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Serum lactogens possessed normal bioactivity in patients with lactation insufficiency

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Summary

OBJECTIVE Insufficient breast milk is the most common reason for premature termination of breast-feeding. The causes of lactation insufficiency are usually multifactorial; in a small percentage of cases it is due to primary lactation failure of unknown origin. The aim of this study was to investigate whether lactation insufficiency of unknown origin could be caused by serum lactogens that had reduced biological activity.

DESIGN Women with lactation insufficiency of unknown origin and normal lactating controls were subjected to a standardized breast-feeding test for assessment of milk production. Thirty minutes later, serum samples were obtained for determination of total lactogen bioactivity, using an in-vitro bioassay, and levels of prolactin (PRL) and growth hormone (GH) using radioimmunoassay (RIA).

PATIENTS Twelve lactating mothers with a clinical diagnosis of lactation insufficiency of unknown origin were compared with 12 matched mothers with normal lactation.

MEASUREMENTS The Nb2 lymphoma cell bioassay was used to measure total lactogen bioactivity in sera. Conventional RIA kits were used to estimate serum PRL and GH concentrations.

RESULTS Mean milk yield on standardized test feed was 21-6 ml for patients and 146-5 ml for controls. In both patient and control groups the total serum lactogen bioactivity ranged from about 150 to 5000 mlU/l, while the serum RIA (PRL + GH) levels ranged from about 350 to over 7000 Miu/l. There was no evidence of lactogens with reduced bioactivity in the patients' sera.

CONCLUSION Lactation insufficiency in the women studied cannot be explained by serum lactogens that possess unusually low bioactivity.

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Breast-milk is the ideal food for young infants. Its unique biologically active ingredients cannot be obtained by artificial means. Despite a high initiation rate of breast-feeding, many mothers abandon breast-feeding prematurely. The most common reason given is insufficient breast-milk (Tanaka *et al.*, 1987). The insufficient milk syndrome has many causes. It involves one or a combination of at least four poorly understood problems: inadequate mammogenesis, failure of lactogenesis, insufficient galactopoiesis and impaired oxytocin secretion (De Coopman, 1993; Livingstone, 1994). Lactation insufficiency (LI) of

unknown origin is becoming recognized more frequently as breast-feeding rates increase. It can be a significant cause of infant failure to thrive (Davies, 1979; Neifert & Seacat, 1986; Neifert *et al.*, 1990).

Inadequate mammogenesis, i.e. lack of appropriate glandular development as indicated by lack of breast enlargement during pregnancy, may result in a slow rate of milk synthesis (Neifert *et al.*, 1985; 1990). The mammary tissue may not respond to stimulation by the high levels of oestrogens, progesterone and prolactin (PRL) normally present in the blood during pregnancy; alternatively, the levels of these hormones may not increase sufficiently to ensure stimulation of the mammary tissue although they are adequate to maintain pregnancy. Initiation of lactation, or lactogenesis, requires a significant decrease in the levels of oestrogens and progesterone made by the placenta, a normal occurrence as the placenta is expelled (Neifert *et al.*, 1981). In addition, lactation is initiated in the presence of relatively high levels of PRL resulting from early, effective and frequent suckling (Aono *et at.*, 1977). Galactopoiesis, the ongoing production of milk, appears to depend initially on suckling-related PRL surges but is subsequently maintained without high levels of PRL (Tyson *et at.*, 1978; Gross & Eastman, 1979; Weichert, 1980; Hartmann & Kent, 1988). It is facilitated by the removal of local inhibitor peptides that accumulate in the breast and decrease milk synthesis (Prentice *et al.*, 1989). Finally, suckling-related surges of oxytocin induce the expulsion of milk from the mammary alveoli and ducts. Inhibition of oxytocin secretion decreases the volume of milk ejected (Neifert & Seacat, 1986).

There has been little systematic work done to investigate the multiple potential aetiologies for LI. Since PRL plays an important role in lactation and its levels in the circulation can be readily measured by radioimmunoassay (RIA), numerous studies have concentrated on the serum RIA levels of PRL in lactation and LI (Aono *et al.*, 1977; Bigg & Yen, 1977; Delvoye *et al.*, 1977; Bunner *et al.*, 1978; Tyson *et al.*, 1978; Gross & Eastman, 1979; Howie *et al.*, 1980; Weichert, 1980; Weitzman *et al.*, 1980; Hennart *et al.*, 1981; Martin, 1983). However, hormone levels measured by RIA do not always correlate with the biological activities of the hormone (Smith & Norman, 1990), and while in certain pathological cases serum PRL was found to have normal activity when measured by RIA, it had markedly decreased activity when measured by the Nb2 lymphoma cell proliferation bioassay (BA) (Nagy *et al.*, 1991).

