Effects of fasting, elevated plasma glucose and plasma insulin concentrations on milk secretion in women

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ABSTRACT
In order to determine whether short term variations in plasma glucose and/or insulin influence milk lactose secretion in women, the effects of fasting and increased blood insulin and glucose on milk volume and composition were studied with glucose clamp methodology in exclusively and partially breast-feeding women. Twenty hours of fasting had no discernable effect on the output of milk or its macronutrient composition. Four hours of increased plasma insulin, studied under conditions where plasma glucose was maintained at the fasting level, had no effect on milk volume, milk glucose concentration, total fat content or lactose secretion rate. Increased plasma glucose, maintained at twice fasting levels for 4 to 6 h, produced a threefold increase in milk glucose concentration but had no significant effect on the rate of lactose synthesis. In partially breast-feeding women producing no more than 200 ml milk per day, a similar degree of hyperglycaemia increased milk glucose more than fourfold but did not significantly increase the milk secretion rate. It is concluded that human milk production is isolated from the homeostatic mechanisms that regulate glucose metabolism in the rest of the body, in part because the lactose synthetase system has a \( K_m \) for glucose lower than the concentration available in the Golgi compartment. Journal of Endocrinology (1993) 139, 165–173

INTRODUCTION
Human milk is secreted at the rate of 500–1000 ml/day in exclusively breast-feeding women (Neville et al. 1988). Lactose and lipid together contribute 520 kcal/day or about 95% of the calorie content of milk and require 75 g glucose for their synthesis (Prentice & Prentice, 1988). In rats and goats it has been shown that systemic glucose and lipid homeostatic mechanisms are regulated during lactation to ensure a flux of substrate to the mammary gland (Baumann & Elliot, 1983; Burnol et al. 1983; Vernon et al. 1990). Furthermore, in these same species (Linzell, 1967b; Carrick & Kuhn, 1978; Wilde & Kuhn, 1979; Bussmann et al. 1984), 24-h food deprivation profoundly depresses the rate of milk production. These observations provide good evidence for the existence of reciprocal interactions between whole body energy metabolism and lactation, at least in some species.

Research in women on the relationship between maternal nutrition and lactational performance has been limited largely to studies designed to elucidate the effects of undernutrition on milk composition (for reviews, see Lönnerrdal, 1986; Prentice & Whitehead, 1987) and the effects of diabetes on milk composition (Butte et al. 1987; Ratzmann et al. 1988; Jovanovic-Peterson et al. 1991). Although providing valuable information, such studies do not allow definition of the nature, or even the existence, of regulatory interactions between metabolism and lactation. For this reason we initiated experimental studies in women designed to examine whether the regulation of systemic glucose metabolism influences the synthesis and secretion of human milk. We used glucose clamp methodology and hourly milk extraction to approach this question.

In the glucose clamp method (DeFronzo et al. 1979), plasma glucose is maintained (clamped) at a predetermined level by intravenous infusion of 20% dextrose at a variable rate adjusted on the basis of frequent analyses of plasma glucose; insulin or other hormones may be manipulated as desired. The power of the technique is that it allows uncoupling of the plasma levels of glucose and insulin. The method has been used in pregnant and lactating rats (Leturque et al. 1984; Girard et al. 1987) and goats (Tesseraud et al. 1992) and in pregnant women (Ryan et al. 1985) to demonstrate effects of reproductive state on glucose
homeostasis. It has not been used in lactating women. In the experiments described in this paper we used a euglycaemic, hyperinsulinaemic clamp to examine the effects of elevated plasma insulin concentration and a hyperglycaemic clamp to examine the effects of increased plasma glucose concentration on milk volume and lactose content. These procedures were initiated after about 16 h of fasting and had a duration of 4 h. Control studies were performed in fasting subjects in order to determine the effect of food deprivation alone.

In the present experiments milk secretion was measured using a technique adapted from studies in goats (Linzell, 1967a) in which newly synthesized milk was extracted hourly from the breasts using an electric breast pump. Nasal oxytocin given after 10 min of pumping during each extraction period assured maximal emptying of the breast. This technique is evaluated in the first part of the results.

