Relationship Between Serum Prolactin Levels and Protein Composition of Breast Secretions in Nonlactating Women

Francisco Vizoso, Irene Diez-Itza, Luis M. Sanchez, Ana F. Tuya, Alvaro Ruibal, and Carlos López-Otín

Servicio de Cirugia (F. V., A.F. T), Hospital de Jove; Departamento de Biologia Funcional (I.D-I., L.M.S., C.L-0.) Facultad de Medicina, Universidad de Oviedo; and Servicio de Medicina Nuclear (A.R.), Hospital Central de Asturias, 33006 Oviedo, Spain

ABSTRACT

The potential relationship between serum PRL levels and protein composition of breast secretions was evaluated in 54 premenopausal nonlactating women during the luteal phase of their menstrual cycle. Women were classified into four groups according to the presence or absence of breast pathology and to the protein pattern of their breast secretions. Type I mammary fluids contain Zn-α_2-glycoprotein, apolipoprotein D, and gross cystic disease fluid protein-15, whereas Type II fluids are characterized by the presence of some milk proteins such as lactoferrin, lysozyme, and ol-lactalbumin. Basal serum levels of PRL, as well as of progesterone, LH, FSH, TSH, Tn, and T4 were within normal range, and no significant differences were found between the different groups of women under study. However, after a TRH stimulation test, the maximum PRL response was significantly higher ($P < 0.02$) in normal women with Type II secretions than in those with Type I (64 ± 6.8 µg/L vs. 43.7 ± 3.9 µg/L). Similarly, when PRL concentrations in patients with benign breast disease were considered, those with breast fluids containing milk proteins had a rise in PRL secretion after TRH stimulation significantly higher ($P < 0.05$) than those with fluids lacking these proteins (77.1 ± 6.2 vs. 58.8 ± 5.1 µg/L). These results indicate that the occurrence of milk proteins in breast secretions from nonlactating women is associated with an increase in serum PRL concentrations after TRH stimulation, and opens the possibility of using breast fluid protein analysis as a simple and noninvasive procedure for studies on the putative role of PRL in the development of benign and malignant breast diseases.

Introduction

The mammary gland is one of the most complex endocrine target organs. Growth, differentiation, lactogenesis, and galactopoiesis need the interplay of ovarian and adrenal steroids, pituitary, thyroid, and pancreatic hormones (1, 2). Among this wide variety of hormones, PRL seems to play a major role in stimulating mammary gland activity in both physiological and pathological conditions (3). Consequently, a large number of clinical studies have tried to elucidate the possible role of this pituitary hormone in the physiopathology of benign breast disease (BBD) and breast cancer (4-7).

However, at present, reports on the significance of PRL in both conditions are conflicting. Thus, whereas some studies have reported increased serum basal levels of PRL in fibrocystic breast disease (4), others have found normal concentrations of the hormone (5). On the other hand, and with regard to breast cancer, it is remarkable that, although different studies have shown that PRL is a major factor in the development and growth of mammary tumors in animals (6), a similar role for this hormone in human breast cancer remains controversial. In this way, some epidemiological studies have found higher serum PRL levels in breast cancer patients (7) whereas other groups have failed to find such an association (8). According to these data, it seems clear that additional biological markers, and especially those reflecting the activity of breast microenvironments, are required to better understand the potential role of PRL in breast pathology.

Over recent years, analysis of nipple aspirates from nonlactating women has attracted considerable interest as a rapid and noninvasive method to assess the environment and metabolic activity within the mammary gland (9, 10). The cytological studies of these secretions have revealed the presence of abnormal epithelial cells in fluids from women with breast diseases, allowing the identification of women who are at increased risk of developing breast cancer (11). Similarly, biochemical analysis of breast secretions has demonstrated the presence of a variety of substances, including hormones (12-15), proteolytic enzymes (16), mutagenic agents (17, 18), and toxic compounds (19) which may be involved in the malignant transformation. As part of our studies on breast fluid composition, we have recently described that these secretions can be classified into two types according to their major polypeptide components (20). Interestingly, one of these subtypes, denominated Type II and characterized by the presence of some milk proteins, is detected in 47% of breast cancer patients but only in 8% of control women and in 16% of women with BBD (21). At present, the source of these milk proteins in breast fluids from nonlactating women, including a large percentage of breast cancer patients, remains unclear but we have proposed 12% gels (0.5 mm thickness) at 50 mA for 30 min in a Bio-Rad apparatus that it could be a consequence of abnormal stimulation of (Hercules, CA). Samples were treated with nonreducing buffer sample breast epithelium by PRL (21). To address this question, in this work we have examined the possible relationship between serum PRL levels and protein composition of breast fluid from nonlactating women or from patients with BBD.