We therefore postulated that LI of unknown origin in patients with a normal PRL secretory response to suckling (as measured by RIA) might, in some cases, be due to lactogens possessing unusually low bioactivity. To examine this possibility we measured the biologically active (BA) and RIA lactogen levels in sera from breast-feeding women with LI of unknown origin and in sera from normally lactating controls. PRL and GH were assessed together since, in humans, GH also has lactogenic activity (Milsom *et al.*, 1992).

Materials and methods

Subject selection

Each breast-feeding mother and infant referred to the breast-feeding centre is routinely assessed clinically for evidence of LI. This assessment includes a detailed maternal and infant history, complete physical examinations of both mother and infant, and observation of breast-feeding. When a clinical diagnosis of LI of unknown cause is made, as described by Weichert (1980) and Neifert and Seacat (1986), it is validated by performing a standardized infant test weighing to evaluate the breast-milk intake (Neville, 1987). For this purpose, infants are nursed to satiety. When total feeding time is less than 30 minutes, breasts are emptied using a Medella electric breast pump to determine the amount of residual milk. Pre and post-feed infant weights are assessed using a Sartorius integrating electronic scale with computer averaging of 40 separate readings and associated with an error of ± 1 g.

A diagnosis of LI of unknown origin was confirmed when the rate of milk synthesis failed to increase despite frequent, effective breast stimulation and breast drainage, and when the test feeds remained below 45 ml, over a 2 3-week period.

In addition, lack of lactation reflex recovery (absent areolar distension before feeding, absent tingling sensation during feeding, absent contralateral milk ejection) after the administration of a galactagogue (domperidone) confirmed the initial diagnosis (Weichert, 1979; Petraglia *et al.*, 1985).

Subjects for the study were selected consecutively from women diagnosed with LI of unknown origin. Entry criteria included all of the following: primiparity, absent or minimum breast enlargement during pregnancy, lack of post-partum breast changes, normal puerperium and maximum test feed of less than 45 ml. Patients were excluded if their LI was associated with puerperal complications or problems, including post-parturn haemorrhage, a delay greater than 12 hours in initiating breast feeding, inadequate early breast-feeding technique, prolonged maternal or infant ill health, infant prematurity or significant past breast surgery (augmentation or reduction). Matched controls were chosen from mothers attending the breast-feeding centre who had well established lactation. Entry criteria included primiparity, normal breast enlargement (> 2 bra size) in pregnancy, uncomplicated puerperium, post-partum breast enlargement and infant test feeds greater than 110 ml. All breast-feeding subjects and controls had physiological lactational amenorrhoea. Ethical approval was obtained and all women gave informed consent.

Serum samples

Blood samples were drawn from rested, relaxed subjects and controls 30 minutes after a standardized test feed. Following serum isolation, each sample was divided into two portions: one portion was stored at —20°C for subsequent lactogen bioassay, the other was used immediately for RIA of PRL and GH.

In-vitro bioassay of lactogenic hormones

The Nb2 lymphoma cell bioassay for lactogens was used to measure the levels in sera of total lactogen bioactivity, as previously described (Tanaka *et al.*, 1980). Lactogen bioactivity in sera from non-pregnant individuals is due to PRL as well as GH. This well established in-vitro assay is based on the ability of lactogens to stimulate the proliferation of cultured, lactogen dependent Nb2 lymphoma cells. Lactogen-induced increase in cell population over a 48-hour period is proportional to the concentration of the hormone in the range 0.06-6 mIU/l (Fig. 1). Sera from patients and control subjects were each assayed in a series of dilutions to cover the growth response of the cultures to the lactogen standard at the useful working range 0.5—6 mIU/l. Lactogen bioactivity of a serum sample was derived from parallel sections of the growth response curves of the sample and the lactogen standard (see Fig. 1) and, in each assay, is the average of at least three values. The interassay coefficient of variation of the bioassay is < l0.8%. The lactogen standard used in all bioassays was ovine PRL (31 lU/mg; Sigma Chemical Co., St Louis, MO, USA). This preparation had about the same potency in the bioassay as a preparation of human PRL (batch 88—1—20, a generous gift from Dr H. G. Friesen's laboratory, Manitoba, Canada) which was stated to be equipotent with hPRL standard WHO 75/504 (32.5 lU/mg). The lactogen-free horse (gelding) serum used as a medium supplement was purchased from the National Biological Laboratory Ltd (Dugald, Manitoba, Canada). Cells were counted with a Coulter electronic cell counter (Hialeah, FL, USA).

