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STUDIES ON CIRCADIAN VARIATIONS OF PLASMA TSH, THYROXINE AND TRIIODOTHYRONINE IN MAN

By

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ABSTRACT

To evaluate the existence of circadian variations in thyroid hormone and TSH levels, blood was drawn every 20 min for 24 h in four and for 14 h in one volunteer. Hormones were measured by sensitive radioimmunoassays.

TSH: A diurnal rhythmicity could be demonstrated with peaks from 8 p. m. to 2 a. m. and a nadir from 7 a. m. to 2 p. m. Superimposed on the diurnal rhythm multiple shortlived fluctuations were observed.

Thyroxine: Pooled data showed peak values from 8 a. m. to 12 a. m. and lowest levels from 11 p. m. to 3 a. m. Again, shortlived fluctuations were superimposed on the diurnal rhythm in all instances.

Triiodothyronine: Hormone levels were highest from 7 a. m. to 1 p. m. and lowest from 11 p. m. to 3 a. m. Fluctuations in T_3 levels were less marked than those of T_4.

Diurnal variations in hormone levels can be demonstrated in most, but not in all instances. Fluctuations are minor and do not exceed the normal range. Therefore these changes are of no relevance in routine testing of these hormones.

There is ample evidence for the existence of diurnal secretory rhythmicity for various hormones (see *Weitzman* 1974 for review). Apparently, diurnal fluctuations of several hormones (GH, prolactin) are sleep-related, while no mechanism

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of this kind is known for other hormones like ACTH and cortisol, testosterone and others.

Until recently there was no evidence of diurnal rhythmicity of the thyroid hormones (*Schatz & Volpé* 1959; *Lemarchand-Béraud & Vannotti* 1969) and their trophic hormone TSH (*Utiger* 1965; *Odell et al.* 1967; *Hershman & Pittman* 1971; *Webster et al.* 1972). Improved assay techniques, however, indicated the existence of minor fluctuations of TSH (*Nicoloff* 1970; *Vanhaelst et al.* 1972; *Patel et al.* 1972; *Weeke* 1973; *van Cauter et al.* 1974) as well as thyroxine (*de Costre et al.* 1971; *Vernikos-Danellis et al.* 1972; *O'Connor et al.* 1974; *Balsam et al.* 1975). Recently these fluctuations have been shown to exist in patients with hypothyroidism, too (*Weeke & Laurberg* 1976).

There is considerable difference in results, probably due to different assay techniques as well as infrequent sampling of blood. The present study was performed to re-evaluate this matter using sensitive radioimmunoassays (RIA) as well as frequent sampling of specimens. In addition, fluctuations of thyroid hormones and TSH were determined in the same group of persons and correlated with each other.

MATERIAL AND METHODS

Studies were performed in five healthy, fully informed male volunteers 22 to 27 years of age. They came to the laboratory at 1 p. m.: a Branula® cannula was inserted into an antecubital vein to obtain specimens without disturbance. Starting at 2 p. m. about 5 ml of blood were drawn every 20 min. Volunteers were allowed to entertain themselves reading, looking television and playing cards. They slept from about 23.00 to 6.30 h. One person developed a local phlebitis close to the area of venipuncture. His study was terminated after 14 h.

The specimens were centrifuged immediately and kept frozen at –20°C until assayed. TSH was determined by RIA as previously described (*von zur Mühlen & Emrich* 1971). T_4 and T_3 were measured by RIA (*Hesch et al.* 1974). The intraassay variance was less than 5 % for TSH, 4.1 % for the T_3-assay and 7.1 % for the T_4-assay.

All specimens from one volunteer were measured in one assay. For simplicity of the graphs, hormone values from one person obtained within one hour were pooled and given as means with standard deviations (± SEM).

F-tests were used to calculate for the presence of diurnal variations. In addition, mean values of hormone acrophases were compared with means of the nadir phases (paired *t*-test).

RESULTS

TSH: In five subjects TSH-levels ranged from 4.98 ± 0.04 to 6.14 ± 0.07 μU/ml (± SEM) (Table 1). Fig. 1 demonstrates pooled data from these persons. TSH-levels are maximal from about 20.00–2.00 and lowest from about 7.00 to 14.00. Comparing the acrophase with the lowest phase, levels were significantly higher at night in 3 of 4 volunteers (paired *t*-test; Table 1).

Table 1.