MATERIALS AND METHODS

Subjects

Subjects were 23 lactating Caucasian women between the ages of 20 and 34 years who were fully breast-feeding a 1- to 4-month-old infant. Some were studied more than once, either in different experiments or at different times during lactation. Four additional subjects were studied during weaning when they were producing 200 to 300 g milk per day. No subjects had a history of diabetes, either gestational or otherwise, and all had fasting plasma glucose and serum insulin concentrations of less than 5.3 mmol/l and 11 μU/ml respectively. All subjects gave written informed consent for these studies which were approved by the Human Subjects Committee of the University of Colorado Health Sciences Center.

Experimental protocol

Subjects fasted after a moderate meal completed before 21.00 h on the evening prior to the experiment, but were encouraged to take caffeine-free, non-calorie containing fluids which were available ad libitum. They were admitted to the Clinical Research Center at the University Hospital at 07.00 h, at which time both breasts were pumped and two intravenous catheters were placed. One catheter, placed retrograde in a dorsal hand vein, was used to obtain blood samples; this catheter was kept open with a slow infusion of normal saline (0-9% w/v in water). A heating pad around the arm ensured arterialization of the venous blood from this arm. The second catheter, in a contralateral antecubital vein, was used for infusion of glucose (as 20% w/v dextrose in water), insulin where indicated and KCl, infused with insulin to prevent hypokalaemia. All experiments commenced at approximately 08.00 h.

In most experiments 2 h of control measurements of plasma glucose and serum insulin concentrations were followed by infusions of glucose and insulin for an additional 4 h. Blood samples (3 ml) were taken every 5 min during the glucose infusion for the measurement of plasma glucose. The protocol was modified to study fasting women: only one catheter was placed for blood sampling and no glucose or insulin infusions were given. In five of these subjects the experiment was continued until 16.00 h giving at least 21 h of fasting.

Infusions

For the hyperglycaemic clamp, after the control period the amount of glucose (20% dextrose in water) necessary to raise the plasma glucose to 8 mmol/l (assuming a glucose distribution space of 200 ml/kg body weight) was rapidly infused. Then glucose was infused at the rate necessary to maintain this concentration level using a Harvard infusion pump. The rate of infusion was adjusted every 5 min in response to the measured arterialized plasma glucose concentration. When insulin was used, a loading dose (calculated assuming an insulin distribution space of 150 ml/kg body weight) was administered in a logarithmically decreasing manner over the first 10 min of the infusion, according to the method of DeFronzo et al. (1979), followed immediately by a constant insulin infusion of either 0.3 or 1.0 μU/min per kg body weight. The insulin used was purified porcine insulin (Novo Nordisk Pharmaceuticals, Princeton, NJ, U.S.A.). Sage (model 355) infusion pumps were carefully calibrated and used for all infusions except glucose. The mean coefficient of variation of the glucose concentration was 7-9% during the euglycaemic clamps and 6-1% in the hyperglycaemic clamps.

Milk extraction and sampling

Once every hour the breasts were pumped for 10 min using a Medela (Crystal Lake, IL, U.S.A.) breast pump fitted with a Y-tubing so that both breasts could be pumped simultaneously. One drop of synthetic oxytocin (Syntocinon; Sandoz Pharmaceutical Company, Switzerland) was then given intranasally and the pumping continued for another 5 min. In the fasting experiments as well as the second series for both the euglycaemic and hyperglycaemic clamps, the breasts were emptied by pumping and syntocinon prior to the beginning of the experiments to decrease the contribution of residual milk to early time points. Longer pumping was sometimes necessary at the first
and second intervals before milk stopped flowing from the breasts. Pre- and post-oxytocin samples were combined and divided into aliquots for storage at −70 °C. Three capillary tubes were filled and spun within 4 h for determination of milk lipid content by the creamatocrit method (Lucas et al. 1978).

**Blood and milk analysis**

Blood samples were centrifuged for 30 s to obtain plasma for glucose analysis immediately after the blood sample was drawn. A Beckman (Fullerton, CA, U.S.A.) glucose analyser (stated accuracy ± 0.05 mmol/l) was used to measure the plasma glucose. Blood samples for insulin were allowed to clot and were centrifuged. Serum was frozen immediately for later analysis. Insulin was determined by radioimmunoassay using a kit obtained from Pharmacia (Piscataway, NJ, U.S.A.).

