JOURNAL OF APPLIED PHYSIOLOGY
Vol. 39, No. 2, August 1975. *Printed in U.S.A.*

# Circadian variations in concentrations of plasma thyroxine and triiodothyronine in man

ALAN BALSAM, CARROLL R. DOBBS, AND LYNN E. LEPPO
*Biochemical Assessments Branch, Environmental Sciences Division, USAF School of Aerospace Medicine, Brooks Air Force Base, Texas 78235*

BALSAM, ALAN, CARROLL R. DOBBS, AND LYNN E. LEPPO. *Circadian variations in concentrations of plasma thyroxine and triiodothyronine in man.* J. Appl. Physiol. 39(2): 297–299. 1975.—The plasma concentrations of thyroxine ($T_4$), triiodothyronine ($T_3$), and total protein (Pr) were measured at 2-h intervals in 8 male subjects during two 24-h periods. Plasma $T_4$ and $T_3$ levels varied significantly during the day. $T_4$ values were highest at 0900 hours and thereafter declined rapidly reaching lowest levels at 1500–1700 hours (mean decrement, 13.2% of 0900-hour value). Plasma $T_3$ was highest at 0900 hours and lowest at 1700–1900 hours (mean decrement, 16.7% of 0900-hour value). Fluctuations observed in Pr were not significant. Variations in plasma $T_4$ and $T_3$ appeared concordant with respect to time, since no significant variation was detected in $T_3$:$T_4$ plasma concentration ratios. In view of previous studies that have demonstrated circadian variations in the binding of thyroid hormones by plasma proteins, it is suggested that the observed temporal variations in plasma concentrations of $T_3$ and $T_4$ reflect parallel changes in the capacity or affinity of specific plasma binding proteins of these iodothyronines.

plasma iodothyronine rhythms; thyroid hormones

CONFLICTING DATA have been reported regarding the existence of circadian rhythmicity in the plasma concentration of thyroxine ($T_4$) (3, 4, 6, 10, 15, 16), and little information is available regarding daily fluctuations in the plasma concentration of triiodothyronine ($T_3$). Recent studies have suggested that circulating $T_4$ and $T_3$ originate largely from dissimilar sources (2, 9, 11, 12, 14). Whereas $T_4$ is secreted by the thyroid gland, $T_3$ appears to be derived principally from the deiodination of $T_4$ by peripheral tissues. The current study was designed to evaluate *1*) whether periodic changes in the levels of $T_4$ and $T_3$ could be detected using assay techniques considered specific for these iodothyronines, *2*) interrelationships between circadian variations in plasma levels of $T_4$ and $T_3$, and *3*) the correlation between fluctuations in plasma levels of iodothyronines and plasma total protein concentration, an index of plasma volume alterations.

## MATERIALS AND METHODS

Eight male volunteers aged 18–21 yr participated in a study to assess the levels of circulating $T_4$ and $T_3$ during two 24-h periods. The subjects were housed in a metabolic unit during the study. Room temperature was maintained at 70°F, and overhead lights were turned on at 0700 and off at 2300. Participants were served a regular diet consisting of three meals, with unrestricted fluid intake, and were allowed out of bed during the study between 0700 and 2300. At 0800 indwelling intravenous butterfly units were inserted in the forearm and maintained patent with small quantities of heparinized saline. Heparinized blood samples were obtained from the 8 subjects at 2-h intervals from 0900 through 0700 of the following day. Blood was sampled from a three-way stopcock attached to the intravenous unit using a two-syringe technique. A small quantity of blood was first withdrawn and discarded to clear the contents of the tubing; then 10 ml of heparinized blood were withdrawn in a second syringe. Plasma specimens were promptly frozen for subsequent analysis. The entire sequence of blood sampling was repeated 48 h after completion of the first study. The plasma concentration of triiodothyronine was measured by radioimmunoassay[1] (13): $T_4$ iodine, by competitive protein binding assay at Bio-Science Laboratories, Van Nuys, Calif.; and total protein, by the Biuret method. All determinations were performed in duplicate. An approximate three-factor analysis of variance procedure was used to analyze the data for each measurement separately (8). The factors were subjects (8), periods (2 days), and times of day (12). Both subjects and periods were considered random effects since a repetition of the experiment would bring in new randomly selected subjects to be observed in randomly selected periods.