Mean levels of TSH, T_4 and T_3 in five subjects. Acrophases and nadirphases represent periods of elevated or suppressed mean values of all five subjects. Not in all instances were individual peaks or nadirs seen at these times, explaining discrepancies in *P*-values and F-tests.

	24 h-mean (± SEM)	Acrophase (20.00–2.00)	Nadir-phase (7.00–13.00)	*P*	F-test
		TSH (μU/ml)			
I	6.14 ± 0.07	6.60 ± 0.11	5.63 ± 0.06	< 0.001	0.01
II	5.49 ± 0.10	6.42 ± 0.18	5.09 ± 0.10	< 0.001	0.01
III	5.59 ± 0.06	5.95 ± 0.39	5.64 ± 0.38	< 0.01	N. S.
IV	5.42 ± 0.04	5.42 ± 0.08	5.47 ± 0.08	N. S.	0.01
V	4.98 ± 0.04				

Thyroxine (μg/ml)

	24 h-mean (± SEM)	Nadir-phase (23.00–3.00)	Acrophase (8.00–12.00)	*P*	F-test
I	6.57 ± 0.10	6.06 ± 0.12	7.42 ± 0.29	< 0.01	0.01
II	6.62 ± 0.08	6.45 ± 0.15	6.65 ± 0.16	N. S.	0.05
III	5.03 ± 0.06	4.49 ± 0.09	5.08 ± 0.10	< 0.05	0.01
IV	5.33 ± 0.06	5.32 ± 0.15	5.42 ± 0.10	N. S.	N. S.
V	5.35 ± 0.13				

Triiodothyronine (ng/ml)

	24 h-mean (± SEM)	Nadir-phase (23.00–3.00)	Acrophase (8.00–12.00)	*P*	F-test
I	1.17 ± 0.01	1.17 ± 0.03	1.14 ± 0.03	N. S.	0.01
II	1.65 ± 0.01	1.65 ± 0.04	1.67 ± 0.03	N. S.	0.01
III	1.29 ± 0.01	1.21 ± 0.02	1.34 ± 0.01	< 0.001	N. S.
IV	1.12 ± 0.01	1.22 ± 0.08	1.18 ± 0.02	N. S.	0.05
V	0.72 ± 0.02				

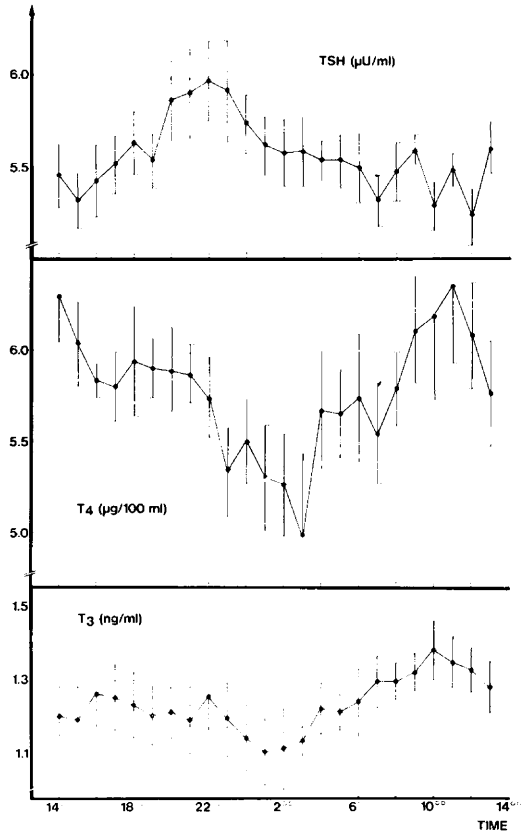

Fig. 1.
Mean levels of TSH, T_4 and T_3 in a 24 h period. A TSH acrophase can be seen between 20.00 and 2.00 and a nadir daytime. T_3 and T_4 levels were lowest from 23.00 to 3.00 and highest from about 8.00 to noon.

Superimposed on the diurnal rhythm multiple shortlived fluctuations in TSH-levels were observed in all instances. These peaks were seen even in the absence of a diurnal rhythm.

