SPONTANEOUS VARIATIONS IN SERUM GROWTH HORMONE LEVELS

By

J. M. H. Buckler

ABSTRACT

Serum growth hormone (HGH) levels have been estimated in hourly samples for 24 hours in 2 women and 1 man. The highest levels in all subjects were found in association with the onset of sleep at whatever time that occurred. Other spontaneous peaks were found particularly in 1 woman. The rise in serum HGH levels in women following the stimuli of exercise or Bovril was greater or more rapid when these stimuli were applied mid-morning compared with early in the morning. Serum HGH levels at the same times on different days showed a great variation in the mid-morning levels from day to day in a female subject. No such variation was found in a man, nor in the female subject in the early morning.

There has been considerable discussion in the literature with regard to the merits of tests employing various stimuli to promote the release of growth hormone (HGH). For example, Best et al. (1968) reported that the response to intravenous arginine in ambulatory subjects was not significantly different from that of controls who received saline. In contrast, reports of Hembree & Ross (1969) and MacGillivray et al. (1969) suggested that arginine was an effective stimulus in fully rested subjects and that the discrepancy with the findings of Best et al. (1968) depended on the degree of activity of the subjects. There has also been difference of opinion with regard to the usefulness of vasopressin (Gagliardino et al. 1967; Czarny et al. 1968) and of Bovril (Jackson et al. 1968; Snodgrass 1970) as stimuli to HGH release. Some writers suggest that elevated HGH levels may result from factors which are independent of applied stimuli. Such factors are feeding or fasting, and physical or psychological stress, and their influence is much more frequently observed in women than in men. The present report describes a study of circumstances under which elevations of
serum HGH occur which are independent of specifically applied stimuli, and demonstrates the difficulty in assessing the value of stimulatory tests for HGH release.

METHODS AND MATERIALS

Serum HGH levels were estimated by a double antibody radio-immunoassay in which anti-HGH antibody prepared in a guinea pig was precipitated by rabbit anti-guinea pig serum (Jackson et al. 1968). The Medical Research Council Human Growth Hormone Research Standard A was used as standard and results are expressed in nanograms (ng)/ml of this preparation. One ng of this standard has been estimated to be equivalent to 1.2 units of the International Reference Preparation of HGH. Serial samples on the same subjects were either estimated in the same assay or in consecutive assays which included control samples.

Repeated estimations of the HGH content of the same serum sample over the period of these studies gave a mean of 11.4 ng/ml ± sp 1.73. In addition with each assay a serum from the previous batch was reassayed and the results did not differ by more than 27%. This variation in serum HGH results due to technical factors of the assay is considerably less than the differences described in these studies which are attributed to physiological mechanisms. The recovery experiments performed during these series of studies have given results similar to those reported previously (Jackson et al. 1968).

Oral Bovril* was used as a stimulus to HGH release as described by Jackson et al. (1968) but with modifications of the test procedure as indicated.

Repeated blood samples were usually taken by indwelling intravenous needle thus avoiding unnecessary stress, though occasionally by fingerprick if the subject preferred. Serial daily samples were either by fingerprick or venepuncture, but the method remained constant for each individual subject.

SUBJECTS

The studies were undertaken on women (aged 29 to 38 years) and one man (aged 34 years) who were in good health and not receiving any form of medication. They were members of hospital or laboratory staff who were accustomed to experimental procedures.

INVESTIGATIONS AND RESULTS

(a) Serum HGH levels throughout 24 hours

In studies performed on 2 women (J. S. aged 29 years and D. J. aged 38 years) and 1 man (J. B. aged 34 years) serum HGH levels were estimated on

* Bovril (on sale in Britain; Bovril Ltd., Alperton, Wembley, Middlesex) is a meat drink whose ingredients consist of various extracts of beef together with yeast, salt, caramel, starch and spices.
blood samples taken at hourly intervals throughout 24 h. The subjects conducted as normal a weekend routine as possible, including meals and activity, though unfortunately it was not possible to take samples at night without the subjects waking on each occasion.

The results for the 2 women are shown in Fig. 1 (a) and (b). Subject J. S. showed several peaks of serum HGH during the daytime, not apparently related to any particular factor. The most marked of these HGH peaks was at 9.30 a.m. In contrast, subject D. J. showed less peaks during the daytime, the only significant elevation occurring before lunch at 2.0 p.m. Both subjects failed to show any rise in serum HGH levels in the hours following a large evening meal, but both showed the largest HGH output at the time of onset of sleep.

