Hypertension, I. H. Page and J. W. McCubbin, Eds. (Year Book Medical, Chicago, 1968), pp. 89–91.
8. D. T. Pals, F. D. Masucci, G. S. Denning, Jr., F. Sipos, D. C. Fessler, *Circ. Res.* **29**, 673 (1971).
9. R. F. Furchgott and S. Bhadrakom, *J. Pharmacol. Exp. Ther.* **108**, 129 (1953).
10. H. O. Schild, *Pharmacol. Rev.* **9**, 242 (1957).
11. O. Arunlakshana and H. O. Schild, *Brit. J. Pharmacol. Chemother.* **14**, 48 (1959).
12. G. R. Marshall, W. Vine, P. Needlemann, *Proc. Nat. Acad. Sci. U.S.A.* **67**, 1624 (1970).
13. K. Arakawa, R. R. Smeby, F. M. Bumpus, *J. Amer. Chem. Soc.* **84**, 1424 (1962).
14. Supported in part by NIH grant HL-6835.
23 May 1972

Human Prolactin: 24-Hour Pattern with Increased Release during Sleep

Abstract. *Human prolactin was measured in plasma by radioimmunoassay at 20-minute intervals for a 24-hour period in each of six normal adults, whose sleep-wake cycles were monitored polygraphically. A marked diurnal variation in plasma concentrations was demonstrated, with highest values during sleep; periods of episodic release occurred throughout the 24 hours.*

Many anterior pituitary hormones in humans are released episodically over the 24-hour day, and the frequency and magnitude of the episodes are controlled in part by central nervous system rhythms related to time of day and the sleep-wake cycle. These include the sleep-onset release of human growth hormone (*1*), the circadian rhythm of episodic secretion of adrenocorticotrophic hormone (ACTH) and cortisol (*2*), and the episodic release of gonadotrophins (*3*). The demonstration that human prolactin is distinct from growth hormone and circulates in plasma (*4*) and the development of a radioimmunoassay technique for its measurement (*5*) made possible this study of 24-hour release patterns of prolactin. We show that plasma prolactin concentrations in normal young men and women undergo episodic changes and that there is a diurnal variation in release, with highest concentrations during the nocturnal sleep period and the lowest during waking hours.

Three men and three nulliparous women, ages 21 to 30 years, all within 10 percent of ideal body weight, were each acclimated to the sleep laboratory for a period of 24 hours. None had ever received hormone therapy or was taking drugs. During the succeeding 24 hours, between 10 a.m. and 2 p.m., a catheter was inserted into an antecubital vein and blood was sampled every 20 minutes from an adjoining room (*6*). Plasma was immediately separated and frozen until radioimmunoassays of prolactin and human growth hormone were performed.

Surface electrodes for electroencephalography, chin muscle electromyography, and electrooculography were applied by standard techniques at approximately 10 p.m. to monitor sleep polygraphically (*7*). Subjects remained in a sound-attenuated room and ate meals at 8 a.m., 12 noon, and 6 p.m. Lights were turned off and subjects allowed to go to sleep at 11 p.m. Five of the six fell asleep within 40 minutes of the time when lights were turned out and were awakened at 7 a.m.; the sixth did not fall asleep until 1:30 a.m. and was allowed to sleep until 9 a.m.

Polygraphic sleep data were scored according to standardized criteria (*7*). Sleep stage percentages were within expected normal limits on the night when the catheter was in place. Total sleep time ranged from 6 hours, 20 minutes up to 7 hours, 40 minutes,