The volume of milk extracted at each pumping was determined by weighing the pump container before and after milk extraction. Milk glucose and lactose concentrations were determined in aliquots of defatted milk samples using the Yellow Springs Instruments glucose analyser Model 23A (Yellow Springs Instrument Company, Yellow Springs, OH, U.S.A.) with enzyme impregnated membranes appropriate for either glucose or lactose. Milk sodium and potassium concentrations were determined by ion selective electrodes using a Beckman (Fullerton, CA, U.S.A.) E2A analyser and urine standards (Neville et al. 1984). Milk chloride concentration was analysed colorimetrically as described previously (Neville et al. 1984). Milk protein content was measured by the bicinchoninic acid method, using reagents from Pierce Chemicals (Rockford, IL, U.S.A.; Keller & Neville, 1986).

**Test-weighing**

To determine the amount of milk produced under normal day-to-day conditions, subjects were asked to test-weigh their infants before and after every feed for a 24-h interval as described previously (Neville et al. 1988). Women who were weaning their infants fed fewer times per day; in order to obtain a representative milk output, they test-weighed at all feeds for three successive days. Test-weighing was carried out in the week prior to the clamp procedure. Extensive evidence that the test-weighing procedure gives representative milk volumes has been described earlier (Neville et al. 1988).

**Data analysis**

Data from samples from right and left breasts were averaged prior to further analysis. All data are presented as means ± s.e.m. Student's t-test was used to determine the significance of observed differences with P values.

**RESULTS**

**Hourly pumping**

Because most subjects did not have time for a full breast-feed before entering the hospital at 07:00 h the first two pump episodes gave large volumes, reflecting residual milk. The mean milk output 5 and 6 h after the start of the experiment for all 28 exclusively breast-feeding subjects was 34.9 ± 1.5 (s.e.m.) g/h compared with a mean value of 31.5 ± 0.8 g/h from 24-h test-weighing carried out as described in detail previously (Neville et al. 1988). When data from the first six subjects whose breasts were incompletely emptied prior to beginning the hourly pumping protocol were excluded from analysis, the corresponding values were 32.3 ± 1.25 g/h and 31.3 ± 1.16 g/h (not significantly different). In all subjects the milk fat content increased significantly (P<0.001) at the second pumping from an average of 5.6 ± 0.6% to 8.5 ± 0.5%, reflecting the well-known observation that the fat content of hindmilk is higher than that of foremilk. Thereafter the fat content fell, gradually levelling out at the last two pumpings and reaching a value of 4.6 ± 0.4% at 7 h. This value is close to the mean fat content of about 4.5% measured in a number of studies of well-nourished women (Allen et al. 1991). The sodium, chloride and protein concentrations of the milk fell slightly but significantly (P<0.05 during the course of the experiment and the potassium concentration rose. The fact that the milk sodium and chloride concentrations did not rise indicates that the dose of oxytocin used did not open the junctional complexes between mammary alveolar cells (Linzell, 1967a). Milk chloride and potassium levels were within the normal ranges previously observed in this laboratory (Allen et al. 1991). However, after the initial sample, the sodium concentration averaged 3.6 mmol/l, slightly below previously observed values of 4.0 to 9.0 mmol/l. There were no differences in any of these variables between women who received infusions of glucose, insulin and glucose, or no infusions. These data indicate that hourly pumping provides milk of normal composition at a rate similar to the daily milk output of the breast-feeding woman.

**Effect of fasting**

Fasting subjects refrained from eating from 20.00 h on the night before the experiment until the
Experiment terminated between 14.00 and 16.00 h on the following day. The plasma glucose concentration showed a tendency to fall over the course of the experiment from 4.4 ± 0.3 mmol/l at about 08.00 h to 3.9 ± 0.3 mmol/l at 16.00 h (Fig. 1, dotted lines). This small change was significant by paired analysis (P < 0.05). The serum insulin fell from a mean of 6 µU/ml to 3 µU/ml over the same interval. Milk glucose and lactose concentrations, on the other hand, remained constant at about 1.4 ± 0.1 mmol/l and 207 ± 3 mmol/l respectively. The mean milk output was 32.8 ± 2.0 (s.e.) g/h from 3 to 8 h and the corresponding lactose secretion rate remained constant at 6.8 ± 0.7 (s.e.) mmol/h. Milk glucose, protein, lactose and citrate were within the ranges reported previously (Allen et al. 1991). These data showed that 20 h of fasting had no discernable effect on the rate of milk secretion or the concentration of macronutrients in the milk.

