

# Effects of a 7-Day Treatment with a Novel, Orally Active, Growth Hormone (GH) Secretagogue, MK-677, on 24-Hour GH Profiles, Insulin-Like Growth Factor I, and Adrenocortical Function in Normal Young Men\*

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## ABSTRACT

To assess the effects of prolonged administration of a novel analog of GH-releasing peptide (MK-677), nine healthy young men participated in a randomized, double blind, three-period cross-over comparison of orally administered placebo and 5- and 25-mg doses of MK-677. Each period involved bedtime administration of the drug for 7 consecutive days. At the end of each period, plasma levels of insulin-like growth factor I (IGF-I) and IGF-binding protein-3 (IGFBP-3) were measured at 0745 h, and 24-h profiles of plasma GH and cortisol were obtained at 15-min intervals together with the 24-h urinary excretion of free cortisol. Profiles of plasma free cortisol were calculated at hourly intervals.

The amounts of GH secreted were similar in all three conditions, but GH pulse frequency was increased with both dosages of the drug,

primarily because of an increase in the number of low amplitude pulses. Plasma IGF-I levels were increased in a dose-dependent manner, whereas IGFBP-3 levels were increased only with the highest dosage. There was a positive relationship between GH pulse frequency and IGF-I increase. Except for an advance in the nocturnal nadir and in the morning elevation, MK-677 had no effect on cortisol profiles. In particular, 24-h mean levels of plasma total and free cortisol and urinary excretion of free cortisol were similar under all conditions.

The present data suggest that the use of MK-677 for the treatment of relative somatotrophic deficiency, particularly in older adults compromised by such deficiency, deserves further investigation. (*J Clin Endocrinol Metab* 81: 2776–2782, 1996)

GH IS RELEASED in a pulsatile fashion from the anterior pituitary under both positive and negative regulation by hypothalamic factors. GHRH stimulates GH secretion, whereas somatostatin exerts an inhibitory influence (1). The initiation of GH pulses could be due to GHRH pulses occurring during periods of low somatostatinergic tone (2).

During the past few years, a family of potent GH secretagogues, known as GH-releasing peptides (GHRPs), has been developed (3). The most studied of these compounds is GHRP-6. Although the mechanism of action of GHRP and related compounds is not completely elucidated, several lines of evidence suggest that specific receptors are involved at both the pituitary and the hypothalamic level (3). Current data support the concept that GHRP acts as a somatostatin antagonist via postreceptor mechanisms (4) and stimulates

GH release synergistically with GHRH (5). *In vitro*, chronic exposure to GHRP or GHRH results in homologous desensitization, but does not inhibit the response to the alternate secretagogue (6, 7). In young adults, single doses of GHRP were shown to stimulate GH secretion in a dose-related manner (8), whereas continuous prolonged GHRP infusions enhanced pulsatile GH secretion and increased plasma insulin-like growth factor I (IGF-I) concentrations (9, 10). Bolus injection of GHRP at the end of the infusion resulted in attenuated GH response, but bolus injection of GHRH under similar conditions was associated with an enhanced GH response, indicating that the prolonged infusion of GHRP resulted in a partial desensitization to this compound, rather than depletion of GH stores (9, 10). The effects of daily administration of GHRP and related compounds for prolonged periods of time remain to be assessed. GHRP has no effect on LH, FSH, TSH, insulin, or glucose concentrations (11–13), but may increase cortisol and PRL levels (8, 13).

Recently, novel compounds acting as functional agonists of the putative GHRP receptor have been synthesized and shown to have endocrine effects similar to those of GHRP (14–17). One of these compounds, MK-677 (also known as L-163,191), was found to be a potent GH secretagogue *in vitro*

Received December 4, 1995. Revision received February 8, 1996. Accepted March 18, 1996.

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\* This work was supported by a grant from the Belgian Fonds de la Recherche Scientifique Médicale, a research grant from the Université Libre de Bruxelles, and a grant from Merck Research Laboratories.

(17) and after administration of single doses in animals (17) and man (C. M. Mendel, personal communication). Unlike other GHRP-related molecules, MK-677 has excellent oral availability. The present placebo-controlled study was designed to examine in normal young men the effects of repeated administration for 7 consecutive days of two dosages of MK-677 on pulsatile GH secretion, IGF-I levels, and adrenocortical function. The drug was administered at bedtime to determine whether it would enhance the physiological release of GH that normally occurs after the onset of sleep.

