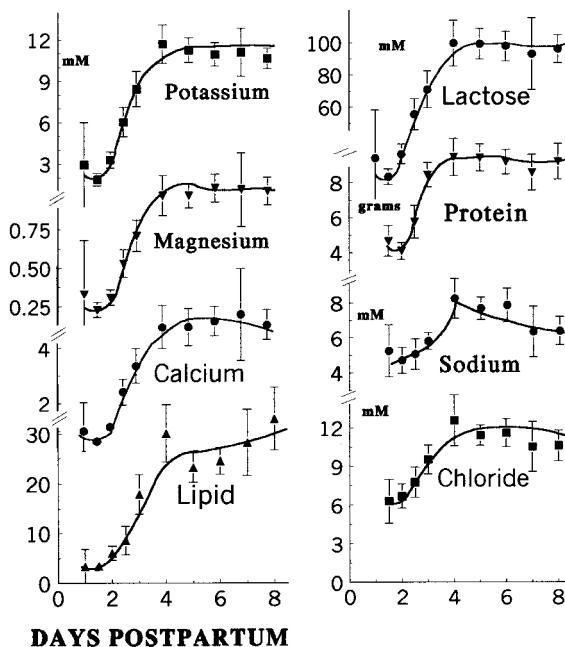


partum (9–11). Milk transfer to the suckling infants starts at a volume of <100 mL/d on d 1 postpartum, begins to increase ~36 h after birth and levels off at an average of 500 mL at ~4 d. Milk composition also changes dramatically during this period, with a fall in the sodium and chloride concentrations and an increase in the lactose concentration that start immediately after birth and are largely complete by 72 h postpartum (12). These changes precede the onset of the large increase in milk volume by at least 24 h and are explained by closure of the tight junctions that block the paracellular pathway (7). Next, the concentrations of secretory immunoglobulin A and lactoferrin increase dramatically and remain high to ~48 h after birth (13). Their concentrations fall rapidly after d 2, in part because of dilution as milk volume secretion increases, but their secretion rate is still substantial (2–3 g/d for each protein throughout lactation). Oligosaccharide concentrations are also high in early lactation, comprising as much as 20 g/kg of milk on d 4 (14,15), falling significantly to a level of ~14 g/L on d 30. These complex sugars are also considered to have substantial protective effect against a variety of infections (16). Thus, during the first 2 d postpartum, large molecules with significant protective power dominate in the mammary secretion; the total nutrient value is low, simply because the amount of milk transferred to the infant is small.

The substantial volume increase occurring between 36 and 96 h postpartum is perceived as the coming in of the milk and reflects a massive increase in the rates of synthesis and/or secretion of almost all the components of mature milk (12), including but not limited to lactose, protein (primarily casein) (17,18), lipid, calcium, sodium, magnesium and potassium (Fig. 1). Considering the secretion patterns for each of the milk components shown in Figure 1, the coordination achieved by the mammary epithelial cell among the activity of the various pathways that contribute all these different milk components is a marvel. The question we need to ask next is: “How is this remarkable degree of coordination achieved?”

Regulation of stage II lactogenesis

It has long been known that abrupt changes in the plasma concentrations of the hormones of pregnancy set lactogenesis



in motion, although the precise hormones that accomplish the process have, in the past, been a subject of some debate. It is clear that a developed mammary epithelium, the continuing presence of levels of prolactin near 200 ng/mL and a fall in progesterone are necessary for the onset of copious milk secretion after parturition (19). That the fall in progesterone is the lactogenic trigger is supported by evidence from many species. For example, exogenous progesterone prevents lactose and lipid synthesis in the mammary gland after removal of the source of progesterone, namely, the ovary, in pregnant rats (20,21), mice (22) and ewes (23). In humans removal of the placenta, the source of progesterone during pregnancy in this species, has long been known to be necessary for the initiation of milk secretion (24,25). Furthermore, retained placental fragments with the potential to secrete progesterone have been reported to delay lactogenesis in humans (25). Thus, without a fall in progesterone, lactogenesis does not occur.

However, other hormones must be present for this trigger to be effective. Either prolactin or placental lactogen are necessary for mammary development in pregnancy, and, with the fall in placental lactogen after removal of the placenta, prolactin is necessary for sustained lactation in most species, although cows may be an exception. Bromocriptine and other analogs of dopamine (drugs that effectively prevent prolactin secretion) inhibit lactogenesis when given in appropriate doses (26,27). Furthermore, prolactin was not necessary for lactogenesis in mice that had not yet given birth, the placental lactogen from the placenta providing the necessary stimulation of prolactin receptors (22). These data support the concept that a surge in prolactin is not the trigger for lactogenesis. It has long been known that glucocorticoids are necessary for milk secretion and lactogenesis, a postulate recently confirmed in our laboratory in mice (22). However, a surge of glucocorticoids is not necessary and a high dose of glucocorticoid does not promote lactogenesis. Insulin is generally required for induction and maintenance of milk protein gene expression in cultured mammary cells and glands, and deficiencies in plasma insulin led to decreased milk production in rats and goats. However, short-term deficiencies in insulin did not interfere with lactogenesis in rats (28). Thus, the available literature

rules out acute changes in the concentrations of prolactin, glucocorticoids or insulin as triggering lactogenesis, although glucocorticoids and prolactin are necessary at some level for a fall in progesterone to act as the lactogenic trigger.