Thyroxine: T_4-levels ranged from 5.03 ± 0.06 to 6.62 ± 0.08 μg/100 ml ($\pm$ SEM) (Tabl 1). Pooled data from all volunteers are shown in Fig. 1; there is a definite diurnal rhythm with maximal levels from 8.00 to 12.00 and low levels from about 23.00 to 3.00. While daytime levels were higher in all instances, this difference reached statistical significance in only two persons (Table 1). A diurnal rhythm – using the F-test – could be demonstrated in three volunteers. Again, superimposed on the diurnal rhythm, shortlived elevations of

T_4 were observed in all instances. These fluctuations usually lasted no longer than two hours. Fig. 2 demonstrates the diurnal rhythm as well as shortlived fluctuations.

Triiodothyronine: T_3 levels ranged from 0.72 ± 0.02 to 1.65 ± 0.01 ng/ml (± SEM) (Table 1). Pooled data are shown in Fig. 1. T_3 reached maximal levels from 7.00 to 13.00 and was at minimum from 23.00 to 3.00. Again daytime levels are higher than those at night, but the difference was significant in only one person. Using the F-test, a diurnal rhythm could be demonstrated in 3 persons. Shortlived fluctuations in T_3-levels were superimposed on the diurnal rhythm (Fig. 3). Frequently, they paralleled those of T_4.

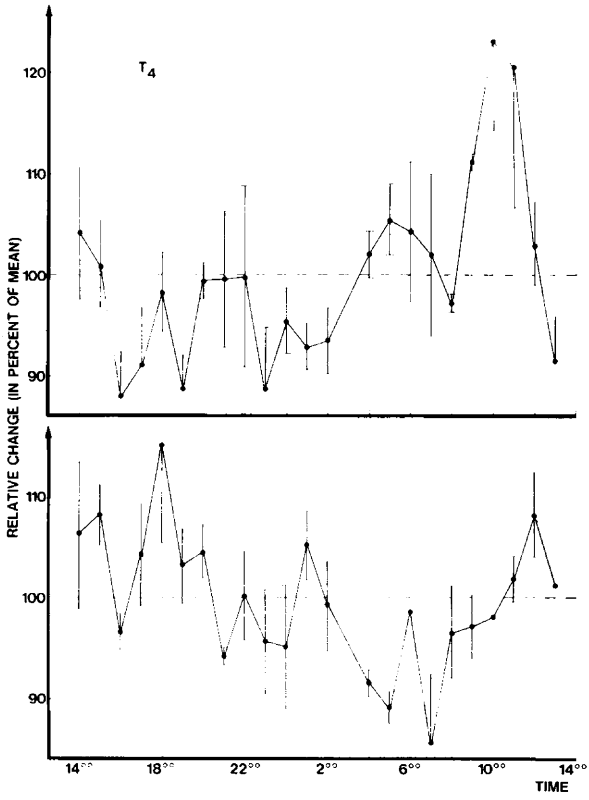

Fig. 2.
Plasma T_4 levels during a 24 h period in two subjects. Note episodic secretion of the hormone. Individual curves do not obviously follow one secretory pattern.

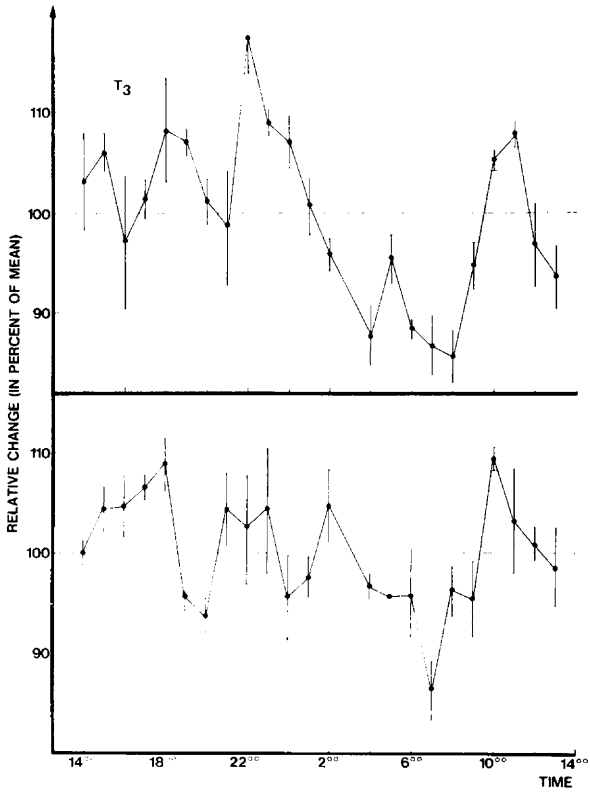

Fig. 3.
Plasma T_3 levels during a 24 h period in two subjects. Note episodic secretion of the hormone. Hormones are lowest at nighttime.