Materials and Methods

Study population

This study included 54 premenopausal nonlactating women (21-50 yr of age), belonging to a large study population whose breast fluid had been analyzed in our previous work (21). Women were classified into four different subgroups according to the protein pattern of their breast secretions and breast pathology (Table 1). There were 26 women with Type I secretion and 28 with Type II (see below). With regard to breast pathology,

there were 39 women with BBD diagnosed on the basis of clinical, mammographic, echographic, cytologic, and histologic studies. Among these women with BBD, 19 had Type I secretion, and the specific histologic diagnosis was as follows: 1 with epithelial hyperplasia with atypia, 3 with sclerosing adenosis, 2 with florid adenosis, 11 with macrocysts, 1 with intraductal papilloma solitary, and 1 with periductal fibrosis. The histologic diagnosis of the 20 women with BBD and Type II secretion was as follows: 4 with epithelial hyperplasia with atypia, 6 with sclerosing adenosis, 3 with florid adenosis, 4 with macrocysts, 1 with intraductal papilloma multiple, and 2 with periductal fibrosis. The remaining 15 women included in the study were healthy volunteers from the family planning clinics or from the general medical clinics. None of them had complaints or significant clinical findings referrable to the breast. All women gave their informed consent to participate in the study, which was approved by the ethical committee of our hospital. In addition, all women participating in the study were given a detailed questionnaire which focused on medical and reproductive information. Mean age and reproductive history of women belonging to the different groups did not show significant differences, All women had regular menstrual cycles, and none were taking hormonal or antidepressive medication at the time of the study or during the preceding 6 months, nor had any been pregnant or lactating at least 4 yr before the study.

Breast fluid collection

Breast fluids were obtained by manual compression of the four periareolar quadrants from the nipple, collected by means of a capillary tube, and transferred to a microcentrifuge tube. Nipple aspirates were always collected before any diagnostic study or surgical procedure on the breast. In addition, after hormonal stimulation tests, breast fluids were immediately collected following the same procedure. After collection, breast secretions (ranging in volume from l-250 μl) were frozen and stored at -20 C until subsequent analysis.

Classification of breast secretions by sodium dodecyl sulfate (SDS)-polyacrylnmide gel electrophoresis.

Samples of breast fluids collected before and after hormonal stimulation test were analyzed by SDS-polyacrylamide gel electrophoresis in 12% gels (0.5 mm thickness) at 50 mA for 30 min in a Bio-Rad apparatus (Hercules, CA). Samples were treated with nonreducing buffer sample before electrophoretical analysis and typed following our previous classification (20). Type I secretions contained distinctive bands of 44, 24, and 17 kilodaltons (kDa) corresponding to Zn-α_2-glycoprotein, apolipoprotein D, and CCDFP-15, respectively. Type II fluids contained distinctive bands of 80, 15, and 14 kDa corresponding to the milk proteins lactofenin, lysozyme, and α -lactalbumin. In addition to these distinctive bands, all samples contained a major band of about 67 kDa which was identified as albumin. The intensity of some bands was variable in different samples (20), however, all three bands characteristic of each of the two patterns were always

detected as major components in each sample. Typing of breast secretions in terms of protein composition was also confirmed by a quantitative criterium based on determination of the apolipoprotein D content of breast secretions in relation to the total amount of albumin present in them. Thus, a secretion was considered Type I if it contained more than 200 µg apolipoprotein D/ mg albumin. Apolipoprotein D and albumin were quantified as previously described (22, 23).