## RESULTS

Representative data are presented in Table 1 for each measurement. The highest plasma $T_4$ mean concentration occurred at 0900 (Fig. 1) and was significantly higher than each of the remaining eleven means ($P < 0.05$). A progressive decline in circulating $T_4$ was noted during the day, reaching a nadir at 1500–1700 hours. The 1700 hour mean was significantly different from all other means ($P < 0.05$) except the means of 1500, 1300, 0500, and 0700. The highest plasma $T_3$ mean concentration occurred at 0900 (Fig. 2) and was significantly higher than each of the remaining eleven means ($P < 0.05$) except for those observed at 1100 and 0300 ($P > 0.05$). A progressive decline in circulating $T_3$ was noted during the day reaching a nadir at

[1] Measured by Drs. M. I. Surks and J. H. Oppenheimer.

1700–1900. No significant difference was observed among the Pr means for the twelve times (Fig. 3). To assess the effects of plasma volume fluctuations, iodothyronine concentrations were indexed to the concentration of Pr. $T_3$:Pr concentration ratios exhibited significant circadian periodicity ($P < 0.05$), with highest measurements at 0900 and lowest at 1700–1900. $T_4$:Pr concentration ratios varied significantly during the day; highest levels occurred at 0900 and lowest levels between 1500 and 1700. Plasma $T_3$ and $T_4$ kept pace with each other throughout the 24-h period; no significant differences were noted in the $T_3$:$T_4$ concentration ratios.

## DISCUSSION

The present study demonstrates significant circadian variations in the plasma concentrations of thyroid hormones. A number of earlier studies failed to demonstrate significant fluctuations of the circulating protein-bound iodine (PBI) during the day (4, 10, 16). Walfish and co-workers (16) detected a decreased plasma disappearance rate of [$^{131}$I]$T_4$ between 0200 and 0800 despite no change in the serum PBI,

TABLE 1. *Variations in plasma $T_4$, $T_3$, and Pr in subject 8**

| Day 1 | | | | Day 2 | | | |
|---|---|---|---|---|---|---|---|
| Hour | $T_4$ | $T_3$ | Pr | Hour | $T_4$ | $T_3$ | Pr |
| 0900 | 4.35 | 148 | 7.782 | 0900 | 4.70 | 142 | 6.235 |
| 1100 | 3.85 | 142 | 7.345 | 1100 | 4.40 | 134 | 6.759 |
| 1300 | 3.65 | 131 | 7.927 | 1300 | 4.40 | 143 | 8.164 |
| 1500 | 3.30 | 127 | 8.000 | 1500 | 4.40 | 134 | 8.055 |
| 1700 | 3.55 | 123 | 7.655 | 1700 | 4.30 | 127 | 6.813 |
| 1900 | 3.55 | 124 | 7.564 | 1900 | 4.60 | 125 | 7.066 |
| 2100 | 3.55 | 128 | 7.836 | 2100 | 4.50 | 125 | 6.723 |
| 2300 | 3.40 | 104 | 7.509 | 2300 | 4.20 | 133 | 6.578 |
| 0100 | 3.55 | 111 | 7.102 | 0100 | 4.55 | 142 | 6.578 |
| 0300 | 3.70 | 111 | 6.651 | 0300 | 4.30 | 139 | 6.380 |
| 0500 | 3.65 | 95 | 6.361 | 0500 | 4.10 | 137 | 6.687 |
| 0700 | 3.45 | 109 | 6.759 | 0700 | 4.45 | 119 | 6.163 |

* Plasma measurements of male subject during two 24-h periods. $T_4$, thyroxine iodine; $T_3$, total triiodothyronine; Pr, total protein.

