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Thyroid Hormones in Human Milk and their Influence on Thyroid Function of Breast-Fed Babies

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Summary

Various assay methods for detection of thyroid hormones in human milk were evaluated in recovery and dilution experiments after which the concentrations of thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3) were measured and compared with those in serum. The effect of breast feeding on pituitary thyroid function of normal babies also was studied. Competitive protein-binding analysis (CPBA) was found to be unsuitable for measurement of T_4 in milk. T_4 was not detected in samples of human milk by four radioimmunoassays (RIA), although more than 100% of T_4 was recovered in the assays. RIA (double antibody-ANS system) seemed to be reliable for detection of T_3 in milk, judging from recovery and dilution experiments. T_3 was detectable in all samples obtained 1–4 months postpartum. The T_3 concentration in milk was not correlated with protein concentration or daily volume. The concentration of T_3 in milk was lower than that in serum and the mean ratio of serum T_3 to milk T_3 was 2.8 ± 1.7 (mean ± S.D.). No correlation was observed between the T_3 concentration or daily T_3 excretion in milk and the T_3 concentration in serum. The total amount of T_3 excreted in milk was estimated as only 5–1000 ng/day. The serum levels of thyrotropin, T_4, free T_4 and T_3 were not significantly different between breast-fed and bottle-fed babies. These results indicate that T_3 excretion in milk cannot be explained by simple diffusion from the blood into the mother's milk and that breast feeding has no influence on the pituitary thyroid axis of normal babies.

Abbreviations

CPBA, competitive protein-binding analysis
RIA, radioimmunoassay
ANS, 8-anilinonaphthaline sulfonic acid
TBG, thyroxine-binding globulin

Although breast-feeding has been suggested to improve the clinical course of congenital hypothyroidism (1), discrepant results have been obtained on the concentrations of thyroid hormones in human milk (1, 9–11, 13–15). Very recently Letarte *et al.* (3) reported that breast feeding had no protective effect against the deleterious effects of congenital hypothyroidism. In this study we examined the performance characteristics of various methods of assaying thyroid hormones in human milk and report the measurements of concentrations of thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3) using these assays. We also examined the relationship of thyroid hormone concentrations in milk and serum and the effect of breast feeding on the pituitary-thyroid axis of normal babies.

MATERIALS AND METHODS

Human milk was obtained from three healthy women and 18 patients with thyroid diseases between 7 days and 7 months after delivery. The patients included 11 women with Graves' disease, 6 with Hashimoto's disease and one with simple goiter. None of the patients with Graves' disease were receiving anti-thyroid drugs at the time of examination. Serum samples were obtained at the same time from all subjects. For estimation of daily T_3 excretion in milk, 24-h samples were collected from 16 patients with thyroid disease 1–4 months postpartum. The T_3 concentration and total volume were measured. Serum samples were collected from breast-fed and 10 bottle-fed normal babies aged about 1 month. All milk and serum samples were frozen at −20°C until assayed.

T_4 concentrations in milk were measured by the following methods using commercial kits: (1) CPBA; *Method A*, ethanol extraction and separation of adsorbing-granules (Thyopac 4, The Radiochemical Centre, Buckinghamshire, England); *Method B*, ethanol extraction and separation of resin sponge (Tetrasorb-125, Dainabott Radioisotope Laboratories, Tokyo, Japan); *Method C*, alkaline extraction and separation of Sephadex column (Tetralute, Ames Company, Elkhart): (2) radioimmunoassay (RIA) (method of bound/free separation and inhibition of T_4-TBG binding are described); *Method D*, double antibody and 8-anilinonaphthalene sulfonic acid (ANS) (T_4 RIA kit, Eiken Immunochemical Laboratory, Tokyo, Japan); *Method E*, adsorbing granules and thiomersalate (T_4 RIA PAC, Radiochemical Center, Buckinghamshire, England); *Method F*, polyethylene glycol and ANS (T_4 RIA KIT II, Dainabott Radioisotope Laboratory, Tokyo, Japan); *Method G*, Sephadex column and alkaline buffer (Seralute, Ames Company, Elkhart).

The standard T_4 solution was prepared by dissolving L-T_4 (sodium salt, Sigma Chemical) in 0.1 N NaOH solution and diluting it with phosphate buffered saline (pH 7.5) containing 0.25% bovine serum albumin. For measurement of milk T_4 using Methods C-G, 1 ml of standard T_4 solution or 1 ml of milk was extracted with 2 ml of 99.5% ethanol, and the mixture was centrifuged at 2000 × *g* at 4°C for 10 min. Then the ethanol fraction obtained was evaporated and the dried sample was dissolved in 1 ml of thyroid hormone-free serum and assayed for T_4. The thyroid hormone-free serum was prepared from pooled normal human serum by removing endogenous T_4 and T_3 by treatment with charcoal (6).