Effect of insulin on milk volume and composition

Euglycaemic, hyperinsulinaemic clamps were used to evaluate the effect of increased plasma insulin on milk volume and milk glucose and lactose concentrations. Two separate series of experiments were carried out using five lactating subjects in the first series and three separate subjects, repeated at one and four months, in the second for a total of 11 experiments. Milk lipid, protein and ion concentrations were not different from concentrations in fasting subjects. The data on plasma and milk glucose and serum insulin are plotted in Fig. 1a and c. In the first series, insulin was infused at a rate of 1 mU/min per kg body weight from 2 to 6 h; in the second series insulin was infused at 0.3 mU/min per kg from hours 2 to 4 and 1.0 mU/min per kg from hours 4 to 6, resulting in a stepwise increase in serum insulin. Because plasma glucose was maintained by infusion, it did not fall as it did in the fasting state (Fig. 1a). The mean milk glucose concentration during the last 2 h of the clamp, 1.47 ± 0.15 mmol/l, was not significantly different from the mean level in the fasting subjects. In spite of the high serum insulin concentration, the milk secretion rate (32.5 ± 3.8 (s.e.) g/h), milk lactose concentration (202 ± 3 mmol/l) and milk lactose secretion rate (6.6 ± 0.8 mmol/h) were not significantly different from the values observed in the fasting subjects. These observations show that serum insulin concentrations of between 2 and 70 µU/ml have no effect on either glucose or lactose secretion into human milk.
Effect of hyperglycaemia on milk volume and composition

In another two series of experiments with six subjects each, glucose was infused at a rate designed to maintain the plasma glucose at twice the fasting concentration for 4 to 6 h (Figs 1b and 1d). The increase in plasma glucose concentration was accompanied by an increase in endogenous insulin secretion that brought the steady state level of serum insulin to approximately 25 μU/ml in both series (Fig. 1d). Milk glucose rose gradually to 3·9 mmol/l, nearly three times the fasting level (Fig 1b) reaching a steady state 3–4 h after the infusion commenced. However, the milk secretion rate (38·4 ± 4·7 g/h in the first series and 32·7 ± 2·1 g/h in the second series), milk lactose concentration (208 ± 3 and 217 ± 3, respectively), and milk lactose secretion rate (8·0 ± 1·0 and 7·1 ± 0·5 mmol/h, respectively) were not significantly different from the fasting controls.

Effect of hyperglycaemia in women weaning their infants

When mean daily milk secretion falls below 400 ml/day, the concentration of glucose in the milk falls proportionately with the milk volume (Allen et al. 1991), suggesting that a fall in glucose availability for lactose synthesis may contribute to the decrease in milk output. To test this hypothesis we performed a hyperglycaemic clamp in three women who, on the basis of test-weighing, were producing between 100 and 210 g milk per day. The mean fasting glucose concentration in the milk of these women was 0·37 ± 0·05 mmol/l compared with 1·4 ± 0·1 mmol/l in subjects in full lactation. During the hyperglycaemic clamp plasma glucose was increased to a steady state level of about 8 mmol/l, resulting in an average steady state serum insulin concentration of about 25 μU/ml, not different from the level observed in the exclusively breast-feeding subjects. The milk glucose concentration increased 4·5-fold to 1·8 ± 0·4 mmol/l (Fig. 2a). While this concentration is lower than that observed in the fully lactating women during hyperglycaemia, it does represent a greater proportional increase. The milk flow rate (10·2 ± 1·4 g/h) was slightly higher during the clamp than obtained previously by test-weighing in these women (7·5 ± 1·2 g/h). It does not seem likely that this increase is the result of increased glucose entry into the mammary cell, because the apparent rate of milk secretion fell continuously after the start of the glucose infusion (Fig. 2c). The lactose concentration increased insignificantly from 189 ± 3 mmol/l at the start of the experiment to 203 ± 9 mmol/l at the end. Together these observations suggest that a fourfold increase in milk glucose is associated with an insignificant increase in lactose secretion.