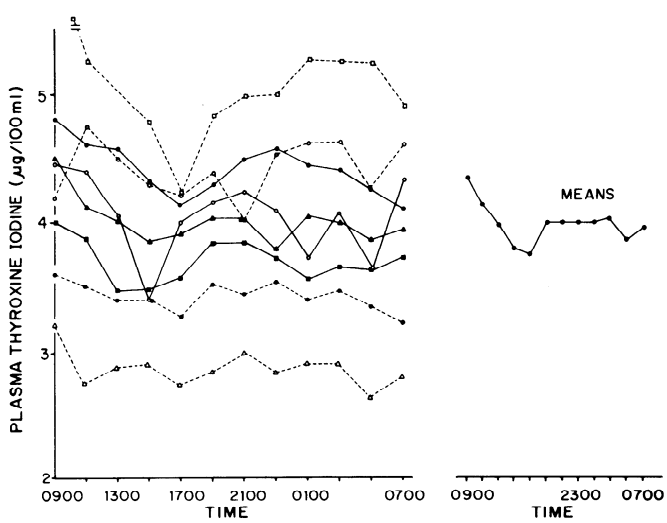


FIG. 1. Plasma concentrations of thyroxine iodine in 8 subjects (average measurements during two 24-h periods). Error term = 0.08586 with 9.5 degrees of freedom (Satterthwaite's approximation).

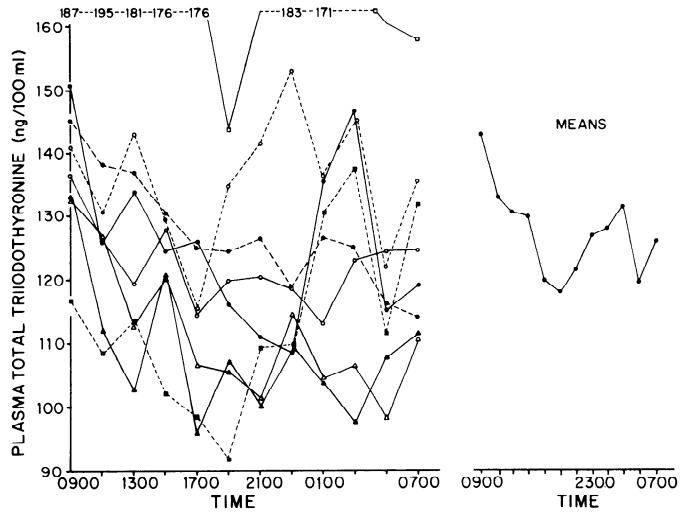


FIG. 2. Plasma concentrations of total triiodothyronine measured as described in legend to Fig. 1. Error term = 126.8 with 3.4 degrees of freedom (Satterthwaite's approximation).

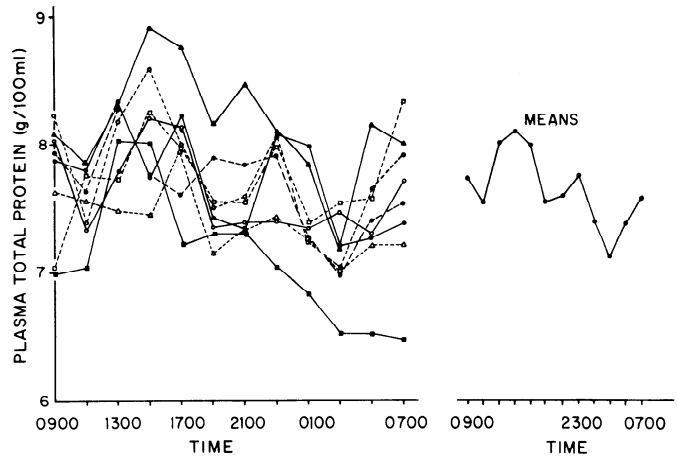


FIG. 3. Plasma concentrations of total protein measured as described in legend to Fig. 1. Error term = 0.6724 with 9.6 degrees of freedom (Satterthwaite's approximation).