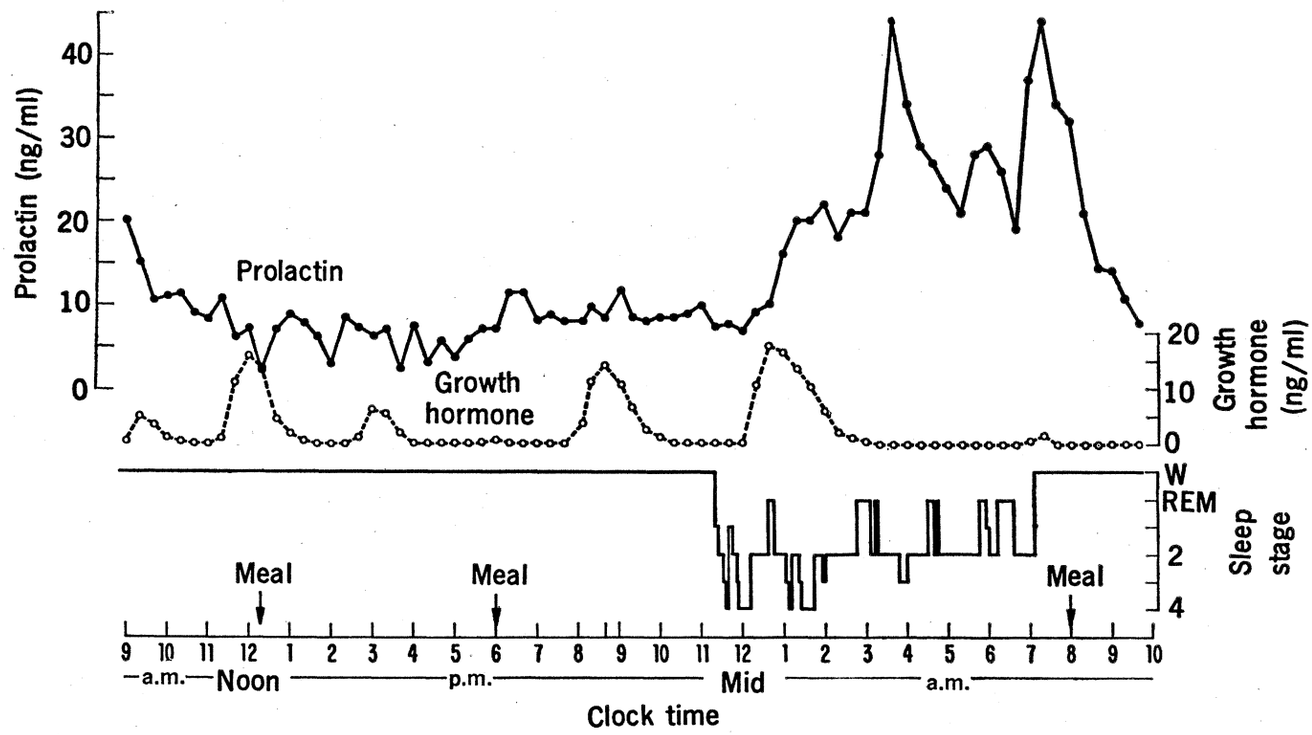

Fig. 1. Prolactin, growth hormone, and sleep stage in a male subject, age 23. The results of this 24-hour catheter study are representative of 24-hour pattern seen in all six subjects; *W*, wake.

averaging 7 hours. The mean percentages of total sleep time for each sleep stage were: stage 1, 4 percent; stage 2, 47 percent; stage 3, 13 percent; stage 4, 11 percent, and stage REM (rapid eye movement), 25 percent.

Growth hormone and prolactin were determined in duplicate on each sample, all samples from one subject being included in the same assay. Prolactin was measured by a double antibody radioimmunoassay employing antibody against human prolactin, and differing from that of Hwang *et al.* (*5*) only in the use of highly purified human, rather than monkey, prolactin for standards and for ^{131}I labeling. The human prolactin preparation, HPr-71-9-4 (*8*), tested by the in vitro mouse breast assay (*4*), had a potency of 30.5 international units (I.U.) per milligram, (95 percent confidence limits, 23.8 to 37.2 I.U./mg), approximately equal to that of the purest ovine preparations. The assay is capable of detecting prolactin in human plasma at concentrations of 1.0 ng/ml, and is unaffected by human growth hormone in amounts well beyond the physiologic range. Results of this assay have correlated well with those of bioassay over a wide range of plasma prolactin concentrations (*9*). Growth hormone was measured by radioimmunoassay with the use of dextran-coated charcoal (*10*).