FIGURE 2. Effect of hyperglycaemia on milk secretion in women who were weaning their infants and had a steady state milk volume of less than 200 ml/day. (a) Plasma and milk glucose levels compared with results of a similar experiment in fully lactating women (shaded area represents 1 s.e.m. above and below the mean values in fully lactating women). (b) Endogenous insulin response to glucose infusion (shaded area represents 1 s.e.m. above and below the mean values obtained in fully lactating women). (c) Milk volume output (shaded area represents 1 s.e.m. above and below the mean hourly milk volume of 7 ml estimated from test-weighing in the women weaning their infants). In all graphs points are means ± s.e.m. The experiment began at 08.00 h (zero time).
DISCUSSION

The most striking result of these studies is that none of the manipulations of the short-term metabolic status of lactating women tested, including fasting of up to 21 h, a tenfold increase in plasma insulin maintained for 4 h or a doubling of plasma glucose maintained up to 6 h, significantly altered any measured parameter of milk secretion except the glucose concentration in the milk. Several aspects of our study require additional discussion. In particular the implications from the results of the hourly pumping methodology for the regulation of milk volume are somewhat at odds with conclusions of Hartmann and his colleagues (Daly et al. 1992, 1993) from measurements of the rate of milk formation in the human breast using a computerized imaging method. In addition, the findings in women differ in some respects from findings in non-human animals, suggesting differences in strategies of metabolic response to food deprivation.

Hourly pumping and milk output

Peaker and his colleagues (Peaker & Wilde, 1987; Wilde et al. 1988) postulate the existence of a feedback inhibitor in milk that regulates milk synthesis. When this inhibitor is removed by more complete emptying of the mammary gland, milk secretion is increased. There is evidence for the presence of the inhibitor in human milk (Prentice et al. 1989). The finding that milk output obtained by hourly complete emptying of the breast for up to 8 h is not significantly different from milk output measured by 24 h test-weighing implies that the time frame within which autocrine regulation of milk output operates is days rather than hours. This conclusion is in accord with earlier data from this laboratory in women (Neville & Oliva-Rasbach, 1987). When a breast pump was used three times daily after breast feeds to remove residual milk a 25% increase in milk output was observed, but only after 7 days. It is not in accord with the conclusion of Hartmann and his colleagues (Daly et al. 1993), based on a computerized morphometric method, that milk production is increased on a feed-by-feed basis by more complete emptying of the breast. There are at least two possible causes for this contradiction. It is possible that, in contrast to fed women, fasting women do not respond to complete emptying of the breast with increased milk output. This seems unlikely given the lack of effect of fasting on milk output described previously (see below). Alternatively the computer imaging technique may be biased in some breasts to give erroneously large volume changes when the breast contains little milk. It is worth noting that a significant relation between the degree of emptying and the rate of milk synthesis was observed in only 6 of 13 breasts in the morphometric study.

Effect of fasting on milk secretion

In goats and rats food deprivation is associated with a rapid decline in many parameters of milk production including total volume (Faulkner & Peaker, 1987), lactose synthesis (Wilde & Kuhn, 1979; Bussmann et al. 1984), lipid synthesis (Robinson & Williamson, 1977; Jones et al. 1984) and glucose oxidation (Robinson & Williamson, 1977). Threadgold et al. (1982) demonstrated a depression of 2-deoxyglucose uptake by rat mammary glands after an overnight fast. However, goats and rats commit over 50% of their nutrient intake to milk production. Sufficient milk production to provide about 750 g of milk per day for a single human infant requires no more than a 25 to 30% increment over the nutrient intake of the non-lactating woman (Prentice & Whitehead, 1987). Furthermore, even under circumstances of reduced nutrition, women build up large energy reserves during pregnancy in the form of fat that can be drawn upon for milk synthesis. Thus it may not come as a surprise that up to 20 h of fasting had no effect on milk volume or composition. A similar observation was made by Prentice et al. (1983) who studied lactation during the day-long fast associated with Ramadan in a Moslem country. Hartmann et al. (1980) reported undiminished milk output in an exclusively breast-feeding woman during a total fast of 7 days. Clearly then, unlike rats and dairy animals, short term fasting in women has no significant impact on lactational performance. The effect of long term food deprivation may be different; an ethical study designed to evaluate this issue is difficult to conceive. However, chronic undernutrition has actually been observed to increase milk volume, possibly in compensation for diminished fat content (Butte et al. 1992).