TRH stimulation test

Testing was carried out between 0900 h and 1100 h, during the luteal phase of the menstrual cycle. The midluteal phase was confirmed by measuring the concentrations of LH, FSH, and progesterone in blood samples. The stimulation test was performed after an overnight fast. An indwelling venous catheter was inserted into a forearm vein, and each 20 women received 200 µg TRH (PREM, Fruntost-Zyma, Barcelona, Spain) as an iv bolus injection. Blood samples were collected at -15 min and at time zero, for basal determinations, and at 15, 30, and 60 min after stimulation. The blood samples were allowed to clot at room temperature for 2 h and the serum was separated by centrifugation and stored at -20 C until used. PRL was determined in all samples, whereas progesterone, LH, FSH, TSH, T3 and TI were only analyzed in specimens before stimulation.

Hormonal assays

All samples from TRH test in each woman were analyzed in duplicate in the same assay. Serum PRL was measured by an immunoradiometric assay (IRMA) using a commercially available kit (CIS Radiochirnie, Gifsur-Yvette, France). The sensitivity of the assay was 0.7 µg/L. The intra- and interassay coefficients of variation (CV) at 8 µg/L PRL were 7.1% and l0%, respectively, whereas the corresponding ones at 94 µg/L PRL were 5.6% and 6.2%. Serum LH and FSH were measured by IRMA using kits obtained from Ire-Medgenix (Fleurus, Belgium). The sensitivity of the assay for both gonadotropins was 0.2 IU/L. The intra- and interassay CV at 68 IU/L of LH were 4.2% and 6.0%, whereas the corresponding values at 27 IU/L FSH were 2.9% and 3.2%, respectively. Serum progesterone levels were measured by IRMA using reagents supplied by CIS Radiochimie, and the assay sensitivity was 0.44 nmol/L. The intra- and interassay CV at 3.18 nmol/L of progesterone were 7.1% and 10.9%, whereas the values for these parameters at 82.68 nmol/L progesterone were 4.9% and 6.8%, respectively. This assay had a cross-reactivity of 4.3% with 20-hydroxyprogesterone, 3.8% with deoxycorticosterone, and less than 0.1% with testosterone, cortisol, and estradiol. Total serum T3 and T4 were determined by RIA using the commercial kit from Abbott Laboratories (Chicago, IL). The intra- and interassay variations were 4.1% and 6.1% at 1.38 nmol/L T3, whereas the corresponding values at 102.9 nmol/L were 3.0% and 5.5%. The intra- and interassay CV were 4.2% and 6.1% at 83.6 nmol/L T4, and 3.9% and 4.4% at 185.3 nmol/L of this hormone. Finally, TSH was assayed by IRMA with the monoclonal antibodies supplied by Behring (Marburg,

Germany). In this case, the intra- and interassay variations were 4.1% and 6.1% at a dose of 0.25 mU/L, and 3.2% and 6.8% at a dose of 6.4 mu/L, respectively.

Statistical analysis

The Student's t test was used to test differences in the baseline serum PRL and different points aster TRH stimulation test, between the different groups of women. Significance was established at the $P < 0.05$ level. Data are presented as mean ± SEM.

Results

To determine the possible relationship between serum PRL levels and protein pattern in breast secretions, women included in the study were classified into four groups according to breast pathology and polypeptide composition of their secretions. Then hormone levels were measured in all cases. Basal serum concentrations of PRL, progesterone, LH, FSH, TSH, T3, and T4 are shown in Table 2. All of them were within normal range, and no significant differences were found between the different groups studied in this work.