In summary, interpretation of the data available from both animal and human studies is that the physiological trigger for lactogenesis is a fall in progesterone; however, maintained prolactin and cortisol are necessary for the trigger to be effective. The caveat is, of course, that the mammary epithelium must be sufficiently prepared by the hormones of pregnancy to respond with milk synthesis. Postpartum prolactin levels are similar in both breastfeeding and nonbreastfeeding women, so that the basic process occurs regardless of whether breastfeeding is initiated (27). Similarly, glucocorticoids are necessary at some level, but their role is currently far from defined and, indeed, has received little study in the past two decades. Likewise, the role of insulin *in vivo* is not well-defined, although it is likely to be important in maintaining a metabolic state that allows flux of nutrients to the mammary gland.

Does milk removal play a role in the timing or extent of lactogenesis?

A delay in the onset of lactogenesis has been reported with poorly controlled diabetes (9,11,29) and stress during parturition (18). The mechanism is unknown but the delay did correlate with high cord blood glucose and cortisol. The delay occurred in women who put the infant to the breast during the first 2 d postpartum, suggesting that poor milk removal is not the cause of stress-related delayed lactogenesis. In another study high breast milk sodium concentrations on or before d 3 were observed in clinical situations in which the infant failed to latch on properly (Fig. 2) (30,31). These high sodium concentrations were statistically related to impending lactation failure and could be reversed by the use of a breast pump to obtain effective milk removal. These observations suggest that milk removal and/or effective suckling are necessary to obtain junctional closure and potentially an increase in milk volume secretion, at least in some women. Evidence on this point is mixed. Careful evaluation of milk composition and

breast-filling in nonbreastfeeding women by Kulski et al. (27) suggested that milk removal is not needed for the programmed physiological changes that bring about lactogenesis stage II. In contrast, Chapman and Perez-Escamilla (32) found that formula feeding before lactogenesis was associated with a delay in the perception of lactogenesis. Furthermore, the time of first feeding and the breastfeeding frequency on d 2 postpartum were positively correlated with milk volume on d 5 postpartum (18), suggesting that milk removal at early times after birth increases the efficiency of milk secretion. In the last decade local factors dependent on milk removal have been implicated in the regulation of milk secretion during lactation (33). The question is: "What are these local factors and when do their effects become apparent?" It is possible to speculate that they are present in high concentration in the scant prepartum secretion product of the breast and that if not removed early in the prepartum period, they contribute to inhibition of lactogenesis stage II, even with adequate hormonal changes. Although Kulski and coworkers were unable to detect any delay in the change in milk composition in their study of nonbreastfeeding women, it is possible that even the small changes in the amount of milk remaining in the breast engendered by removal of 5 or 10 mL of secretion product per day by manual expression could stimulate lactogenesis stage II.

We can conceptualize the problem of failed lactogenesis as preglandular, glandular or postglandular (31). An example of preglandular would be hormonal causes, such as retained placenta or lack of pituitary prolactin. Glandular causes might be surgical procedures, such as reduction mamoplasty or, possibly, insufficient mammary tissue. Postglandular would be any cause for ineffective or infrequent milk removal. This latter aspect has received insufficient attention. A neonatal intensive care nursery where careful correlation between early milk removal and subsequent lactation performance is possible would be ideal for such a study. In these studies milk volume would be most easily measured in women who are using a breast pump to provide milk for their infants. Very small samples could be taken for determination of milk sodium and casein on a daily basis to allow a true distinction to be made between failure of tight junction closure and reopening of the junctions due to lack of milk removal. Such data will be valuable in determining how to manage the initiation of breastfeeding in mothers of sick infants as well as in sick mothers of well infants. It would also be of great value to determine, using techniques developed at the Hannah Research Institute in Ayr, Scotland in collaboration with Prentice et al. (34), whether a chemical present in colostrum inhibits milk secretion in an *in vitro* model system. Identification of such a chemical and precise measurements of its concentration could provide a new index for predicting which women are likely to have problems initiating stage II lactogenesis.

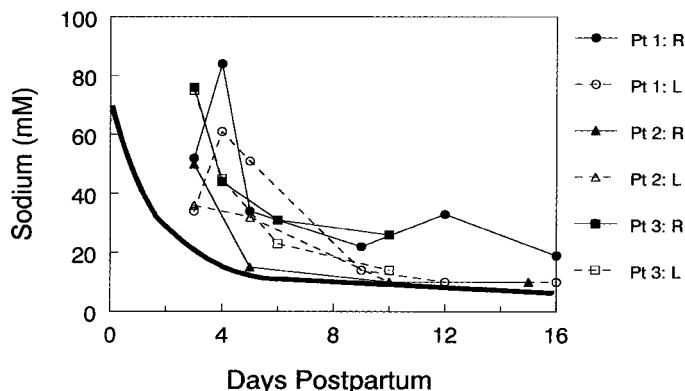


FIGURE 2 Milk sodium in poorly nursing infants. Three mothers of full-term infants visited the clinic on d 3 of lactation complaining of poor milk production and problems with infant latch on. Milk samples were taken for measurement of sodium and the mothers were instructed to use a breast pump several times daily and to take small samples of milk daily, which were later brought to the clinic for analysis of sodium. Sodium concentrations in right and left samples are plotted. Note that once pumping was initiated the sodium concentrations declined with a time course similar to that in reference women, shown as a heavy black line.

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