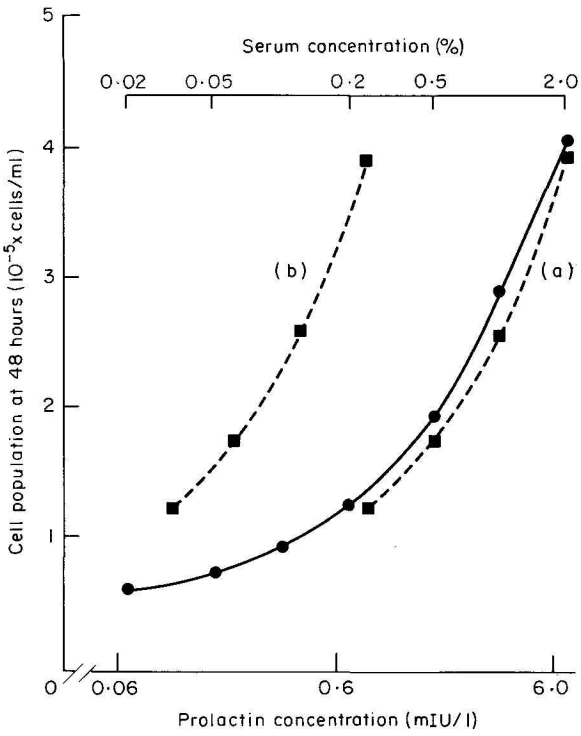

Fig. 1 Effect of g , human sera and n , a prolactin standard, used at various concentrations, on the population growth of Nb2 lymphoma cells during a 48 hour period. LI patient's serum (a) and control serum (b) had lactogen biological activity (BA) levels of 214 and 1860 mIU/1, respectively. Ovine prolactin was used as a standard (see Materials and methods).

RIA

Serum PRL concentrations were estimated in duplicate using a conventional hPRL RIA kit (Becton-Dickinson, Orangeburg, NY, USA). Similarly, serum GH levels were estimated using an RIA kit for hGH (Kallestad Diagnostics, Austin, TX, USA). For both RIAs the interassay coefficient of variation was < l0.8%. The hPRL and hGH standards of the kits had been calibrated against WHO hPRL 81/541 (35 lU/mg) and WHO hGH 66/217 (2 lU/mg), respectively.

Results

Forty-three of 309 consecutive lactating subjects (14%), seen at the breast-feeding clinic within the last 2 years, had inadequate lactation. In the majority of these cases, there was no readily identifiable aetiology. Twelve recent patients with typical LI of unknown origin, who fitted entry and exclusion criteria, were evaluated. Twelve matched breast-feeding controls were enrolled. A clinical description of the cases is given in Table 1.

Table 1 Clinical data of cases

	Subjects with lactation insufficiency	Controls
Number	12	12
Mean parity	1	1·3
Mean age infant days	46	48
Mammogenesis		
absent	7	0
minimum < 1 bra cup	5	0
definite > 2 bra cups	0	12
Breast anatomy		
normal	5	12
hypoplastic, normal nipple/areola	2	0
hypoplastic, puffy areola	4	0
hypoplastic, long nipples	1	0
Uncomplicated puerperium, including spontaneous vaginal delivery	12	12
Initiation of breast-feeding, less than 12 hours	12	12
Lactogenesis		
absent	9	0
minimum engorgement (< 1 bra cup)	3	0
definite engorgement (> 2 bra cups)	0	12
Galactopoiesis		
adequate (exclusive breast-feeding)	0	12
inadequate regular supplements	12	0

As shown in Table 2, breast-milk yield during a 30-minute infant standardized test feed was substantially lower in the group of LI patients than in the control group, showing mean milk volumes of 216 and 1465 ml, respectively.

Table 2 Milk yield during infant test feedings*

	Subjects with lactational insufficiency ($n=12$)	Controls ($n=12$)
Mean volume (ml)	21·6	146·5
Range (ml)	3–43	107–210

* Infants were nursed to satiety. When total feeding time was less than 30 minutes, breasts were emptied using a Medella electric breast pump. Pre and post-feed weights were assessed using a Sartorius integrating electronic scale with computer averaging of 40 separate readings and an error of ±1 g (Neville 1987).