We also considered the possible occurrence of variations in the PRL response to TRH. To examine this possibility, we performed a TRH stimulation test, and serum PRL concentrations were determined at the different time points. The results obtained in the four groups of women included in the present work are shown in Figs. 1 and 2. A significant rise ($P < 0.001$) in PRL levels was observed in all groups at 15, 30, and 60 min after TRH stimulation. In all cases, the maximum PRL response was reached 15 min after TRH stimulation. This rise in PRL secretion was significantly higher ($P < 0.05$) in women with benign breast pathology (n = 39; mean = 68.2 ± 3.8 µg/L) than in normal women (n = 15; mean = 54.5 ± 4.8 µg/L). In addition, significant differences were also observed when PRL values were examined in relation to the different protein patterns of breast secretions. Thus, the maximum PRL response to TRH stimulation was significantly higher ($P < 0.02$) in normal women with Type II secretions (group II) than in those with Type I (group I) (64 ± 6.8 µg/L vs. 43.7 ± 3.9 µg/L). It is also noteworthy that PRL levels remained significantly higher ($P < 0.01$) 30 and 60 min after stimulation test (Fig. 1). Similarly, when PRL levels in patients with BBD were considered, those with breast fluids containing milk proteins (group IV) showed a rise in PRL secretion after TRH stimulation significantly higher ($P < 0.03$) than those with fluids lacking these proteins (group III) (77.1 ± 6.2 µg/L vs. 58.8 ± 5.1 µg/L, respectively). This increase in PRL concentrations also remained significantly higher in both groups at 60 min after the stimulation test ($P < 0.02$) (Fig. 2). Finally, it should be mentioned that when the overall study population was considered, women with Type II secretion (n = 28) had a PRL response to TRH stimulation significantly higher ($P < 0.005$) than those with Type I secretion (n = 26) (73.4 ± 4.8 µg/L vs. 54.8 ± 4.9 µg/ L, respectively).

Discussion

In this work we have presented evidence indicating that the occurrence of milk proteins in breast secretions from nonlactating women is associated with an increase in PRL serum levels after TRH stimulation. Since the presence of milk proteins in breast secretions is statistically associated with premalignant or malignant lesions (20, 21), the results presented in this work support previous findings suggesting a role for PRL in the development of benign (4, 24-26) and malignant breast diseases (6, 7).

After our recent finding showing that a significant percentage of breast secretions from nonlactating women with benign and malignant breast diseases are characterized by the presence of milk proteins (20, 21), this work was initially aimed at evaluating the possibility that this abnormal secretion of milk proteins could be mediated by PRL. According with this hypothesis, we first examined the possible occurrence of variations in the serum basal levels of PRL. However, we did not detect any significant difference between the different groups of women classified in terms of breast pathology or as a function of the protein pattern of their breast secretions. By contrast, we observed a significantly increased PRL response to TRH stimulation in those women whose breast secretions contained milk proteins. This rise in PRL levels was detected in both normal women and patients with BBD and suggests the existence of an increased storage of PRL-secreting lactotropes of pituitary origin which could be responsible for an elevated daily PRL release in these women. This assumption is supported by previous findings of Peters et al. (27, 28) showing that maximum TRH-induced PRL response in patients with fibrocystic mastopathy are significantly correlated to mean 24-h serum concentrations and to the sleep-induced PRL rise. Since PRL plays a primary role in the transcription of milk protein genes (l), the chronic exposure of epithelial mammary cells to these elevated concentrations of PRL might account for the abnormal production and subsequent secretion of milk proteins in nonlactating women. In this regard, it is noteworthy that Rose et al. (29) have found that women affected with BBD have high concentrations of PRL in breast fluid, despite having normal serum levels of this pituitary hormone. In addition to this increased PRL stimulation of breast epithelium as a source of milk proteins in breast secretions from nonlactating women, the occurrence of an abnormal response of the breast itself to PRL is also possible. This response could be mediated through the high-affinity membrane PRL receptors whose existence has been demonstrated in both normal and pathological mammary epithelial cells (30, 31). Finally, it should be considered that alternative mechanisms, including an increase in TRH receptors or variations in the signal transduction pathway, could also contribute to explain the increased PRL response to TRH stimulation, and they can not be definitively ruled out at present.