Each of the six subjects demonstrated a similar pattern of prolactin release during the 24-hour period (Fig. 1). Plasma prolactin concentrations were lowest during the waking period, particularly between noon and 5 p.m., but never fell low enough to be undetectable. Prolactin concentration began to increase in each subject 60 to 90 minutes (mean, 73 minutes) after sleep onset and rose to an initial nocturnal peak an average of 30 minutes later. This initial elevation was then followed by a series of secretory episodes resulting in progressively higher plasma concentrations during the remaining hours of sleep. Maximal concentrations were reached in the early morning hours at approximately 5 to 7 a.m., toward the end of the sleep period. During the hour after awakening, a rapid fall in concentration began. The lowest plasma values were reached between 10 a.m. and noon and approximated concentrations obtained at the same clock time the previous day (Fig. 1). For the subject who did not fall asleep until 1:30 a.m., the initial nocturnal elevation of prolactin was at 2:30 a.m. and the decrease at the end of sleep was at 9 a.m., a delay of about 2 hours compared with the other subjects.

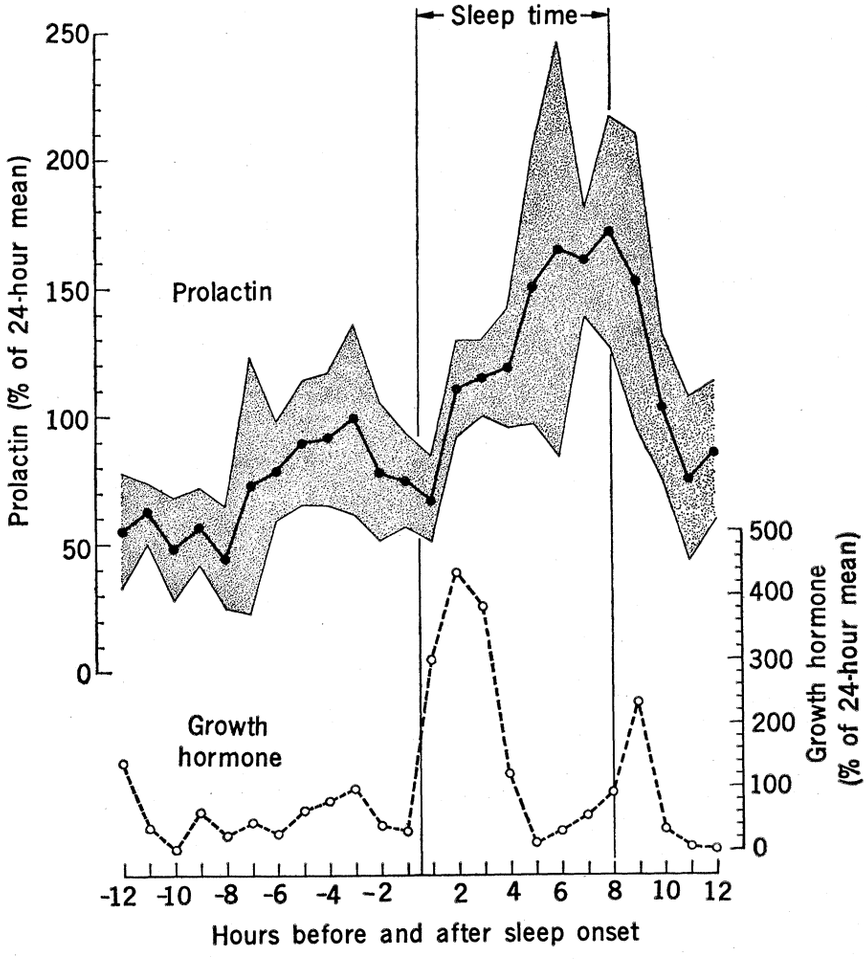

Fig. 2. Mean concentrations of prolactin and growth hormone in plasma, averaged for six subjects. Results for each hour before and after sleep onset are expressed as the percentage of the 24-hour mean. Each point represents the mean percentage computed from 18 plasma samples, 3 samples collected every 20 minutes for each of the six subjects. The shaded area in the prolactin graph indicates 1 standard deviation.