## Subjects and Methods

### Subjects

Nine normal young men, 18–30 yr old (mean  $\pm$  SD,  $27 \pm 3$  yr), were selected after a careful clinical and biological evaluation. Body weight ranged between 60–85 kg. With the exception of subject 4, who had a body mass index of  $29.1 \text{ kg/m}^2$ , the body mass index ranged from  $20.3\text{--}22.9 \text{ kg/m}^2$ . All subjects were in good health and had normal medical history, physical examination, and electrocardiogram. Screening laboratory measurements (blood cell count, plasma or serum urea, creatinine, total bilirubin, transaminases,  $\gamma$ -glutamyl-transpeptidase, alkaline phosphatase, fasting blood sugar, Na, K, Ca, albumin, TSH, and IGF-I, and 24-h urinary free cortisol) were all within the normal range. The subjects were nonsmokers, did not take any drugs, and had no history of substance abuse. Shift workers, subjects who had travelled across time zones during the 2 weeks preceding the study, and subjects with sleep complaints were excluded. The subjects agreed to avoid strenuous physical activity throughout the duration of the study. The protocol was approved by the institutional review board, and all subjects gave written informed consent after receiving a complete explanation of the aims and means of the study.

### Experimental protocol

The protocol was designed as a double blind, placebo-controlled, three-period cross-over study. The subjects were randomly assigned to a sequence of three treatment periods according to a computer-generated allocation schedule. In each of the three treatment periods, subjects were given a single oral dose at bedtime (around 2245 h) for 7 consecutive days. Doses were 5 and 25 mg MK-677 and matching placebo. Subjects were required to abstain from food and drink (except water) from 2045 h (*i.e.* 2 h before the evening dose) until 0745 h the next morning, *i.e.* until 9 h after drug ingestion. The treatment periods were separated by at least 14 days. All experiments were performed in the Sleep Laboratory of the Center for the Study of Biological Rhythms of Erasme Hospital, Université Libre de Bruxelles (Brussels, Belgium).

Before the study, the subjects were required to sleep 2 consecutive nights in the laboratory to habituate them to the hospital environment and recording procedures. Sleep was polygraphically recorded during the second night. Throughout the study, the subjects were asked to maintain regular sleep-wake cycles (bedtime, 2300–0700 h in total darkness) and meal schedules (breakfast, 0800 h; lunch, 1230 h; dinner, 1900 h).

Each treatment period lasted 7 days. On the morning of day 1, before the treatment, the subjects came to the laboratory after an overnight fast, and blood samples for measurement of serum IGF-I, IGF-binding protein-3 (IGFBP-3) and dehydroepiandrosterone sulfate (DHEAS) were obtained. Each evening of days 1–5, a member of the investigators' staff delivered the drug or placebo at the volunteer's home and supervised drug ingestion at 2245 h. On day 6, the subjects were required to report to the sleep laboratory at 1900 h. The drug or placebo was administered under supervised conditions at 2245 h. Bedtime was 2300–0700 h in total darkness. The subjects were discharged in the morning of day 7 and readmitted between 1600–1700 h on the same day. They remained hospitalized until 1900 h on day 8. During that period, meals were served at 0800, 1230, and 1900 h; bedtime was 2300–0700 h in total darkness; and sleep was polygraphically recorded. The drug or placebo was administered under supervision at 2245 h. Snacks between meals, alcoholic beverages, daytime recumbency, and naps were prohibited. At 1700 h,

a catheter was inserted into a forearm vein, and 1-mL blood samples for GH and total cortisol determinations were obtained at 15-min intervals for 25 consecutive h starting at 1800 h. In addition, blood samples were collected for cortisol-binding globulin (CBG) and albumin determinations at 60- and 120-min intervals, respectively. Data collected between 1800–1900 h on day 7 were not included in the analysis to avoid artifacts due to the venipuncture stress. The iv line was kept patent by a slow drip of heparinized saline (750 IU heparin in 0.9 g NaCl/dL). Blood samples were collected using a plastic syringe connected to the lateral arm of a three-way stopcock. During waking hours, the stopcock was directly attached to the antecubital catheter. During the sleep period, the indwelling catheter was connected to plastic tubing (length, 250 cm; diameter, 0.18 mm; dead space, 6 mL) extending to an adjacent room through a hole in the wall. To prevent sample dilution, before collecting a blood sample through the plastic tubing, 6 mL saline solution were removed together with 2 mL blood. After having removed the blood sample, the dead space saline mixed with blood was returned to the subject, and the tubing was slowly rinsed using the heparinized saline drip. The overall rate of iv fluid administration was maintained at 10 mL/h.

An additional blood sample for the measurement of serum IGF-I, IGFBP-3, and DHEAS was collected at 0745 h on day 8. Twenty-four-hour urine collection for the measurement of urinary free cortisol and creatinine started at 1900 h on day 7.