T_3 concentrations in milk was measured by double antibody radioimmunoassay, which used ANS for inhibition of T_3-TBG binding (T_3 RIA kit Eiken Immunochemical Laboratory, Tokyo, Japan). The standard T_3 solution was prepared by dissolving L-T_3 (sodium salt, Sigma Chemical) in 0.1 N NaOH solution and diluting it with thyroid hormone-free serum. T_3 standard solutions

or milk samples were extracted with ethanol as described above and the dried extracts were dissolved in thyroid hormone-free serum for T_3 assay.

Recovery experiments were conducted by mixing aliquots of standard T_4 or T_3 solution with milk and then measuring the T_4 or T_3 concentrations as described above. After skimming off the lipid, protein concentrations in milk were measured by the method of Lowry *et al.* (14).

Serum concentrations of thyrotropin (TSH RIA kit, Daiichi Radioisotope Laboratories, Tokyo, Japan), T_4 and T_3 (T_4 RIA kit, T_3 RIA kit Eiken Immunochemical Laboratory, Tokyo, Japan) were measured by double antibody RIA and free T_4 was measured by solid-phase RIA (Gamma Coat Free T_4 RIA kit, Travenol Laboratories, Massachusetts, USA).

RESULTS

The recoveries of [^{125}I]-T_4 and [^{125}I]-T_3 in the ethanol extraction were 91.9 ± 0.05% and 89.5 ± 0.63% (mean ± S.D.), respectively.

Table 1. *Concentration of T_4 in human milk measured by various methods*

Case No.	Diagnosis	Time of sampling (postpartum)	Serum concentration[1] (μg/dl)	Milk concentration (μg/dl)						
				Competitive protein-binding assay			Radioimmunoassay			
				Method[2] A	Method B	Method C	Method D	Method E	Method F	Method G
1	Normal	6 days	8.8	49.7	25.1	3.8	<0.7	<0.7	<0.7	<1.5
2	Normal	25 days	6.0	46.3	33.7	4.9	<0.7	<0.7	<0.7	<1.5
3	Normal	2 months	7.7	49.3	9.3	8.6	<0.7	<0.7	<0.7	<1.5
4	Graves' disease	7 days	24.0	33.9	49.9	5.2	<0.7	0.7	0.7	<1.5
5	Hashimoto's disease	7 months	4.5	46.7	29.8	3.2	<0.7	<0.7	0.7	<1.5

[1] Serum T_4 was measured by Method D (see "Materials and Methods" in the text). Normal range 5.0–11.0 μg/dl.
[2] Method A-G, see "Materials and Methods" in the text.

Table 2. *Recovery of T_4 added to human milk in various assays*

Assay method[1]	T_4 concentration in milk (μg/dl)	T_4 added to milk (μg/dl)	Measured value (μg/dl)	Recovery (%)[2]	
				Individual	Mean ± S.D.
Competitive protein-binding analysis					
t A	49.4	3.2	50.4	31	
	49.4	6.3	54.3	78	94 ± 49
	49.4	12.5	65.8	131	
	49.4	25.0	83.3	135	
B	9.3	3.2	19.6	329	
	9.3	6.3	27.9	297	333 ± 24
	9.3	12.5	52.5	343	
	9.3	25.0	>100	>363[3]	
C	8.6	3.2	11.5	92	
	8.6	6.3	13.8	83	93 ± 9.8
	8.6	12.5	21.8	105	
	8.6	25.0	34.0	101	
Radioimmunoassay					
D	<0.7	3.2	7.7	240	
	<0.7	6.3	11.0	176	161 ± 64
	<0.7	12.5	17.6	141	
	<0.7	25.0	22.0	88	
E	<0.7	3.2	4.2	131	
	<0.7	6.3	6.9	112	
	<0.7	12.5	16.6	132	129 ± 13
	<0.7	25.0	34.5	142	
F	<0.7	3.2	4.8	153	
	<0.7	6.3	5.7	90	115 ± 29
	<0.7	12.5	15.3	122	
	<0.7	25.0	23.6	94	
G	<1.5	3.1	3.3	107	
	<1.5	6.2	10.0	160	158 ± 37
	<1.5	12.5	21.7	173	
	<1.5	25.0	48.2	193	

[1] See "Materials and Methods."
[2] In calculation of recovery, undetectable levels were taken as 0 ng/dl.
[3] Percent recovery was more than 363% and in calculation of mean % recovery 363% was taken as recovery.