Prolactin release during the waking period was also characterized by episodes of release, although for the most part the concentrations attained were smaller than during sleep. Analysis of values for the six subjects over the entire 24 hours revealed a total of 36 instances (2 to 8 per subject) in which plasma prolactin concentration rose to a peak at least 50 percent greater than the value at the beginning of each of the episodes. Seventeen (47 percent) of these episodes occurred during the sleep period. The mean difference between initial and peak prolactin concentrations in these episodes was 10.8 ng/ml. No clear relation between prolactin peaks and sleep stages could be readily detected.

Plasma half times for prolactin, calculated on the most rapid disappearance rates after peak values had been reached, ranged from 15 to 25 minutes, approximating previous estimates (*5*). Much longer half times, however, were more common, which indicates that prolactin was being secreted in small amounts in addition to the major secretory episodes described.

The 24-hour mean concentrations of prolactin of these subjects varied between 5.0 and 25.8 ng/ml (mean, 14.4 ng/ml). No clear sex difference was evident in this small sample. There are no prior reports of 24-hour release patterns with which to compare these estimates of the 24-hour means.

Each subject's plasma prolactin values at 20-minute intervals were

averaged for 24 time periods of 1 hour each (three samples per subject per period); sleep onset was zero time. These hourly means were expressed as a percentage of that subject's mean prolactin concentration, and the percentages were averaged for all six subjects (Fig. 2). This calculation demonstrated for the group as a whole the prominent diurnal variation in relation to nocturnal sleep which was seen in each subject individually. A similar hour-by-hour analysis of human growth hormone concentrations in the same samples contrasted with that of prolactin (Fig. 2). Each subject had the expected nocturnal rise in growth hormone 1 to 2 hours after sleep onset; elevated concentrations lasted 2 to 3 hours, with an ultimate return to undetectable concentrations (Fig. 1). With the exception of this rise in growth hormone concentration during early sleep and the initial rise in prolactin concentration after sleep onset, no consistent relation between episodes of prolactin and growth hormone release could be identified throughout the 24-hour period. The initial rise in prolactin concentration after sleep onset usually coincided with the first elevation in growth hormone concentration, but the prolactin peak occurred an average of 40 minutes later than the first growth hormone peak.

This study of the 24-hour pattern of release of prolactin in humans provides evidence that prolactin secretion, like that of growth hormone, ACTH, cortisol, and gonadotrophins, is episodic and not constant over the sleep-wake period. The pattern of prolactin release, however, could not be directly correlated in temporal sequence with those of the other hormonal systems.

A number of stimuli have been demonstrated to result in prolactin release, including stress of various kinds, hypoglycemia, strenuous exercise, suckling in postpartum women, and administration of psychotropic drugs (*9*). None of these appeared to be operative in producing the patterns seen in our subjects. Rather, the overall 24-hour changes in prolactin in this study appear related to factors underlying the sleep-wake or light-dark cycle. It is not possible to determine from these data whether the initiation of the nocturnal rise in plasma prolactin is entirely dependent on sleep or whether it has free-running circadian properties to some degree independent of physiological state or environmental events. Shifts of the sleep-wake cycle and light-dark patterns are necessary to clarify this issue.

J. F. SASSIN
Department of Neurology, Montefiore Hospital and Medical Center, and *Albert Einstein College of Medicine, Bronx, New York 10467*

A. G. FRANTZ
Department of Medicine, Columbia College of Physicians and Surgeons, and *Presbyterian Hospital, New York 10032*

E. D. WEITZMAN
S. KAPEN
Department of Neurology, Montefiore Hospital and Medical Center, and *Albert Einstein College of Medicine*