### Hormonal assays

GH levels were measured using a commercially available immunoradiometric assay (Sorin Biomedica, Milan, Italy) with a lower limit of sensitivity of  $0.1 \mu\text{g/L}$ . In our laboratory, the mean intraassay coefficient of variation was 7% at concentrations between  $0.2\text{--}1 \mu\text{g/L}$  and 2.5% for concentrations above  $1 \mu\text{g/L}$ . Total cortisol levels were determined by a commercially available RIA (Coat-A-Count, Diagnostics Products Corp., Los Angeles, CA), with a lower limit of sensitivity of  $30 \text{ nmol/L}$ , an average intraassay coefficient of variation of 5%, and an interassay coefficient of variation less than 10%. Throughout the text, the term plasma cortisol levels refers to total (*i.e.* free plus bound) cortisol concentrations in plasma. Plasma CBG levels were determined by a commercially available RIA (Radim, Liege, Belgium) with a lower limit of sensitivity of  $6 \text{ mg/L}$ , an average intraassay coefficient of variation of 3.6%, and an interassay coefficient of variation of 7.5%. Plasma albumin levels were measured by a commercially available bromocresol green method using an automated procedure (BM/Hitachi 704, Tokyo, Japan). Plasma free cortisol levels were calculated from total cortisol, CBG, and albumin values, using a previously described computer program (18). The  $K_a$  values of cortisol for CBG and for albumin were assumed to be  $7.6 \times 10^7$  and  $3 \times 10^3 \text{ mol/L}^{-1}$ , respectively (19). Serum IGF-I levels were measured by a commercially available RIA that includes an acid-ethanol extraction procedure (Nichols Institute Diagnostics). The sensitivity of this assay is  $15 \mu\text{g/L}$ , the average intraassay coefficient of variation is 3%, and the interassay coefficient of variation is less than 5%. Serum IGFBP-3 levels were determined by a commercially available RIA (Nichols Institute Diagnostics), with a lower limit of sensitivity of  $0.6 \text{ mg/L}$ , an intraassay coefficient of variation less than 4%, and an interassay coefficient of variation less than 10%. Serum DHEAS levels were measured by a commercially available RIA (Diagnostic System Laboratories, Webster, TX), with an intraassay coefficient of variation around 8% and an interassay coefficient of variation of approximately 10%. Urinary free cortisol was measured by fluoro-polarized immunoassay after dichloromethane extraction (Abbott Laboratories, Chicago, IL). The intra- and interassay coefficients of variations are 3.5% and 5%, respectively.

### Sleep recording and analysis

Polygraphic sleep recordings were scored at 20-s intervals in stages wake, I, II, III, IV, and rapid eye movement (REM), using standardized criteria (20). The sleep period was defined as the time interval separating sleep onset from morning awakening. Sleep efficiency was calculated as the total recording time minus the time spent awake, expressed as a percentage of the total recording time.

### Data analysis

**GH profiles.** Significant pulses of GH secretion were identified using a modification of the computer algorithm ULTRA (21, 22). The threshold for significance of a pulse was set at 2 times the intraassay coefficient of variation in the relevant range of concentration. For each significant pulse, the amount of GH secreted was estimated by deconvolution based on a one-compartment model for GH clearance and variable individual half-lives, as previously described (23). The half-life was adjusted for each subject by an iterative process designed to minimize the number of negative secretory rates. On the average, the half-disappearance time was  $14.3 \pm 1.7$  min (mean  $\pm$  SD). A volume of distribution of 7% of the body weight was used in these calculations. The SD associated with each estimated secretory rate was calculated by following the theory of error propagation under the assumption of normally distributed errors in plasma levels. The total amount of GH secreted over a given time interval was determined by summing the amounts secreted in each of the significant pulses occurring during that time interval. If a pulse overlapped two periods, the amount of GH secreted was divided proportionally.

**Cortisol profiles.** The wave shape of each individual profile of plasma cortisol was quantified by a best-fit curve obtained using a robust, locally weighted regression procedure proposed by Cleveland (24) with a regression window of 4 h. The acrophases and nadirs were defined as, respectively, the times of occurrence of maxima and minima in the best-fit curve. The amplitude of the best-fit curve was defined as 50% of the difference between the value at the highest acrophase and the value at the lowest nadir and was expressed as a percentage of the overall mean level. Cortisol secretory rates were mathematically derived from the plasma cortisol concentrations using a two-compartment model for cortisol distribution and metabolism (25, 26). The volume of distribution, short half-life, and fraction of decay associated with the short half-life were obtained from previous studies (25, 26) and taken to be 4.1% of body weight, 5 min, and 0.80, respectively, for all subjects. For each subject, the long half-life was adjusted in the previously reported physiological range of 65–90 min (27) by an iterative process designed to minimize the number of negative secretory rates. On the average, the long half-life was  $75.9 \pm 9.5$  min. The error associated with each secretory rate, which results from measurement error on plasma levels, was derived following the theory of error propagation.

