URINARY EXCRETION OF THYROID HORMONES IN NORMAL HUMAN PREGNANCY

by

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Introduction

The accurate clinical assessment of the thyroid function in human pregnancy is difficult, as physiological changes of pregnancy closely simulate features of early thyrotoxicosis. The increased basal oxygen consumption, heat intolerance, widening pulse pressure and sinus tachycardia are often misinterpreted to represent a "hyperthyroid state of pregnancy". A marked rise in circulating levels of total thyroxine (TT_4) and total triiodothyronine (TT_3) is well documented in normal human pregnancy (Rostogi, *et al*, 1974; Man *et al*, 1969; Chan *et al*. 1975; Osthanondh *et al*, 1976). However, reports on the circulating levels of free T_4 (FT_4) and free T_3 (FT_3), which are the only metabolically active fractions available to the tissues have revealed conflicting data. Increased (Schatz *et al*, 1968) decreased (Sterling and Brenner, 1966 and Mitsuma *et al*, 1972) as well as unaltered (Malkasian and Mayberry, 1970; Osthanondh *et al*. 1976) FT_4 and FT_3 levels have been reported during pregnancy. Since thyroid hormone binding proteins are not excreted in the urine, in the absence of proteinuria, the urinary levels of unconjugated T_4 and T_3 have been claimed to reflect circulating levels of free thyroid hormones (Chan *et al*, 1972; Shakespear and Burke, 1976; Rastogi and Sawhney, 1976). The present communication describes relationship between urinary and plasma levels of T_4 and T_3 as well as thyroidal responsiveness to endogenous thyrotropin (TSH) in normal pregnant women at diflerent periods of gestation.

Material and Methods

Healthy euthyroid pregnant women attending the antenatal clinic of the Nehru Hospital of PGI, Chandigarh were studied. Subjects were selected at random and the duration of pregnancy was determined from the date of last menstrual period. It was ensured that none of the subjects selected had either proteinuria or any other evidence of impaired renal, hepatic or endocrine function. Age matched healthy euthyroid non-pregnant women drawn from amongst the hospital and laboratory staff served as controls. None of the subjects investigated received

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medications viz. salicylates, penicillin, dilantin or steroidal centraceptives which are known to alter thyroid hormone binding to binding proteins.

Blood samples were collected from the forearm vein, sera were separated and stored at-20°C till assayed. Urine was collected separately for two consecutive twenty four hours. Completeness of the urinary collection was ensured by interrogation of each subject and urinary creatinine estimations. A 20 ml aliquot was stored at — 20°C using saturated sodium carbonate as a preservative. To assess the sensitivity of the hypothalamic-pituitary-thyroid axis, thyrotropin releasing hormone (TRH) test was performed in 10 pregnant women in late pregnancy (32 to 38 weeks) and in age matched non-pregnant women. Twenty-four hour urine was collected for T_4 and T_3 estimations the day before, on the day of TRH administration (100 ug/iv) and the day after. To monitor rise in serum TSH, blood samples were also obtained before, and 20 and 60 minutes after TRH administration.

Serum and urinary levels of T_4 and T_3 were estimated using specific radioimmunoassay (RIA) techniques (Rastogi and Sawhney, 1976). Upto 100 ng/tube of 3,5-diiodo-l-thyronine (T_2) and 3',5' triiodothyronine (reverse T_3, rT_3) showed no significant displacement of ^{125}I-T_4 or ^{125}I-T_3 in their respective RIAs Lack of significant cross reaction by other thyroid hormone analogues and metabolites has been reported previously (Rastogi and Sawhney, 1976). The inter and interassay variabilities at three different points were less than 10%. Free T_4 (FT_4) was measured by the method of Sephadex binding (Levinson and Reider, 1974). Same methodology was utilized to measure free T_3 (FT_3). The renal clearance of T_3 and T_4 were calculated by the following formula:

$$\frac{\text{Urine excretion ml/min X Urinary concentration of the hormone}}{\text{Plasma concentration of unbound hormone}}$$

Circulating levels of TSH were estimated by a double antibody RIA technique (Kannan *et al*, 1973). Anti TSH guinea pig serum was obtained as a gift from Prof. R. Fraser Hammersmith Hospital, London, U.K. Purified hTSH for radioiodination was generously supplied by the NPA, NIAMDD, Betheseda, Md. hTSH standard (68/38) was kindly supplied by the NIMR. London, U.K. In separate experiments ability of different hormones to displace ^{125}I-TSH from its antisera was also evaluated. hLH (MRC 68/38), FSH (hFSH, MRC 69/39), hCG (IRP2) and human placental lactogen (hPL, NIH) did not significantly interfere with TSH measurements. Addition of human chorionic gonadotropin (hCG) as high as upto 200 iu/ml did not significantly displace ^{125}I-TSH from its antiserum.