Regardless of the precise mechanism of the enhanced production of milk proteins in nonlactating women, the abnormal finding of these proteins in breast secretions may be of interest in relation to the putative significance of PRL in the development and growth of breast cancer. In the present study, patients with breast cancer were not included because of ethical concerns about performing a preoperatory TRH-stimulation test in

these women. However, if we consider that a similar increase in PRL response to TRH stimulation was observed in normal women and BBD patients with Type II secretions, it is tempting to speculate that a similar situation may occur in the case of women with breast carcinoma. In addition, since a large percentage of secretions from breast cancer patients (about 50%) are characterized by the presence of milk proteins, it is also suggestive to propose that the above described abnormal PRL hyperresponse may be somewhat associated to some cases of breast cancer. At present, the putative role of PRL in human breast cancer is controversial because whereas several studies have established a correlation between high PRL levels and tumor progression, therapy resistance, and poor clinical outcome of the disease (7, 32-34), other groups have failed to find significant associations (8, 35). It has been proposed that methodological aspects including those derived from variations in PRL through the menstrual cycle and its secretion in a pulsatile fashion throughout the day (36) could contribute to explain these discrepancies (37). In relation to this, the finding that the presence of milk proteins in breast secretions from nonlactating women reflects the occurrence of a PRL hyperresponsiveness to TRH opens the possibility of using breast fluid protein analysis as a simple and noninvasive procedure for future studies on the putative role of PRL in the development of benign and malignant breast diseases.

Acknowledgments

References

1. Topper YJ, Freeman CS. 1980 Hormones and mammary development Physiol Rev. 60:1049-1 106.

2. Robin C. 1983 Endocrinological aspects of breast physiology. In: Angeli A, Bradlow HL, Dogliotti L, eds. Endocrinology of cystic breast disease. New York: Raven Press; 25-34.

3. Shiu RPC, Friesen HG. 1980 Mechanism of action of prolactin in the control of mammary gland function. Annu Rev Physiol. 42:83- 96.

4. Walsh PV, McDicken IW, Bulbrook RD, Moore JW, Taylor WH, George WD. 1984 Serum oestradiol-170 and prolactin concentrations during the luteal phase in women with benign breast disease. Eur J Cancer. 20:1345-1351.

5. Sitruk-Ware R, Sterkers N, Mauvais-Jarvis P. 1979 Benign breast disease I: Hormonal investigation. Obstet Gynecol. 53:457-460.

6. Welsch CW, Nagasawa H. 1977 Prolactin and murine mammary tumorigenesis: a review. Cancer Res. 37:951-963.

7. Rose DP. Pruitt BT. 1981 Plasma prolactin levels in patients with breast cancer. Cancer. 48:2687-2691.

8. Wang DY, De Stavola BL, Bulbrook RD, et al. 1992 Relationship of blood prolactin levels and the risk of subsequent breast cancer. Int J Epidemiol. 21:214-221.

9. Sartorius OW, Smith HS, Morris P, Benedict D, Friesen L. 1977 Cytologic evaluation of breast fluid in the detection of breast disease. J Natl Cancer Inst. 59:1073-1078.

10. Petrakis NL. 1986 Physiologic, biochemical and cytologic aspects of nipple aspirate fluid. Breast Cancer Res Treat. 8:7-19.

11. Petrakis NL, Wrensch MR, Ernster VL, Miike R, King EB, Goodson WH. 1987 Prognostic significance of atypical epithelial hyperplasia in nipple aspirates of breast fluid. Lancet. ii:505.

12. Wvnder EL, Hill P. 1977 Prolactin. oestrogens, and lipids in breast fluid. Lance;. ii:840.

13. Miller WR, Humeniuk V, Forrest APM. 1981 Factors affecting dehydroepiandrosterone sulphate levels in human breast secretions. Breast Cancer Res Treat. 1:267-272.

14. Hill P, Garbaczewski L, Wynder EL. 1983 Testosterone in breast fluid. Lancet. i:761.

15. Rose DP. 1986 Hormones in breast fluid. Breast Cancer Res Treat. 8:25-28.

16. Sanchez LM, Ferrando AA, Diez-Itza I, Vizoso F, Ruibal A, López-Otín C. 1993 Cathepsin D in breast secretions from women with breast cancer. Br J Cancer. 67:1076-1081.

17. Petrakis NL, Maack CA, Lee RE, Lyon M. 1980 Mutagenic activity in nipple aspirates of human breast fluid. Cancer Res. 40:188-189.

18. Scott WN, Miller WR. 1990 The mutagenic activity of human breast secretions. J Cancer Res Clin Oncol. 116:499-502.

19. Petrakis NL, Gruenke LD, Beelen TC, Castagnoli N, Craig JC. 1978 Nicotine in breast fluid of non lactating women. Science. 199:303-305.