### Statistical tests

All comparisons among placebo, low MK-677 dose, and high MK-677 dose were performed by ANOVA for repeated measures, with pairwise contrasts tested by the Fisher procedure. All statistical calculations were performed using the Statview<sup>SE+</sup> software (Abacus Concepts, Berkeley, CA). Unless otherwise stated, all group results are expressed as the mean  $\pm$  SD.

## Results

### 24-h GH profiles

Mean profiles of plasma GH concentrations and of GH secretory rates, calculated across subjects for each treatment condition, are shown in Fig. 1. Representative individual profiles of one subject are shown in Fig. 2. For each subject, the number of GH pulses and the amount of GH secretion are reported in Table 1. After 7 days of treatment with either the low or the high MK-677 dosage, the total amounts of GH secreted over the 24-h span were not statistically different from those observed after placebo treatment. Moreover, the temporal profile of GH secretion was similar in all three conditions, with most of the secretion ( $80 \pm 20\%$ ,  $86 \pm 18\%$ , and  $85 \pm 12\%$  during placebo, low dose, and high dose treatments, respectively) occurring during sleep. There were, however, significant effects of both dosages of the active drug on the apparent frequency of GH pulses. After treatment with the low and the high MK-677 doses, the number of

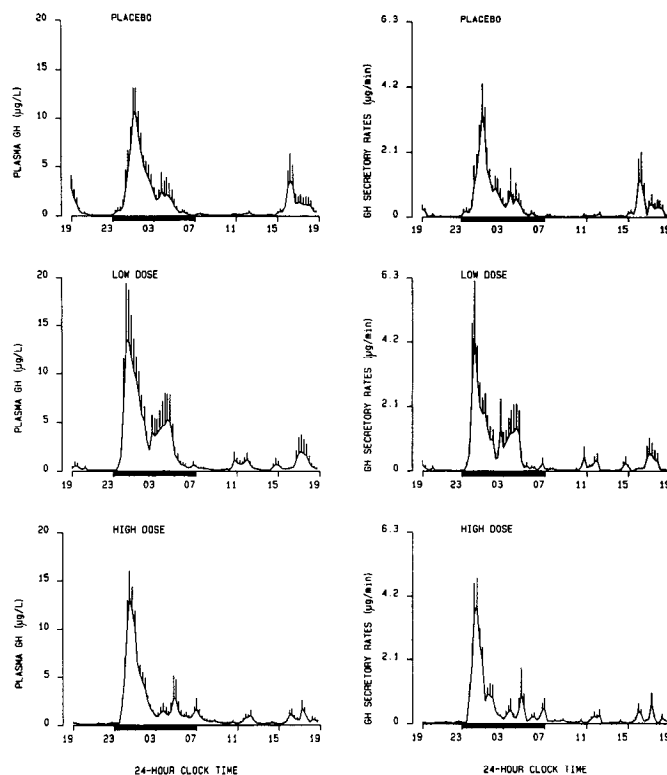


FIG. 1. Mean ( $\pm$ sem) profiles of plasma GH (left panels) and GH secretory rates (right panels) after 7 days of treatment with placebo and low and high doses of MK-677. Black bars represent the scheduled sleep periods.

detectable GH pulses per 24 h averaged  $11 \pm 4$  and  $11 \pm 3$ , respectively, compared to  $7 \pm 4$  after placebo treatment ( $P < 0.02$  for both dosages compared to placebo). This apparent higher pulse frequency resulted primarily from an increase in the number of low amplitude (peak GH levels  $< 5$   $\mu\text{g/L}$ ) pulses. Indeed, the frequency of large amplitude pulses was not affected by MK-677 treatment. Taking into account all individual profiles, a total of 16 pulses with a peak plasma value of 5  $\mu\text{g/L}$  or more were observed with the placebo treatment *vs.* 18 pulses with the low dose and 16 pulses with the high dose of MK-677. This increase in the number of low amplitude pulses resulted in a significant elevation of the amount of GH secreted in the intervals between the large amplitude pulses (*i.e.* pulses with peak GH levels  $\geq 5$   $\mu\text{g/L}$ ) after treatment with both the low ( $P < 0.04$ ) and the high ( $P < 0.002$ ) doses of MK-677 compared with placebo.