Results

Table I shows serum and urinary levels of T_4 and T_3 in control and pregnant women. In 10 normal women the mean excretion of T_4 and T_3 were 1.39 ± 0.09 ug/24h and 0.78 ± 0.11 ug/24h respectively. Inspite of elevated serum TT_4 and TT_3 levels, the urinary excretion of T_4 and T_3 in different trimesters of pregnancy was not significantly different ($P > 0.05$) from that of non-pregnant women.

Table II shows renal clearance of T_4 and

TABLE I

Mean ± SE and Range in Parenthesis of Serum and Urinary T_4 and T_3 During Pregnancy and in Control Women

	N	Serum levels of		Urinary excretion of	
		T_4 μ/dl	T_3 ng/dl	T_4 ug/24h	T_3 ug/24h
CONTROLS (C)	10	8.7 ± 0.2 (6.8-10.0)	131 ± 4 (100-180)	1.39 ± 0 09 (0.95-1.8)	0.78 ± 0.11 (0.44-1.8)
PREGNANCY (PG)					
1st Trimester	10	15.9 ± 0.3 (12-18)	171 ± 13 (100-240)	1.59 ± 0.09 (1.15-1.94)	0.81 ± 0.034 (0.58-0.95)
2nd Trimester	10	17.6 ± 0.8 (12-22)	190 ± 9 (160-240)	1.6 ± 0.06 (1.35-1.97)	0.8 ± 0.05 (0.53-1.13)
3rd Trimester	10	17.0 ± 0.6 (14-20)	270 ± 29 (180-440)	1.44 ± 0.11 (0.81-2.08)	0.85 ± 0.066 (0.56-1.28)
P C Vs PG		<0.01	<0.01	>0.05	>0.05

T_3 in control and pregnant women. The mean renal clearance of T_4 and T_3 in 20 pregnant women were 36 ± 1.9 ml/minute (range 18.4-54.1) and 159 ± 10.8 ml/minute (101.6-254) respectively. These values were not significantly different ($P > 0.05$) than the mean value of 34.5 ± 1.67 ml/minute (23.3-51.7) and 169.3 ± 11.4 ml/minute (100-282) in non-pregnant women. A positive correlation was forthcoming between serum free T_3 and urinary T_3 ($r = + 0.92$, $Y = 1.31 \times + 0.23$, $P < 0.01$) as well serum free T_4 and urinary excretion of T_4 ($r = + 0.88$, $Y = 0.72 x - 0.38$, $P < 0.01$) in pregnant women, Although basal TSH levels were slightly but significantly increased ($P < 0.01$) during pregnancy, the peak, the overall TSH response as reflected by the sum of serum TSH levels of 20 and 60 minutes as well as maximum rise over the basal (ΔTSH) were not significantly different ($P > 0.05$) than those of control women.

Administration of 100 ug of TRH intravenously consistently increased serum TSH and there was a significant increase ($P < 0.01$) in the urinary excretion of both T_4 and T_3 on the day of TRH administration. The peak as well as maximum rise over the basal levels (ΔT_4 or ΔT_3) in pregnant women was not significantly different ($P > 0.05$) than those of control subjects.

Discussion

The present study demonstrated that inspite of elevated circulating levels of TT_4 and TT_3, the urinary excretion of T_4 and T_3 as well as serum levels of free T_4 and T_3 remained unaltered during normal human pregnancy. Since the thyroid hormones binding proteins are not excreted in the urine, in the absence of proteinuria urinary excretion of unconjugated T_4 and T_3 would be an index of circulating levels of free thyroid hormones. This was infact revealed by the observation of a positive correlation between serum free levels and urinary excretion of thyroid hormones. Although both T_4 and T_3 excretion per 24 hour bear a good correlation with T_4 and T_3 values expressed per

TABLE II

Showing Mean ± SE of Renal Clearance of T_4 and T_3 in Pregnant and Non-pregnant Women

	Urine excretion	Urinary levels of		Serum levels of		Renal clearance of	
	ml/min	T_4 ng/ml	T_3 ng/ml	FT_4 pg/ml	FT_3 pg/ml	T_4 ml/min	T_3 ml/min
Controls (C)	0.82 ± 0.07	1.97 ± 0.18	0.96 ± 0.07	40.4 ± 2	4.6 ± 0.33	34.5 ± 1.67	169.3 ± 11.4
n = 20	(0.45 — 1.6)	(0.8 — 3.5)	(0.58 — 1.6)	(24.0 — 59)	(2.9 — 8.3)	(23.3 — 51.7)	(100 — 282)
Pregnancy (PG)	0.79 ± 0.044	1.48 ± 0.09	0.91 ± 0.2	34.7 ± 0.66	4.6 ± 0.29	36.0 ± 1.9	159 ± 10.8
n = 22	(0.55 — 1.4)	(0.84 — 2.6)	(0.6 — 1.2)	(21.6 — 62.5)	(2.7 — 8.2)	(18.4 — 54.1)	(101.6 — 254)
P C Vs PG	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

gram of creatinine, the former way of expressing the results was preferred in view of the fact that urinary creatinine excretion is altered during pregnancy and thus might introduce an another variable (Hytten and Leitch, 1972).