The effect of insulin on milk secretion

An increase in plasma insulin to about ten times basal levels for 4 h had no effect on lactose synthesis, milk volume or milk glucose concentration. This finding is consistent with results of a glucose clamp study in goats in which insulin did not alter the arteriovenous difference in glucose concentration (Tesseraud et al. 1992). Because the lactating mammary gland appears to possess only one type of glucose transporter, the insulin-insensitive GLUT I (Burnol et al. 1990; Madon et al. 1990), the observation is not unexpected. An increase in the synthesis of medium chain fatty acids in response to insulin is expected from the


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effects of insulin on fatty acid synthesis in the mammary glands of rats (Munday & Hardie, 1987). Preliminary observations (Neville et al. 1987) suggest this to be the case in women as well.

The effect of increased plasma glucose on milk secretion

Increased plasma glucose resulted in an increase in milk glucose that attained a new steady state level after a delay of 3 h. A similar delay was reported when plasma glucose was raised to 17 mmol/l in diabetic women (Jovanovic-Peterson et al. 1991). Because this length of time was required for isotopic glucose to equilibrate with milk glucose (Neville et al. 1990), it probably reflects equilibration of residual milk in the alveoli and ducts of the human breast.

We have reviewed elsewhere evidence from goats and rats that the concentration of glucose in milk is equivalent to that in the mammary cell (Neville et al. 1990). The rate of isotopic enrichment of milk glucose and lactose is consistent with this concept in women as well. If the Golgi compartment, the site of lactose synthesis, is freely accessible to cell glucose, the rise in milk glucose observed with hyperglycaemia almost certainly reflects an increase in substrate availability to the lactose synthetase system. In-vitro studies (Kuhn & White, 1975; Kuhn, 1983) suggested that the glucose within the Golgi compartment equilibrates rapidly with cytoplasmic glucose. However the mechanism remains controversial. Leong et al. (1990) found the transport of sugar into isolated Golgi vesicles to be consistent with the presence of a pore that selects monosaccharides purely on the basis of size. Faulkner & Peaker (1987) studied hexose transport across the apical membrane of the goat mammary epithelium and concluded that the transport specificity was similar to that of the baso-lateral membrane and that transport was rapid. They postulated the source of these transporters to be secretory vesicle membranes. More recently Madon et al. (1990) presented evidence for the presence of the GLUT I transporter in both plasma membrane and Golgi vesicle fractions from lactating mammary glands. If glucose transport into the Golgi compartment is rapid as seems reasonable based on the above evidence, the $K_m$ for glucose incorporation into lactose must be less than 0·1 mmol/l. Thus increases in the cell glucose above 0·4 mmol/l appeared to have no effect on the rate of lactose synthesis. Although most studies using the isolated enzyme complex show a $K_m$ for lactose synthesis from glucose in the millimolar range, Kuhn and his coworkers recently showed that more physiological activators of the lactose synthetase reaction than the 10 mmol manganese/l usually used reduce the $K_m$ to 0·2 mmol/l or below (Navaratnam et al. 1988).

In fasted goats an increase in blood glucose concentration significantly stimulated milk secretion (Faulkner & Peaker, 1987). The independence of the pathways for human lactose synthesis from insulin and the apparently low $K_m$ for the lactose synthetase system insure that lactose synthesis in the human breast is protected from fluctuations in maternal glucose metabolism. This protection appears to constitute one of several adaptive mechanisms that isolate human milk production from changes in maternal metabolic state (Prentice & Whitehead, 1987).

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Glucose, insulin and milk secretion