20. Sanchez LM, Vizoso F, Diez-Itza I, López-Otín C. 1992 Identification of the major protein components in breast secretions from women with benign and malignant breast diseases. Cancer Res. 52:95-100.

21. Vizoso F, Sanchez LM, Diez-Itza I, Lamelas ML, López-Otín C. 1992 Factors affecting protein composition of breast secretions from nonlactating women. Breast Cancer Res Treat. 23:251-258.

22. Sanchez LM, Diez-Itza I, Vizoso F, López-Otín C. 1992 Cholesterol and apolipoprotein D in gross cystic disease of the breast. Clin Chem. 38:695-698.

23. Balbin M, Vizoso F, Sanchez LM, Venta R, Ruibal A, Fueyo A, López-Otín C. 1991 GCDFP-70 protein in cyst fluid identified as albumin and used to classify cysts in women with breast gross cystic disease. Clin Chem. 37:547-551.

24. Halbreich U, Assael M, Ben-David M, Bornstein R. 1976 Serumprolactin in women with premenstrual syndrome. Lancet. ii:654- 656.

25. Cole EN, Sellwood RA, England PC, Griffiths K. 1977 Serum prolactin concentrations in benign breast disease throughout the menstrual cycle. Eur J Cancer. 13:597-603.

26. Watt-Boolsen S, Anderson AN, Blichert-Toft M. 1981 Serum prolactin and estradiol levels in women with cyclical mastalgia. Horm Metab Res. 13:700-702.

27. Peters F, Pickardt CR, Zimmermann G, Breckwoldt M. 1981 TSH and thyroid hormones in benign breast diseases. Klin Wochenschr. 59:403-407.

28. Peters F, Schuth W, Scheurich B, Breckwoldt M. 1984 Serum prolactin levels in patients with fibrocystic breast disease. Obstet Gynecol. 64:381-385.

29. Rose DP, Berke B, Cohen LA, Lahti H. 1987 A comparison of serum and breast duct fluid-immunoassayable prolactin and growth hormone with bioassayable lactogenic hormones in healthy women and patients with cystic breast disease. Cancer. 60:2761-2765.

30. Di Carlo R, Muccioli G, Bellusi G, Mussa A. 1984 Presence and characterization of prolactin receptors in human benign breast tumors. Eur J Cancer. 20:635-641.

31. Bonneterre J, Peyrat JP, Beuscart R, Lefebvre J, Demaille A. 1987 Prognostic significance of prolactin receptors in human breast cancer. Cancer Res. 4714724-4728.

32. Dowsett M, McGarrick GE, Harris AL, Coombes RC, Smith IE, Jeffcoate SL. 1983 Prognostic significance of serum prolactin levels in advanced breast cancer. Br J Cancer. 47:763-769.

33. Holtkamp W, Nagel GA, Wander HE, Rauschecker HF, von Heyden D. 1984 Hyperprolactinemia is an indicator of progressive disease and poor prognosis in advanced breast cancer. Int J Cancer 34:323-328.

34. Bhatavdekar JM, Shah NG, Balar DB, et al. 1990 Plasma prolactin as an indicator of disease progression in advanced breast cancer. Cancer. 65:2028-2032.

35. Wang DY, Hampson S, Kwa HG, et al. 1986 Serum prolactin levels in women with breast cancer and their relationship to survival. Eur J Cancer. 22:487-492.

36. Vekemans M, Robyn C. 1987 The influence of exogenous estrogen on the circadian periodicity of circulating prolactin in women. J Clin Endocrinol Metab. 64:111-118.

37. L'Hermite M, L'Hermite-Baleriaux M. 1988 Prolactin and breast cancer. Eur J Cancer. 24:955-958.

Table 1. Distribution of the study population according to breast pathology and protein pattern in breast secretions.