### Levels of serum IGF-I and IGFBP-3

On day 1, before the treatment, serum levels of IGF-I (placebo,  $279 \pm 101$   $\mu\text{g/L}$ ; low dose,  $302 \pm 97$   $\mu\text{g/L}$ ; high dose,  $255 \pm 98$   $\mu\text{g/L}$ ) and IGFBP-3 (placebo,  $2.48 \pm 0.69$  mg/L; low dose,  $2.52 \pm 0.48$  mg/L; high dose,  $2.53 \pm 0.59$  mg/L) were similar in the three studies. On day 8, serum levels of IGF-I were consistently higher after treatment with both doses of MK-677 than after placebo, as shown in the upper panel of Fig. 3 (placebo,  $248 \pm 101$   $\mu\text{g/L}$ ; low dose,  $325 \pm 110$   $\mu\text{g/L}$ ; high dose,  $360 \pm 95$   $\mu\text{g/L}$ ;  $P < 0.01$  for both dosages *vs.* placebo). In one subject (no. 8), the IGF-I level

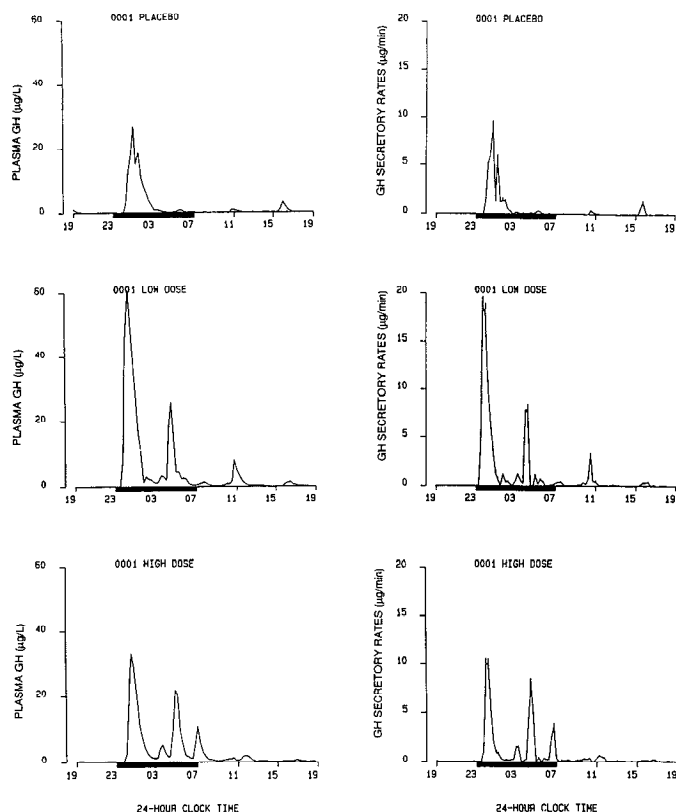


FIG. 2. Plasma GH (left panels) and GH secretory rates (right panels) profiles in a representative individual (subject 1) after 7 days of treatment with placebo and low and high doses of MK-677. Black bars represent the scheduled sleep periods. Vertical lines at each time point in the right panels represent the SE associated with the estimation of secretory rates from plasma levels.

dropped from 326  $\mu\text{g/L}$  with the low dose to 207  $\mu\text{g/L}$  with the high dose of MK-677; hence, the difference in IGF-I between the low and the high dose fell outside the 99% confidence interval for all other subjects. If this outlier was excluded from the analysis, the increment in IGF-I values between the low and the high dose of MK-677 averaged  $55 \pm 49 \mu\text{g/L}$  and was statistically significant ( $P < 0.03$ ), indicating that MK-677 administration increased IGF-I levels in a dose-dependent manner. As illustrated in the lower panel of Fig. 3, serum IGF-BP-3 levels on day 8 were modestly, but significantly, higher after treatment with 25 mg MK-677 than after low dose or placebo treatment ( $P < 0.01$ ).

We investigated whether the elevation in IGF-I levels observed with both dosages of the MK-677 treatment could be related to the increase in GH pulse frequency. The correlation between the number of GH pulses on day 8 and the corresponding IGF-I level was significant after adjustment for each subject's mean level ( $r = 0.52$ ;  $df = 16$ ;  $P < 0.05$ ).