The results of measuring T_4 concentration in human milk by various methods are summarized in Table 1. Values from 3.2–49.9 μg/dl were obtained by CPBA, but values obtained using RIA were ≤0.7 μg/dl. Recovery data are shown in Table 2. Results varied considerably: mean values ranging from 93–333%. Furthermore, the dilution curves of milk samples were not parallel with the standard curve in any of the three CPBA assays. Results were also unsatisfactory when skimmed milk was used instead of whole milk.

On the other hand, T_3 concentration in milk was consistently measured by RIA. The mean % recoveries of T_3 added to two samples of human milk were 100.7 and 93.7% (Table 3). The dilution curves of milk samples were parallel with the standard curve (Fig. 1). Results of the T_3 concentrations in various samples of human milk are shown in Table 4. All the milk samples obtained 1–4 months postpartum had detectable T_3, but the concentrations were lower than those in serum, and the ratio of serum T_3 to milk T_3 was 2.8 ± 1.7 (mean ± S.D.). No correlation was observed between the T_3 concentration or daily T_3 excretion in milk and the T_3 concentration or free T_3 index in serum. The T_3 concentration in milk was not correlated with protein concentration or daily volume of milk. The total daily amounts of T_3 excreted in milk were estimated as 5–1000 ng.

As shown in Table 5, there were no significant differences in serum levels of thyrotropin, T_4, free T_4 and T_3 between breast-fed and bottle-fed babies.

DISCUSSION

Discrepant results have been reported for the thyroid hormone concentration in human milk. Earlier publications (13, 14) reported T_4 concentrations in milk proportional to those in human serum measured by CPBA (*Method B* in this study). Sack *et al.* (9, 10) reported similar levels of T_4 in milk obtained 8–48 days after delivery, he used a RIA similar to *Method F* in this study. In later studies, little or no T_4 was detected in human milk by RIA (1, 11, 15). These discrepancies are due probably to methodologic differences. The reliability of T_4 and T_3 assay in milk was examined by recovery experiments in only one earlier study (7).

Our study showed that the CPBA methods were unsuitable for the measurement of T_4 in milk because the % recovery of added T_4 varied among the several methods and the dilution curves were not parallel with the standard curves. On the other hand, the reliability of the RIA method was also not clear because the recoveries of T_4 added to milk exceeded 100%, although the recovery of [^{125}I]-T_4 in the ethanol extraction was about 90%. Fatty acid seems to interfere in CPBA but not in RIA (12). The mechanism of positive interference in RIA is not clear. Because T_4 was not detectable in milk by RIA, the data suggest that the T_4 level In milk is very low. This suggestion is in agreement with results of animal experiments, which showed that little T_4 was transferred from the mother to sucklings (8, 16) and with human data showing extremely low levels of butanol-extractable iodine in human milk (5).

Reported data on milk T_3 concentrations also have been conflicting. Bode *et al.* (1) and Varma *et al.* (15) reported higher T_3 levels in milk than in normal serum: measurements were made using ethanol extraction. On the contrary, Sato and Suzuki (11) reported very low levels of T_3 in unextracted milk. The mean values approximated one-tenth the serum concentrations. In the present study, T_3 RIA (double antibody-ANS system) seemed to be reliable for measurement of milk T_3 judging from the recovery and dilution experiments. We used dried samples after extraction with ethanol as reported by Bode *et al.* (1). In earlier reports (1, 10, 15) the reliability of the method for detection of milk T_3 was not examined by recovery experiments; in contrast, we obtained good results in recovery and dilution experiments.

The mechanism(s) of T_3 secretion in milk is not clear. Iodine is known to be actively concentrated by mammary tissue and considerable quantities of iodine are secreted in milk (2, 5, 8). It has been shown that more than 99% of the thyroid hormones in serum are bound to thyroxine-binding proteins. More than 60% of the iodine in rat milk is bound to protein (8). Our preliminary study revealed no detectable TBG in human milk (unpublished data). Furthermore, we found no correlation between the T_3 and protein concentration in human milk. It is unlikely that T_3 is actively concentrated from blood, synthesized from iodine, or derived from T_4 in the mammary gland because the T_3 concentration in milk was lower than that in serum in all samples. Assuming that serum T_3 is transferred to milk by simple diffusion, the milk T_3 concentration might be expected to relate to the T_3 or free T_3 level in serum. To assess this possibility we compared T_3 and free T_3 index levels with milk T_3 concentrations in lactating patients with var-