a measurement that reflects largely circulating $T_4$. The increased nocturnal concentration of [$^{131}$I]$T_4$ was attributed to plasma volume contraction since a similar slowing in the rate of disappearance of [$^{131}$I]albumin from plasma during this period was documented. In contrast to earlier reports of absent diurnal variation of the serum PBI, Vernikos-Danellis et al. (15) reported significant circadian fluctuations in plasma levels of thyroxine measured by competitive protein-binding assay. $T_4$ levels were assessed at 4-h intervals with maximal levels occurring at 0730. The magnitude of the maximal variation in plasma $T_4$ detected by Vernikos-Danellis was approximately 10–20%. O'Connor and co-workers (6) observed slightly higher mean plasma $T_4$ concentrations during wakefulness than during sleep in a group of four euthyroid men.

The determinants of the plasma concentration of total $T_4$ and $T_3$ are threefold: *1*) secretion or production, *2*) degradation, and *3*) binding by plasma proteins. The scope of the present study does not permit rigorous separation of these determinants as etiological factors in the observed circadian variations in plasma hormone levels. Nevertheless, it appears likely that the plasma $T_4$ and $T_3$ fluctuations

are related to alterations in the capacity or affinity of the binding proteins for the hormones. Vernikos-Danellis et al. (15) noted increased serum binding of tracer $T_3$ concomitantly with the early morning elevation of plasma $T_4$. Lemarchand-Beraud and Vannotti (4) observed a circadian rhythm in hormonal binding by plasma proteins. Assessing plasma thyroxine binding by equilibrium dialysis, these investigators observed that the dialyzable fraction of $T_4$, which is inversely related to the strength of $T_4$ binding by plasma, was lowest in the early morning and significantly higher at 1600 hours. The basis of changes in plasma hormone binding is uncertain. In the present study simultaneous monitoring of plasma $T_3$ and $T_4$ concentrations and plasma total protein concentration, a commonly accepted index of plasma volume changes, suggested that iodothyronine variations were not attributable to plasma volume alterations. Significant variations were detected in $T_4$:Pr and $T_3$:Pr concentration ratios. Although the principal binding protein of $T_4$ and $T_3$, thyroxine-binding globulin, is a minor constituent of the total plasma protein, hydrational changes in the plasma compartment would be expected to influence the concentrations of specific binding protein and total protein in a similar manner.

Changes in hormone secretion, production, or degradation would appear to be much less likely explanations of the circadian variation in plasma levels of thyroid hormones. Conflicting data have been reported regarding the detection of a circadian rhythm of plasma TSH in man (1, 4, 7). Nicoloff (5) demonstrated circadian release of radioiodine from the prelabeled thyroid gland in euthyroid man. However, these fluctuations appeared related to the release of intrathyroidal inorganic iodine and not to varying secretion of iodothyronines. The biological half-life of $T_4$ is approximately 7 days and that of $T_3$ is about 1–1.5 days in man. Only major changes in hormone production, secretion, or degradation during the day would likely affect plasma hormone levels. Moreover, in view of the current evidence of disparate origins of $T_4$ and $T_3$ (i.e., $T_4$ is secreted by the thyroid gland, while $T_3$ is derived largely from the deiodination of $T_4$ in peripheral tissues), circadian variation in the production or degradation of these hormones might be expected to produce asynchronous changes in the plasma levels of $T_3$ and $T_4$. The current study demonstrates, to the contrary, that the variation patterns of the two hormones with respect to time of day are remarkably synchronous. This synchrony in plasma $T_3$ and $T_4$ rhythms, on the other hand, appears consistent with varying hormone binding by plasma proteins during the day: according to current concepts, $T_3$ and $T_4$ share the same binding sites on carrier proteins, and alterations in the binding proteins should affect the levels of the two hormones similarly.

The present study confirms the existence of a circadian rhythm of plasma $T_4$ and demonstrates the presence of a parallel rhythm of plasma $T_3$. It is suggested that fluctuations in hormonal binding by plasma proteins may be responsible for the observed variation in plasma total hormone measurements.

The authors are indebted to Mr. Alton J. Rahe of the Biometrics Division, USAF School of Aerospace Medicine, for statistical consultations.