#### Adrenocortical function

Mean profiles of plasma cortisol concentrations, calculated across subjects for each treatment condition, and individual profiles from one representative subject are shown in Fig. 4. Group parameters quantifying cortisol secretion in each treatment condition are given in Table 2. The 24-h mean levels of plasma cortisol, the values of the acrophase and the

nadir, and the amplitude of the circadian variation were similar in all three conditions. However, after treatment with the high dose, but not the low dose, of MK-677, the timing of the nadir of the circadian cortisol variation was advanced by more than 1 h compared with that after placebo ( $P < 0.05$ ). This advance of the nadir was associated with an earlier timing of the morning circadian rise (Fig. 4). Despite the differences in the timing of the nadir and that of the morning rise, the timing of the acrophase was similar in the three treatment conditions. Similarly, the profiles of plasma CBG and albumin and that of the calculated plasma free cortisol were unaffected by treatment with MK-677. The 24-h cortisol secretion and urinary excretion of free cortisol were also similar in all three conditions. In contrast, serum DHEAS levels (which were similar in the three studies on day 1, before treatment) were higher on day 8 ( $P < 0.05$ ) with both doses of MK-677 than with placebo (Table 2). Differences in DHEAS values between the low and the high MK-677 dosages were not statistically significant.

#### Sleep

On day 8, after 7 days of treatment, the timing of sleep onset, the duration of the sleep period, the sleep efficiency, and the durations of stages wake, I plus II, and III were similar in the three study conditions. However, high dose treatment with MK-677 was associated with significant increases in the durations of stages IV (placebo,  $37 \pm 19$  min; high dose,  $54 \pm 29$  min;  $P < 0.05$ ) and REM (placebo,  $85 \pm 19$  min; high dose,  $103 \pm 10$  min;  $P < 0.05$ ).

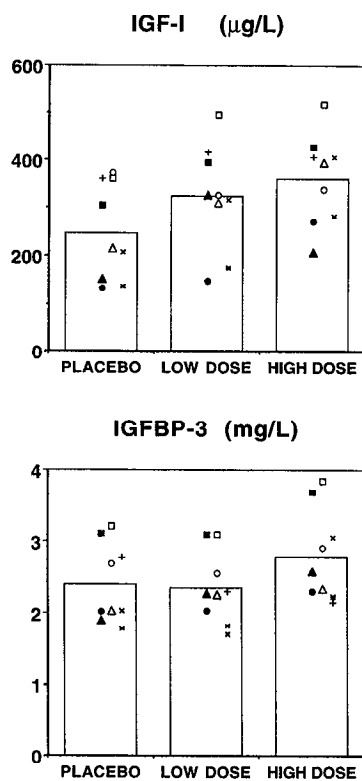
#### Discussion

GH secretion decreases markedly with aging, so that the daily GH output in adults over 60 yr of age is typically less than one third of that in 20- to 30-yr-old subjects (28). The health consequences of reduced GH secretion include increased fat tissue and abdominal obesity, reduced muscle mass and strength, reduced exercise capacity, and reduced bone mineral content (29, 30). Clinical trials with recombinant human GH have indicated that some of these effects of aging can be reversed by GH replacement (31–33). The development of orally active GH secretagogues able to enhance the endogenous pulsatile secretion of GH may constitute a more practical and physiological approach to the treatment of relative somatotropin deficiency than daily sc injections of recombinant human GH, which result in single and prolonged elevations of peripheral GH concentrations. The recent observations that acute administration of a newly developed mimic of GHRP consistently stimulates GH secretion in old (34) as well as in young (16) healthy subjects offer promise in that area. Whether increased GH secretion will persist after chronic, rather than acute, treatment with such compounds is unclear because chronic exposure to GHRP may result in homologous desensitization (9, 10), and it is not known whether GHRP stimulates *de novo* GH synthesis.

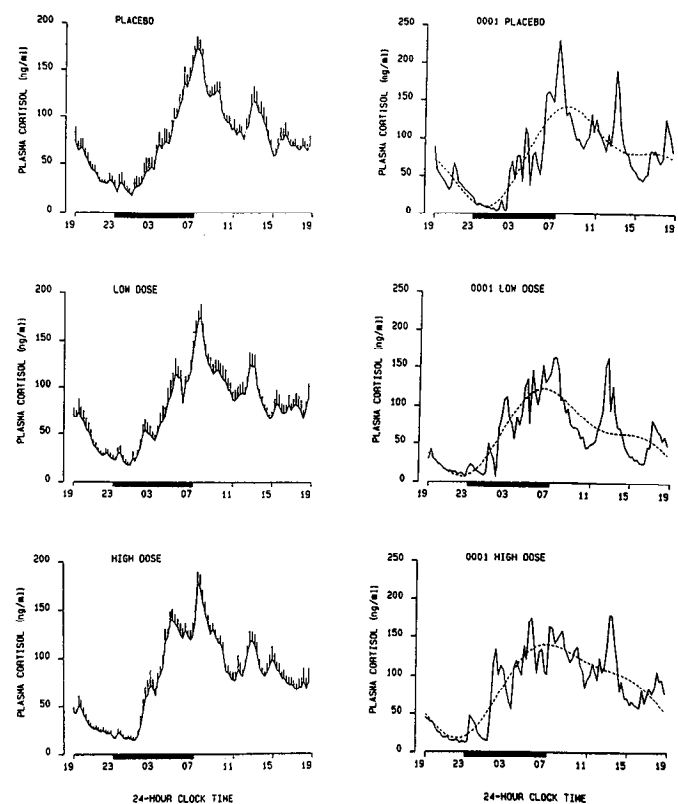
The present report describes for the first time the effects of repeated administration for 7 consecutive days of two dosages of a novel orally active mimic of GHRP, MK-677, in healthy young men. These dosages have been shown to elicit