Groups	Number of women	Age (years) (mean ± SEM)	Breast pathology	Secretion patterns
I	7	38.8 ± 2.7	none	I
II	8	39.2 ± 3.4	none	II
III	19	41.5 ± 1.3	benign	I
IV	20	38.3 ± 0.9	benign	II

TABLE 2. Baseline serum PRL, LH, FSH, progesterone, TSH, TB, and T, in the four studied groups of women

Hormones	Women groups			
	I (7)	II (8)	III (19)	IV (20)
PRL (μg/L)	9.7 ± 1.8	6.8 ± 1.3	8.8 ± 0.9	8.6 ± 1.4
LH (IU/L)	3.4 ± 0.5	3.3 ± 0.4	3.7 ± 0.3	3.5 ± 0.5
FSH (IU/L)	5.2 ± 0.6	3.5 ± 0.5	4.1 ± 0.3	4.3 ± 0.4
Progesterone (nmol/L)	36.3 ± 9.3	36.9 ± 4.8	41.4 ± 4.5	38.4 ± 3.9
TSH (mU/L)	0.8 ± 0.1	0.9 ± 0.1	1.4 ± 0.2	1.2 ± 0.8
T_3 (nmol/L)	1.5 ± 0.08	1.6 ± 0.1	1.5 ± 0.07	1.5 ± 0.06
T_4 (nmol/L)	113.4 ± 5.2	112.1 ± 6.9	114.5 ± 3.9	113.2 ± 4.3

Results are the mean ± SEM. Number of women is in parenthesis.

FIG. 1. Serum PRL levels before and after TRH iv infusions in 15 normal women during the midluteal phase of the menstrual cycle. Women were divided into two groups according to the protein pattern of their breast secretions. Of the 15 women, 7 had Type I secretions (group 1) and 8 showed Type II (group II).
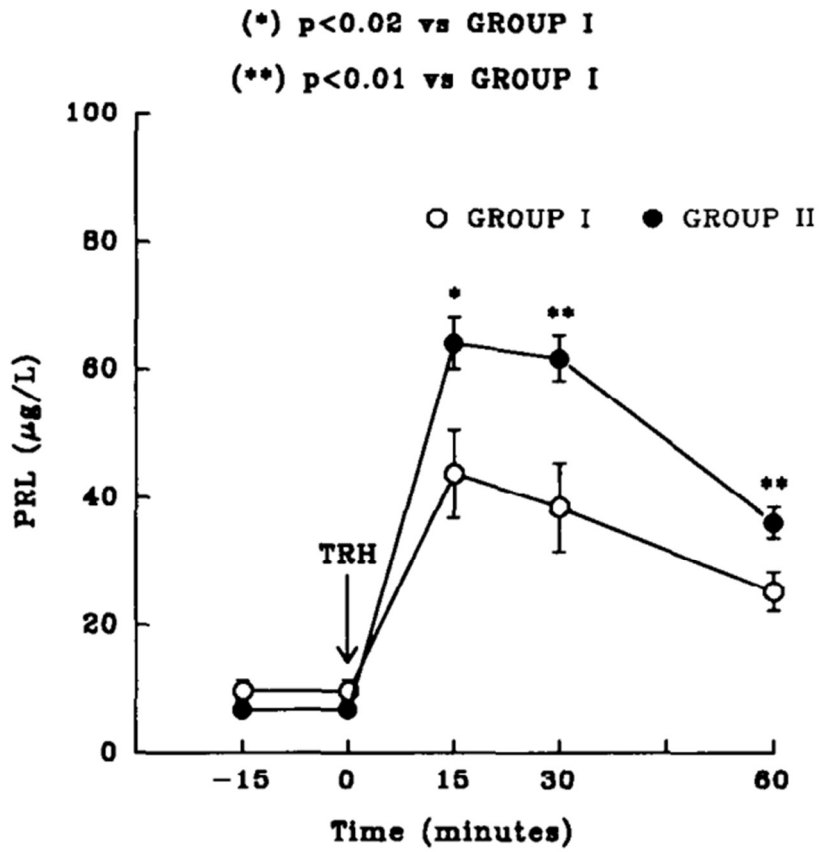
FIG. 2. Serum PRL levels before and after TRH iv infusions in 39 patients with benign breast diseases. Determinations were performed during the midluteal phase of the menstrual cycle. Patients were divided into two groups according to the protein pattern of their breast secretions. Of the 39 patients, 19 had Type I secretions (group III) and 20 showed Type II (group IV).