The research reported in this paper was conducted by personnel of the Environmental Sciences Division, USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, United States Air Force, Brooks Air Force Base, Texas.

The voluntary informed consent of the subjects used in this research was obtained in accordance with AFR 80-33.

Address reprint requests to: Alan Balsam, Department of Internal Medicine, Veterans Administration Hospital, 4500 S. Lancaster, Dallas, Texas 75216.

Received for publication 11 October 1974.

## REFERENCES

1. Blum, A. S., F. S. Greenspan, and J. Magnum. Circadian rhythm of serum TSH in normal human subjects (Abstract). *Proc. Intern. Congr. Endocrinol., 3rd, Mexico City, 1968*, p. 14.
2. Braverman, L. E., S. H. Ingbar, and K. Sterling. Conversion of thyroxine to triiodothyronine in athyreotic human subjects. *J. Clin. Invest.* 49: 855–864, 1970.
3. De Costre, P., U. Buhler, L. J. DeGroot, and S. Refetoff. Diurnal rhythm in total serum thyroxine levels. *Metabolism* 20: 782–791, 1971.
4. Lemarchand-Beraud, T., and A. Vannotti. Relationships between blood thyrotrophin level, protein bound iodine and free thyroxine concentration in man under normal physiological conditions. *Acta Endocrinol.* 60: 315–326, 1969.
5. Nicoloff, J. T. A new method for the measurement of thyroidal iodine release in man. *J. Clin. Invest.* 49: 1912–1921, 1970.
6. O'Connor, J. F., G. Y. Wu, T. F. Gallagher, and L. Hellman. The 24-hour plasma thyroxine profile in normal man. *J. Clin. Endocrinol. Metab.* 39: 765–771, 1974.
7. Odell, W. D., J. F. Wilber, and R. D. Utiger. Studies of thyrotropin physiology by means of radioimmunoassay. *Recent Progr. Hormone Res.* 23: 47–78. 1967.
8. Ostle, B. *Statistics in Research.* Iowa State Univ. Press, 1963, p. 325–329.
9. Pittman, C. A., J. B. Chambers, and V. H. Read. The rate of extrathyroidal conversion of thyroxine to triiodothyronine in man (Abstract). *J. Clin. Invest.* 49: 75a, 1970.
10. Schatz, D. L., and R. Volpe. Lack of diurnal variation in the level of serum protein-bound iodine. *J. Clin. Endocrinol. Metab.* 19: 1495–1497, 1959.
11. Schwartz, H. L., M. I. Surks, and J. H. Oppenheimer. Quantitation of extrathyroidal conversion of L-thyroxine to 3,5,3′-triiodo-L-thyronine in the rat. *J. Clin. Invest.* 50: 1124–1130, 1971.
12. Sterling, K., M. A. Brenner, and E. S. Newman. Conversion of thyroxine to triidothyronine in normal human subjects. *Science* 169: 1099–1100, 1970.
13. Surks, M. I., A. R. Schadlow, and J. H. Oppenheimer. A new radioimmunoassay for plasma L-triiodothyronine: measurements in thyroid disease and in patients maintained on hormonal replacement. *J. Clin. Invest.* 51: 3104–3113, 1972.
14. Surks, M. I., A. R. Schadlow, J. M. Stock, and J. H. Oppenheimer. Determination of iodothyronine absorption and conversion of L-thyroxine ($T_4$) to L-triiodothyronine ($T_3$) using turnover rate techniques. *J. Clin. Invest.* 52: 805–811, 1973.
15. Vernikos-Danellis, J., C. S. Leach, C. M. Winget, P. C. Rambaut, and P. B. Mack. Thyroid and adrenal cortical rhythmicity during bed rest. *J. Appl. Physiol.* 33: 644–648, 1972.
16. Walfish, P. G., A. Britton, P. H. Melville, and C. Ezrin. A diurnal pattern in the rate of disappearance of $I^{131}$ labeled L-thyroxine from the serum. *J. Clin. Endocrinol. Metab.* 21: 582–586, 1961.