The serum HGH levels in the male subject during a normal day are shown in Fig. 2 (a). Despite moderate exertion for much of the day, there was no marked HGH elevation during the waking hours, except after a large evening meal (in contrast to the female subjects). Again the most striking elevation was at night on going to sleep, although there had also been a mild increase in HGH output at an earlier time of night when the subject had become drowsy, though not fallen asleep. In order to determine whether this HGH response was associated with night time or specifically with sleep, studies were conducted on the same subject with a reversal of the 24 h activity, as illustrated in Fig. 2 (b). After a normal day, the subject remained sitting awake through the night, then

![Fig. 1.](image-url)

Serum HGH levels in two women throughout a normal 24 h.
undertook moderate activity the following morning and went to bed and slept in the afternoon. Apart from raised serum HGH levels which again followed the evening meal, the only other serum HGH peak was at the time of going to sleep, and there was no elevation in serum HGH levels during a night spent awake. This clearly suggests that sleep is the major factor responsible for HGH output at night.

(b) **Comparison of the serum HGH pattern in the same woman at different times of the morning and under different experimental conditions**

The serum HGH levels during 6 studies on different mornings in a fasted female subject (J. S.) who was accustomed to experimental procedures are illustrated in Fig. 3. These studies were not conducted around the time of ovulation, and were separated in time by several days.

Fig. 3 (a) demonstrates a high basal fasting HGH level at 9.30 a.m. following moderate activity. A dose of Bovril subsequently did not result in any further increment in serum HGH levels. Similar findings are demonstrated in Fig. 3 (c) when a spontaneous peak of serum HGH occurred at 9.30 a.m. when the subject was ambulatory. However, on another occasion when she remained in bed, this spontaneous output of HGH was small and delayed until 11.0 a.m. as shown.
Fig. 3.
Effect of various factors on the timing of the peak morning level of serum HGH in the same woman:

(a) Moderate exercise between 8 a.m. and 9 a.m. Bovril at 10 a.m.
(b) Bedrest until 11 a.m. No Bovril.
(c) Bedrest until 11 a.m. Bovril at 7 a.m.
(d) Moderate exercise between 6 a.m. and 7 a.m. No Bovril.
(e) Mild exercise continuously from 7 a.m. No Bovril.
(f) Bedrest until 11 a.m. Fainting episode at 8.15 a.m. No. Bovril.

in Fig. 3 (b), but nevertheless it did occur prior to rising from bed. With this experiment (Fig. 3 (b)) as a control, Bovril was administered at 7.0 a.m. on another day, with the subject completely at rest (Fig. 3 (c)). A peak of serum HGH occurred at 10.0 a.m. which was earlier than in the control experiment, but later after the stimulus than with most female subjects when they had received Bovril at 9.30 a.m. Moderate exercise (walking fast) for 1 h between 6.0 a.m. and 7.0 a.m. resulted in no increased output of HGH as shown in Fig. 3 (d), in contrast to the marked response that occurred in association with lesser degrees of exercise at a later hour of the morning (Fig. 3 (a) and (e)).

In a further experiment which was intended to be a control similar to that shown in Fig. 3 (b), the subject fainted for no apparent reason at 8.15 a.m. Serum HGH levels rapidly rose following this episode and there was no secondary rise at a later time in the morning (Fig. 3 (f)). This demonstrates that stress is a powerful stimulus to HGH release at any time, as most other stimuli do not produce a response at this early hour of the morning.
(c) The significance of the time of morning at which stimulatory tests for HGH release are conducted in women

In addition to the experiments in subject J. S. described above, tests in which Bovril was administered at 7.0 a.m. were conducted in 3 other women who were completely rested. The results for all 4 subjects are shown in Table 1. One of these subjects (D. F.) is the first female of many tested who has failed to show a response to Bovril. The other three showed the peak HGH response 2 h or more after Bovril. In contrast, the mean time of the peak serum HGH levels after Bovril in 13 women who had received the dose at 9.30 a.m. was 67 min ± 27.8 (1 sd). However, these latter subjects were ambulatory.

Moderately strenuous exertion in 4 women (aged 28-33 years) between 6.0 a.m. and 7.0 a.m. resulted in no significant elevation of serum HGH levels in 3 and in the 4th the highest level (which occurred at 7.0 a.m.) was only 9.8 ng/ml. Of these subjects only J. S. repeated the study at a later hour of the morning (as shown in Fig. 3 (a) and (c)). However, lesser degrees of exercise shortly before 9.30-10.0 a.m. have been associated with elevated serum HGH levels at that time in many women, though this is not a consistent finding.