References and Notes

1. Y. Takahashi, D. M. Kipnis, W. H. Daughaday, *J. Clin. Invest.* **47**, 2079 (1968); Y. Honda, K. Takahashi, S. Takahashi, K. Azumi, M. Irie, M. Sakuma, T. Tsushima, K. Shiaume, *J. Clin. Endocrinol. Metab.* **29**, 20 (1969); J. F. Sassin, D. C. Parker, J. W. Mace, R. W. Gotlin, L. C. Johnson, L. G. Rossman, *Science* **165**, 513 (1969).
2. E. Weitzman, H. Schaumberg, W. Fishbein, *J. Clin. Endocrinol. Metab.* **26**, 121 (1966); L. Hellman, F. Nakada, J. Curti, E. Weitzman, J. Kream, H. Roffwarg, S. Ellman, D. Fukushima, T. Gallagher, *ibid.* **30**, 411 (1970); E. Weitzman, D. Fukushima, C. Nogeire, H. Roffwarg, T. Gallagher, L. Hellman, *ibid.* **33**, 14 (1971).
3. S. Kapen, R. Boyar, L. Hellman, E. Weitzman, *Psychophysiology* **7**, 337 (1970); R. Rubin, A. Kales, A. Adler, T. Fagan, W. Odell, *Science* **175**, 196 (1972).
4. A. G. Frantz and D. L. Kleinberg, *Science* **170**, 745 (1970).
5. P. Hwang, H. Guyda, H. Friesen, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 1902 (1971).
6. K. Van Kirk and J. Sassin, *Amer. J. Electroencephalogr. Tech.* **9**, 143 (1969).
7. A. Rechtschaffen and A. Kales, Eds., *A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects* (Government Printing Office, Washington, D.C., 1968).
8. P. Hwang, H. Guyda, H. Friesen, *J. Biol. Chem.* **247**, 1955 (1972).
9. A. G. Frantz, D. L. Kleinberg, G. L. Noel, *Recent Progr. Horm. Res.* **28**, 527 (1972).
10. K. Laue, C. Gottlieb, V. Herbert, *Proc. Soc. Exp. Biol. Med.* **123**, 126 (1966).
11. Supported by PHS grants AM 11294, AM 5397, CA 11704, AM 14282, HD 06209, and OH 00331 and NASA contract 9-BB32-79-2-1461. Sleep studies were done in the Montefiore Hospital and Medical Center Clinical Research Center (PHS grant RR 53). We thank H. Friesen for supplying the human prolactin preparation and antibody used in these studies. R. Sundeen, J. Finney, B. Ward, L. Liotta, K. Tucker, and P. McGregor provided technical assistance.

18 April 1972

Peripheral Motion Detection and Refractive Error

Abstract. *Motion thresholds were determined for the fovea and peripheral retina with and without correction for peripheral refractive error. With correction, motion thresholds decreased and individual differences disappeared. These results imply that under normal observation conditions, peripheral sensitivity is limited mainly by dioptric rather than retinal variables.*

The potential contribution of the periphery to the functional performance of the eye can be appreciated when one considers that the fovea occupies only a small fraction of the entire visual field. Although the resolution of the peripheral regions is much less than that of the fovea, it is generally assumed that off-axis stimulation provides an important cue in directing voluntary eye movements. In spite of the functional importance of peripheral stimulation, little attention has been given to the possible influence of the refractive characteristics of the peripheral visual fields. Since both the resolution of the retina and the quality of the retinal image are degraded with increasing eccentricity, it is not possible to state which factor is limiting peripheral vision. Studies by Ferree, Rand, and Hardy (*1*) have demonstrated that very large refractive errors are present in the peripheral visual fields and furthermore that the direction of these errors—that is, toward hyperopia or myopia—varies considerably among individuals. The purpose of the study reported here was to determine the effect of the correction of peripheral refractive errors on the threshold for motion perception, a function which plays an important role in peripheral vision.

Three male graduate students, experienced in visual psychophysics, served as observers. As an aid to maintaining fixation, a stimulus was imaged in the blind spot so that involuntary movement of the eye would be immediately apparent. While a subject maintained monocular fixation with his dominant (right) eye, thresholds for motion perception were determined for the temporal visual field for a 1.0-second exposure at eccentric angles ranging from 0° to 80° in 10° steps. The stimulus was a white (reflectance 80 percent) square, 1.3 cm on a side, with luminance 4.3 mlam, viewed against a black (reflectance 0.8 percent) background at a distance of 78.7 cm. The subjects reported whether the