**TABLE 1.** Quantitative characteristics of individual GH profiles after 7 days of treatment with placebo (PL) or low dose (LD) or high dose (HD) MK-677

Subject no.	No. of pulses/24 h			24-h secretion ( $\mu\text{g}$ )		
	PL	LD	HD	PL	LD	HD
1	13	12	12	558	1395	965
2	9	8	15	367	146	428
3	2	11	7	341	442	246
4	1	7	8	380	253	347
5	7	11	11	533	546	392
6	4	12	9	510	85	352
7	8	8	13	110	563	416
8	10	19	8	281	1128	302
9	10	13	14	645	337	386
Mean	7	11 <sup>a</sup>	11 <sup>a</sup>	414	544	426
SD	4	4	3	164	443	210

<sup>a</sup>  $P < 0.02$  vs. placebo.**FIG. 3.** Mean (histograms) and individual values (symbols) of IGF-I (upper panel) and IGFBP-3 (lower panel) after 7 days of treatment with placebo and low and high doses of MK-677. Each symbol represents an individual subject. IGF-I values after both dosages of MK-677 and IGFBP-3 values after the higher dosage were significantly ( $P < 0.01$ ) higher than those after placebo. Note that IGF-I levels were also significantly higher after the high dose than after the low dose ( $P < 0.03$ ) if an outlier (subject 8, depicted by black triangles) was excluded from the analysis.

robust GH responses when given as single oral doses to normal young volunteers (C. M. Mendel, personal communication). In contrast, in the present study, the amounts of GH secreted and the temporal profile of GH secretion after 7 days of treatment with either the low or the high MK-677 dosage were similar to those observed after placebo treatment. However, the apparent frequency of GH pulses was increased with both dosages of the drug, primarily because of an increase in the number of detectable low amplitude

**FIG. 4.** Mean ( $\pm$ SEM) profiles (left panels) and individual profiles of subject 1 (right panels) for plasma cortisol after 7 days of treatment with placebo and low and high doses of MK-677. The dotted lines represent the best-fit patterns of circadian variations. Black bars represent the scheduled sleep periods.

pulses. This finding of an increased number of detectable GH pulses after treatment with MK-677 is consistent with the apparent enhancement of GH pulse frequency reported in a previous study during 24-h GHRP infusion (9). These observations could either represent a genuine drug-induced alteration of GH ultradian rhythmicity or reflect an enhancement of the amplitude of preexisting GH micropulses, undetectable by the immunoradiometric assays used in the present and previous (9) studies.

Despite similar values of GH secretion in the three experimental conditions, circulating levels of IGF-I were increased

**TABLE 2.** Parameters quantifying adrenocortical function after 7 days of treatment with placebo (PL) or low dose (LD) or high dose (HD) MK-677: group values

	PL	LD	HD
24-h mean level of plasma total cortisol (nmol/L) <sup>a</sup>	216 ± 39	216 ± 38	219 ± 29
24-h secretion of cortisol (nmol) <sup>a</sup>	30.6 ± 6.1	32.5 ± 7.9	32.5 ± 5.7
24-h mean level of plasma CBG (mg/L) <sup>b</sup>	26.9 ± 2.9	26.7 ± 3.0	26.4 ± 3.2
24-h mean level of plasma albumin (g/L) <sup>c</sup>	4.4 ± 0.2	4.4 ± 0.2	4.3 ± 0.3
24-h mean level of plasma free cortisol (nmol/L) <sup>b</sup>	20.2 ± 4.8	19.3 ± 5.3	20.1 ± 3.1
Cortisol circadian amplitude (% of mean) <sup>a</sup>	86 ± 18	81 ± 15	87 ± 13
Acrophase value of plasma cortisol (nmol/L) <sup>a</sup>	401 ± 49	392 ± 68	397 ± 45
Acrophase timing of plasma cortisol (clocktime ± min) <sup>a</sup>	07.22 ± 55	07.48 ± 71	06.58 ± 33
Nadir value of plasma cortisol (nmol/L) <sup>a</sup>	39 ± 58	48 ± 34	20 ± 18
Nadir timing of plasma cortisol (clocktime ± min) <sup>a</sup>	00.27 ± 58	00.03 ± 72	23.18 ± 56 <sup>d</sup>
Urinary free cortisol (nmol/24 h)	186 ± 115	171 ± 96	153 ± 43
Serum DHEAS at 0745 h (μmol/L)	8.43 ± 3.05	9.85 ± 3.89 <sup>d</sup>	10.32 ± 4.22 <sup>d</sup>

Values are the mean ± SD.