Table 3 shows the RIA and BA levels of lactogens in the sera from the LI patients and their controls 30 minutes after a standardized test feed. The RIA (PRL + OH) levels were similar for both groups and ranged from about 350 to over 7000 mIU/l. Although the total lactogen BA levels of the sera were in most cases lower than their RIA (PRL + OH) levels, there was no significant difference between the two groups in lactogen BA levels. In either group the latter ranged from about 150 to 5000 mIU/l.

Table 3 Lactogen levels (mIU/l) in sera from breast-feeding patients with lactation insufficiency (LI) and normally lactating eontrols*

	LI patients			Controls		
Subject no.	PRL+GH (RIA)	Lactogen BA† Mean	SD	PRL+GH (RIA)	Lactogen BA† Mean	SD
1	356	214	38	335	136	16
2	747	499	78	330	149	41
3	542	527	11	1550	772	109
4	1402	626	101	2908	1172	82
5	1297	741	56	2942	1352	106
6	2173	1076	123	3753	1860	201
7	2190	1156	208	4799	1885	320
8	6129	2669	244	2429	1928	73
9	4414	3112	129	3654	2719	443
10	4767	3398	193	3725	2821	263
11	5546	4340	175	5434	4086	201
12	9804	5468	717	7003	4743	828

* Serum samples were taken from both groups 30 minutes after a standardized test feed.
† Data are presented as means ± standard deviation (SD)

Discussion

There was substantial variation in the serum lactogen levels within both the LI patient and control groups (Table 3). This finding is consistent with a variation in serum PRL levels previously reported for lactating subjects (Martin, 1983) and is probably related to a decreasing PRL response to suckling as a function of time following delivery (Bunner *et al.*, 1978; Gross & Eastman, 1979; Hennart *et al.*, 1981), regardless of the maintenance of an adequate milk supply (Aono *et al.*, 1977).

A rigorous comparison of absolute levels of BA and RIA lactogen levels in the sera is not possible since the BA and RIA assays were made with different standards. However, the results do indicate that there was a significant variability in the BA:RIA ratios within each group (Table 3). The reason for these variations is not known. It may be noted, however, that PRL and GH can be present in the circulation in different biochemical forms which may have different bio and immuno-aetivities (Smith & Norman, 1990). Circulating PRL has been shown to exist in both the glyeosylated and non-glycosylated form; the concentration of the latter increases as pregnancy progresses (Brue *et al.*, 1992). After parturition, the non-glycosylated PRL form tends to persist in nursing, but not in non-nursing mothers (Markoff & Hollingsworth, 1988; Hashim *et al.*, 1990). Furthermore, non-glycosylated PRL has a much greater biological activity than glycosylated PRL (Pellegrini *et al.*, 1988; Haro *et al.*, 1990). These findings raise the possibility that the variation found in the lactogen BA:RIA ratios of the two groups in the present study may stem from differences in the relative amounts of glycosylated and non-glycosylated forms of PRL present in the sera of the subjects.

The lactation difficulties of the women in the present study are unlikely to have been caused by poor bioactivity of their circulating lactogens, even when the levels were comparatively low, since similarly low levels of bioactive lactogens were found in some of the normally lactating controls (Table 3). Even at serum lactogen concentrations as low as 335 mIU/l (RIA), or 136 mIU/l (BA) (control no. 1), lactation could normally proceed. There was no evidence of the presence of lactogens with normal RIA activity, but unusually low bioactivity, which could account for their LI.

Having ruled out biologically inactive lactogens as a cause for LI, it could still have been caused by a humoral inhibitor of the interaction between lactogens and their receptors in mammary tissues. We did not find evidence of any such inhibitory activity. The lactogen induced mitogenesis of the Nb2 cells, which is receptor mediated, did not show signs of inhibition when patients' serum samples were used at increasingly high concentrations, e.g. up to 2% in the assay wells (Fig. la).

The actual cause of lactation failure in these patients is not known. Mammogenesis and lactogenesis were both abnormal in all experimental subjects (Table 1). There were no other obvious compounding factors, such as excessive obstetrical haemorrhage or a delay in initiation of lactation, that could account for the lactation insufficiency. It is possible that hypotension during pregnancy, that is not so severe as to produce circulatory collapse, could lead to decreased posterior pituitary function as well as the previously reported effect on the anterior pituitary (Sheehan & Murdoch, 1938; Morishita *et al.*,, 1985). The present study did not exclude the possibility of deficiencies in oxytocin secretion. Target organ abnormality, such as impaired receptor function or insufficient pubertal glandular development (Neifert *et al.*, 1985), is unlikely to account for all the LI cases. It is clear, however, that abnormally low lactogen bioactivity does not play a role in the LI of the subjects investigated in this study.

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