(d) Variation in serum HGH levels from day to day at the same times of the morning

Serum HGH levels were estimated in blood samples taken at the same times of the morning (7.30 a.m., 9.30 a.m. and 10.0 a.m.) on many different days in the female subject J. S. and at 10.0 a.m. in the male subject J. B. The activity of these subjects did not alter greatly from day to day. The results are shown in Figs. 4 and 5. Serum HGH levels at 7.30 a.m. (about half an hour after rising) in subject J. S. and at 10.0 a.m. in subject J. B. did not show any marked variation throughout a month (Fig. 4). The 9.30 a.m. and 10.0 a.m. serum HGH values in subject J. S., however, showed enormous variation from day to day (Fig. 5). There was no consistent pattern in this behaviour, though

\[
\begin{array}{|c|c|c|c|}
\hline
\text{Subject} & \text{Age (years)} & \text{Serum HGH (ng/ml)} & \text{Time of maximum response after Bovril (min)} \\
& & \text{Fasting level} & \text{Maximum level} & \\
\hline
J. S. & 29 & 3.4 & 64.8 & 180 \\
D. F. & 31 & 3.7 & - & No response by 180 \\
G. S. & 33 & 3.7 & 15.7 & 120 \\
J. B. & 34 & 3.8 & 64.0 & 120 \\
\hline
\end{array}
\]

Table 1.
Results of Bovril tests commencing at 7.0 a.m. in four women.
**DISCUSSION**

The factors which control and influence HGH release are very complex in their interrelationships. The importance of sleep has, however, not often been emphasized. Takahashi et al. (1968) and Honda et al. (1969) showed that the onset of deep sleep was a powerful stimulus to HGH release, and the present studies demonstrate that this effect is manifested at whatever time sleep occurs. Unfortunately these subjects woke when blood samples were taken and therefore an element of stress could contribute to a subsequently elevated serum HGH level, but this is unlikely as samples were taken by indwelling intravenous needle in a similar way throughout the whole study. It seems likely that sleep is the major factor that accounts for the elevated levels of serum HGH that have been reported to occur at night (Hunter & Rigal 1966; Quabbe et al. 1966; Glick & Goldsmith 1968).

It has also become apparent that though subjects of both sexes are capable of releasing HGH at any age, the circumstances under which this release occurs are different in adults of the 2 sexes (Buckler 1969). The present studies and those of other workers demonstrate also that many adult women have spontaneous episodes of HGH release but in men these occur less frequently and are of smaller magnitude (Unger et al. 1965; Quabbe et al. 1966; Hunter et al. 1968). Yet some women show less tendency to this spontaneous release of HGH,
Fig. 5.
Serum HGH levels at the same times on different days in the same woman (a) 9.30 a.m. (b) 10 a.m. Interrupted lines join estimates not on consecutive days. The downward arrows indicate the days of onset of menstruation.

and this behaviour does not appear to be related to age as some older subjects have high fasting HGH levels (Buckler 1969).

It is possible that all women have an inherent diurnal rhythm which affects the threshold for HGH release which is absent or less apparent in men. In some women this may result in a spontaneous outpouring of HGH at certain times of day, notably mid-morning. In others, though elevated HGH levels do not occur spontaneously the threshold of response to other stimuli, e.g. moderate exercise, or Bovril, may be lower at these times. The only woman who failed to respond to Bovril had received the dose at 7.0 a.m. (subject D. F.). Another 3 women tested in this way responded 2 h or more after receiving the Bovril, which was about an hour later than the average time of peak response in subjects to whom Bovril was administered about 9.30 a.m. It thus seems likely that when the threshold is lower at certain times of the day, the same ex-
ternal stimulus may produce its effect more rapidly. Even the apparent small stimulus of being ambulatory in contrast to fully rested caused the spontaneous serum HGH peak to occur considerably earlier in the morning in subject J. S.

The variation in HGH behaviour from day to day was shown strikingly in the studies of the female subject J. S. No clear pattern throughout a month could be demonstrated and interpretation of the results is difficult owing to the speed at which serum HGH peaks develop and subside. Though samples taken at 9.30 a.m. and 10.0 a.m. could miss the peak time, these times are sufficiently close together to indicate that the day to day differences are significant, if only in the timing at which serum HGH peaks occur in this subject.

Such complex and demanding studies as those described cannot reasonably be performed on a large number of subjects. The results obtained, however, indicate the complexity of the control of HGH release and emphasize how difficult it is to assess the effects of specific stimuli for HGH release. Failure to realize this may well account for the conflicting opinions in the literature of the value of the tests, and in particular it must be emphasized that the time of day at which tests are performed is very important.

ACKNOWLEDGMENTS

I wish to thank Dr. Barbara E. Clayton for her advice and interest. I also thank my colleagues who have acted as subjects for these studies, in particular Mrs. Jean Shepherd and Mrs. Doreen Jackson. I gratefully acknowledge financial help from the Smith, Kline and French Foundation and the Joint Research Board of the Hospital for Sick Children and Institute of Child Health.

REFERENCES


Received on February 28th, 1970.