<sup>a</sup> Based on measurements at 15-min intervals.

<sup>b</sup> Based on measurements at 60-min intervals.

<sup>c</sup> Based on measurements at 120-min intervals. For the calculation of plasma free cortisol, hourly values of albumin concentrations, when not measured, were linearly interpolated.

<sup>d</sup>  $P < 0.05$  vs. placebo.

after 1 week of treatment with both dosages of MK-677. This could be due to the increase in the number and/or the amplitude of small GH pulses, resulting in elevated "basal" GH secretion. The finding of a relationship between GH pulse frequency and the increase in IGF-I values is consistent with this hypothesis. The feedback exerted by increased IGF-I levels on GH release is likely to explain the absence of global elevation of GH secretion. An alternative explanation could be that prolonged treatment with MK-677 induced a desensitization of somatotrophic cells, and the increase in IGF-I levels without concomitant elevation of GH secretion resulted from the difference in the half-disappearance times of the two hormones (~15 min and ~15 h, respectively) (35, 36). However, a desensitization phenomenon appears unlikely, as it has recently been shown that the GH response to hexarelin, a synthetic hexapeptide similar to GHRP-6, is preserved after a 15-day treatment with this drug (37). Daily monitoring of serum IGF-I levels during a more prolonged administration of MK-677 and the use of an ultrasensitive chemiluminescence GH assay would probably allow elucidation of these questions.

The elevation of IGFBP-3, which was observed only with the high MK-677 dosage, was more modest than that of IGF-I. This is consistent with data obtained in previous studies investigating the effects of GH administration in adults with and without GH deficiency, indicating that the magnitude of the IGF-I increase is markedly higher than the magnitude of the IGFBP-3 increase, especially when basal levels of IGF-I and IGFBP-3 are in the normal range (38, 39).

The 24-h mean levels of plasma total and free cortisol, the temporal pattern of cortisol secretion, and the 24-h urinary excretion of free cortisol were essentially unaffected by the treatment. The only significant effect observed with the higher dosage of MK-677 was an advance by approximately 1 h of the early morning cortisol rise, which could reflect an influence of the drug on the circadian regulation of corticotropic function and/or be related to its effects on sleep quality. Previous studies have shown that acute administration of GHRP or related compounds induces a short term stimulation of cortisol secretion (8, 13). The relatively modest, but

significant, increase in serum levels of DHEAS (a compound with a half-life of 8–11 h, vs. 1.0–1.5 h for cortisol) (27, 40) could indicate that adrenocortical stimulation occurred during the first few days of treatment, but disappeared with prolonged administration. As recent data suggest that the well known decline of DHEAS with age could be an important component of the aging process (41), the ability of MK-677 to increase DHEAS levels could be beneficial in the elderly.

The durations of sleep stages IV and REM were significantly enhanced after treatment with the higher dosage of MK-677. Although the mechanisms involved in these sleep-promoting effects are not known, the present results are consistent with previously described somnogenic effects of GHRH and indicate that the hypnotic properties of MK-677 should be further examined in subjects with decreased sleep propensity.

In conclusion, daily oral administration for 1 week of MK-677, a novel GH secretagogue acting as a GHRP agonist, increases circulating IGF-I levels together with an apparent enhancement of GH pulse frequency, but without detectable elevation of GH secretion. This treatment does not induce any hypercortisolism. Furthermore, it is associated with an enhancement of both stage IV and REM sleep. Aging is characterized by a marked decrease in circulating IGF-I levels (42, 43), an increase in nighttime cortisol secretion (28), and profound alterations in sleep, including reductions in the amounts of the deeper stages of sleep as well as of REM sleep (28). Therefore, the present data suggest that the use of MK-677 for the treatment of relative GH deficiency and sleep disturbances in older adults deserves further investigation.

### Acknowledgments

The authors are greatly indebted to Dr. Etienne Stanus for invaluable assistance in setting up the sleep laboratory and the sleep recording procedures. They wish to thank Ms. Monique Zanen for expert assistance in performing the hormonal assays, and Dr. Francoise Vertongen for the determination of plasma albumin.

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