Available reports on the effect of pregnancy on FT_4 and FT_3 levels have revealed conflicting data. Increased (O'Halloran, 1966 and Schatz *et al* 1968), decreased (Ingbar. *et al* 1965; Sterling and Brenner, 1966) as well as normal levels (Malkasian and Mayberry, 1970 and John *et al* 1973) have been reported during pregnancy. FT_4 was measured by equilibrium dialysis technique at temperatures ranging from 4-37°C (Oppenheimer *et al* 1963; Sterling and Brenner, 1966 and Schussler and Plager, 1967), in buffers with a wide range of ionic strength (Gordon and Coutsoftides, 1969; Spaulding and Gregerman, 1972) and pH (Oppenheimer *et al*, 1963; Sterling and Brenner, 1966; Schussler and Plager, 1967 and Fang and Selenkow, 1970). Temperature (Tabachnick, 1966; Raz and Goodman, 1969), pH (Oppenheimer, 1968 and Gordon and Coutsoftides, 1969) and ionic strength (Gordon and Coutsoftides, 1969 and Spaulding and Gregerman, 1972) of the medium have been shown to considerably affect the binding of thyroid hormones to binding proteins. Similarly, both decreased (Mitsuma *et al*, 1972) as well as normal (Osthanondh *et al* 1976) FT_3 levels have been reported during pregnancy. Like FT_4 the measurement of FT_3 is also influenced by the temperature, ionic strength and pH of the buffer (Oppenheimer, 1968; Gordon and Coutsoftides, 1969 and Spaulding and Gregerman, 1972). Thus non-physiological assay systems will give FT_4 values which might differ to a large extent from the

in vivo concentration. In the present study FT_4 and FT_3 levels were measured at room temperature (approx. 20°C) at physiological pH (7.5). Although TT_4 and TT_3 levels were increased during pregnancy the FT_4 and FT_3 levels available to the tissues remained unaltered.

There is paucity of data on renal handling of T_4 and T_3. Both T_4 as well as T_3 are excreted in the urine in three forms, unconjugated, as glucuronide and sulphate conjugates and as metabolities. Only the unconjugated one reflects the serum unbound moieties by analogy with steroid hormones (Burke, 1974). To our knowledge this is the first report on renal clearance of T_4 and T_3 in pregnancy. Our values reported for non-pregnant women for renal clearance of T_4 and T_3 are in close agreement to the values reported by Burke and Shakespear, 1976. The renal clearance of both T_4 as well as T_3 remained unaltered during gestation.

It is difficult to define the exact relation between elevated levels of TT_4 or TT_3 on the one hand and slight but significant increase in TSH levels on the other hand during pregnancy. Therefore, a certain degree of insensitivity of the thyroid to circulating TSH was suggested (Rastogi *et al* 1974). The TRH stimulation test was performed in late pregnancy when increase in serum levels of TSH, T_4 and T_3 was maximum. The observations that magnitude of increase in serum TSH and urinary excretion of T_4 and T_3 after TRH administration was essentially similar in pregnant and non-pregnant women, suggested that responsiveness of the pituitarythyroid axis to appropriate stimulation during normal human pregnancy remained unaltered.

Summary

Alterations in urinary levels and renal clearance of thyroxine (T_4) and triiodothyronine (T_3) and sensitivity of the pituitary-thyroid axis were monitored in normal human pregnancy. In 10 non-pregnant women the mean excretion and renal clearance of T_4 and T_3 were 1.39 ± 0.09, SE ug/24h, 0.78 ± 0.11 ug/24h and 34.5 ± 1.67 ml/min and 169 ± 11.4 ml/min respectively. The mean values of these parameters in pregnant women were not significantly different ($P > 0.05$) than that of controls. A positive correlation between serum free T_4 and urinary T_4 ($r = + 0.88$, $P < 0.01$) and serum free T_3 and urinary T_3 ($r = + 0.9$ $P < 0.01$) was recorded in pregnant women. Administration of TRH (100 ug iv) consistently raised urinary excretion of both T_4 and T_3 in all the women. However, the peak as well as maximal rise over the basal level (ΔT_4 and ΔT_3) in pregnant women was not significantly different ($P > 0.05$) than those of control subjects.

It is suggested that urinary excretion and renal clearance of T_4 and T_3 as well as sensitivity of pituitary-throid axis remains unchanged during the gravid state.

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