DISCUSSION

A number of earlier studies failed to demonstrate the presence of diurnal variations in TSH levels. This seems to be due to two factors: lower sensitivity of the assay and infrequent sampling of blood. Recent data quite clearly demonstrate the presence of the diurnal rhythm (*Patel et al.* 1972; *Vanhaelst et al.* 1972; *Weeke* 1973; *van Cauter et al.* 1974), and these data are supported by our findings.

In agreement with reported data we found a TSH acrophase at night and a nadir in the morning. However, we observed peak values between 20.00 and 24.00, while *Patel et al.* (1972) found highest levels between 2.00 and 4.00, *Vanhaelst et al.* (1972) between 4.00 and 6.00, and *van Cauter et al.* (1974) between 3.00 and 8.00. This discrepancy may be explained by the relatively

minor fluctuations observed and the small number of persons studied. Still, from our data and the studies mentioned, it may be concluded, that nighttime values are higher than those during the day.

The mechanisms responsible for diurnal fluctuations in TSH levels are unknown. Probably the nocturnal rise is not sleep-related, as TSH levels increase before the onset of sleep (*Patel et al.* 1972; *Parker et al.* 1976). Using electroencephalographic monitoring of sleep, *Parker et al.* (1976) observed pre-sleep peak values as well as an inhibitory influence of sleep on the secretion of TSH. The similarities in mean 24 h TSH patterns seen by these authors with those reported in this study are striking. In addition, our data seem to support their view of an inhibitory influence of sleep on TSH levels (Fig. 1). *Nicoloff* (1970) suggested that TSH secretion might be regulated by cortisol levels, with inversed relation in TSH and cortisol levels. Recent studies by *van Cauter et al.* (1974) do not favour this hypothesis and imply that TSH and ACTH are secreted rather independently. At present it seems best to postulate independent mechanisms responsible for the fluctuations of both hormones.

Similarly to TSH, early attempts to demonstrate diurnal variations in thyroxine levels were unsuccessful. Several recent studies clearly indicate the presence of diurnal fluctuations in T_4-levels. Using sensitive RIA systems as well as a frequent blood sampling technique, *O'Connor et al.* (1974) found higher T_4 concentrations during wakefulness than during sleep. *Balsam et al.* (1975) observed peak values at 9 a. m. and lowest levels from 5 to 7 p. m.; *de Costre et al.* (1971) found highest levels at noontime and lowest levels at 2 a. m., while we found peaks from 9 to 12 a. m. and lowest levels at 2 a. m. Recently, extensive studies of *Azukizawa et al.* (1976) failed to reveal any persistent fluctuation in thyroid hormone levels. In some of their subjects, however, long term variation suggested the existence of a 24 h cyclic rhythm. Differences in results may be explained in part by a different assay techniques (RIA, PBI) as well as the frequency in blood sampling. Also, it should be kept in mind that diurnal fluctuations are relatively small and superimposed on this rhythm numerous short-lived elevations in T_4-levels of various amplitude are observed. Therefore, highest values may occur at different times, but predominantly during daytime.

The long halflife of the hormone excludes the possibility that diurnal fluctuations of T_4 are due to variation in secretion. *de Costre et al.* (1971) demonstrated T_4 fluctuations closely paralleling total protein, haematocrit, PBI, TBG and prealbumin. Moreover, a reversed cyclic fluctuation could be attained reversing the normal sleep-waking pattern. They concluded that changes in T_4 concentrations represent no cyclic secretory patterns, but are caused by cyclic movements of fluids into and out off the vascular compartment. It is of interest, that the biologic active hormone – free thyroxine – showed no cyclic variations. Hence, there is little reason to believe that fluctuations in TSH secretions

might be triggered by changes in T_4 levels, as the active form shows no fluctuation.

Summarizing our data as well as recent studies mentioned above, some diurnal fluctuation in thyroid hormone levels can be observed in most, but not in all subjects. The underlying mechanism remains unknown, but most likely is not thyroidal in origin.

In contrast to age dependent changes in thyroid hormone levels (*Hesch et al.* 1977), diurnal fluctuations are minor and do not exceed the normal range of healthy persons. Therefore these ranges are of no relevance in routine testing of thyroid hormones.

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