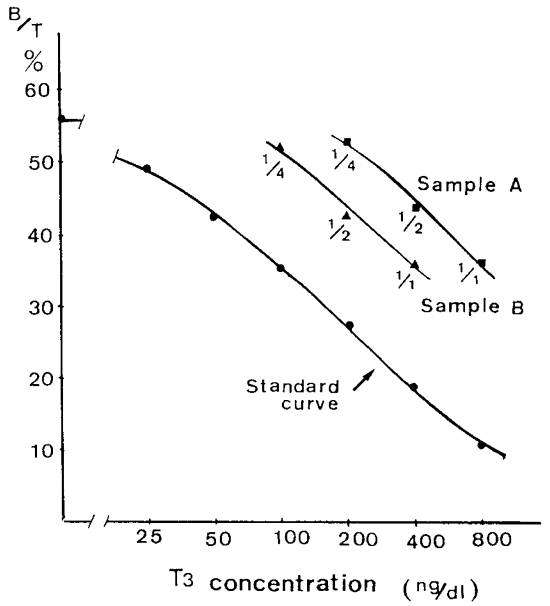

Fig. 1. Standard curve and dilution curve of samples in radioimmunoassay of milk T_3.

Table 3. *Recovery of T_3 added to human milk in radioimmunoassay*

	T_3 Concentration in milk (ng/dl)	T_3 added to milk (ng/dl)	Measured value (ng/dl)	Recovery %	
				Individual	Mean ± S.D.
Sample 1	123	25	144	84	
	123	50	173	100	100.7 ± 12.3
	123	100	229	106	
	123	200	350	113	
Sample 2	146	25	166	80	
	146	50	195	98	93.7 ± 11.5
	146	100	236	90	
	146	200	360	107	

Table 4. *Concentration of T_3 in serum and milk in puerperally lactating patients with thyroid disease*

Case No.[1]	Time of test (postpartum mo.)	Serum concentration[2] T_3 (ng/dl)	Serum concentration[2] Free T_3 index	Milk concentration T_3 (ng/dl)	Milk concentration Protein (mg/dl)	T_3 excretion in milk[3] (ng/day)	Serum T_3 to milk T_3 ratio
1	4	270	381	112	8.0	605	2.4
2	4	368	494	90	8.9	450	4.1
3	2	202	222	26	8.9	156	7.8
4	1½	147	136	72	9.9	396	2.0
5	1	141	116	82	10.1	246	1.7
6	1	140	120	45	15.4	5	3.1
7	3	292	271	47	18.1	33	6.2
8	1	185	178	90	11.2	342	2.1
9	2	125	125	70	11.6	203	1.8
10	1	175	162	95	11.1	380	1.8
11	1½	175	211	111	9.3	200	1.6
12	1	190	183	83	14.3	100	2.3
13	1	150	139	59	8.0	531	2.5
14	3	131	117	60	8.8	300	2.2
15	1	170	146	111	10.7	999	1.5
16	1	160	165	82	9.4	738	2.0

[1] Cases No. 1–10 were patients with Graves' disease, cases No. 11–15 were patients with Hashimoto's disease and case No. 16 was a patient with simple goiter.

[2] Normal range T_3, 110–200 ng/dl; Free T_3 index, 110–188.

[3] Total T_3 excretion in milk per day was calculated as the product of T_3 concentration in milk and the volume of milk secreted in 24 h.

Table 5. *Comparison of serum hormones between breast-fed and bottle-fed babies*[1]

	Breast-fed $n = 24$	Bottle-fed $n = 10$	
Thyrotropin (μU/ml)	6.6 ± 3.0	5.8 ± 3.5	N.S.[2]
T_4 (μg/dl)	11.4 ± 2.4	10.5 ± 2.2	N.S.
Free T_4 (ng/dl)	2.2 ± 0.6	1.9 ± 0.5	N.S.
T_3 (ng/dl)	218 ± 40	215 ± 49	N.S.

[1] Values are mean ± S.D.

[2] Mann Whiteny U test, N.S.: not significant.

ious levels of serum T_3; however, we found no correlation between serum and milk T_3 levels. Daily T_3 excretion in milk also was not related to the serum T_3 concentration. Further studies are necessary to elucidate the mechanism of T_3 excretion in milk.

In contrast with the report by Bode *et al.*, Letarte *et al.* (3) recently reported that the breast feeding had no protective effect on congenital hypothyroidism. In the present study, breast feeding had no influence on the serum levels of thyrotropin and thyroid hormones of normal babies. The adequate dose of T_3 to maintain newborn hypothyroid babies in a euthyroid state was reported to be 50 μg per sq m per day or more (7). We estimate the excretion of T_3 in milk was estimated as less than 1 μg daily. This amount does not seem sufficient to ameliorate the results of mass screening and is adequate to prevent the deleterious effect of congenital hypothyroidism.

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