Growth Hormone (GH) Response to GH-Releasing Peptide-6 and GH-Releasing Hormone in Normal-Weight and Overweight Patients With Non–Insulin-Dependent Diabetes Mellitus

D. Micić, Dj. Macut, V. Popović, A. Kendereški, M. Šumarac-Dumanović, S. Zorić, C. Dieguez, and F. F. Casanueva

The growth hormone (GH) response to GH-releasing hormone (GHRH) in patients with non–insulin-dependent diabetes mellitus (NIDDM) was found to be either decreased or normal. The recent introduction of a new and potent GH stimulus, GH-releasing peptide-6 (GHRP-6), allowed further investigation of the functional properties of somatotropes in a variety of metabolic diseases. The aim of the present study was to investigate the response of GH to GHRP-6, GHRH, and GHRP-6 + GHRH in NIDDM patients. Twenty-one patients with NIDDM were divided into two groups: group A, normal weight (body mass index [BMI], 23.31 ± 0.62 kg/m^2); and group B, overweight (BMI, 27.62 ± 0.72 kg/m^2). Eight normal-weight control subjects (group C) were studied. Each subject received GHRP-6 (90 μg intravenously [IV]), GHRH (100 μg IV), and GHRP-6 + GHRH on three separate occasions. There was no difference between the GH response after GHRP-6 in groups A, B, and C in terms of the GH peak (50.95 ± 11.55, 51.96 ± 7.71, and 70.07 ± 15.59 mU/L, $P > .05$) and the area under the curve (AUC) for GH (2,340.06 ± 617.36, 2,684.54 ± 560.57, 3,462.78 ± 1,223.53 mU/L/120 min, $P > .05$). A decreased GH response to GHRH was found in group B in comparison to group A (B *v* A: peak GH response, 8.25 ± 1.90 *v* 22.19 ± 8.81, $P < .05$; AUC GH, 479.62 ± 84.0 *v* 1,443.21 ± 743.76, $P < .05$). There was no difference in the GH response between group A and group C (peak GH response, 22.19 ± 8.81 *v* 26.42 ± 6.71, $P > .05$; AUC, 1,443.21 ± 743.76 *v* 1,476.51 ± 386.56, $P > .05$). There was a significant difference between the same parameters in group B versus group C (8.25 ± 1.90 *v* 26.42 ± 6.71, $P < .05$; AUC, 479.62 ± 84.0 *v* 1,476.51 ± 386.56, $P < .05$). The combined administration of GHRP-6 + GHRH elicited a synergistic GH response in NIDDM patients and controls. There was a significant difference between groups A and B for the GH peak (96.49 ± 9.80 *v* 68.38 ± 8.25, $P < .05$), whereas there was no difference for the AUC (5,111.13 ± 703.77 *v* 3,425.95 ± 459.67, $P > .05$). There was no difference in the peak GH after the combined test between group A and group C (96.49 ± 9.80 *v* 139.82 ± 24.16, $P > .05$), whereas the peak GH in the same test was significantly decreased in group B in comparison to group C (68.38 ± 8.25 *v* 139.82 ± 24.16, $P < .05$). The AUC for GH after combined GHRP-6 + GHRH in group A versus group C was not significantly different (5,111.13 ± 703.77 *v* 9,274.71 ± 1,541.46, $P > .05$), whereas there was a significant difference for the same test between group B and group C (3,425.95 ± 459.67 *v* 9,274.71 ± 1,541.46, $P < .05$). Our results demonstrate that normal-weight NIDDM patients have a preserved GH response to GHRP-6, GHRH, and GHRP-6 + GHRH, and overweight NIDDM patients have a blunted response to GHRH and GHRP-6 + GHRH. The preserved GH response to GHRP-6 in both diabetic groups suggests that the secretory potential of somatotropes is preserved in NIDDM patients. The impairment of the GH response to GHRH in overweight NIDDM patients could be a functional defect due to the obesity, since it could be overridden by administration of GHRP-6.

INCREASED SPONTANEOUS and stimulated growth hormone (GH) secretion is well documented in insulin-dependent diabetes mellitus.[1] It was suggested that increased plasma concentrations of GH in diabetes may be important for the development of complications.[2] On the contrary, in non–insulin-dependent diabetes mellitus (NIDDM), conflicting results are found in the literature.[3] The GH response to GH-releasing hormone (GHRH) was lower in normal-weight and obese diabetics in comparison to control subjects.[3] In other studies, there was no difference between the GH response to GHRH in normal-weight NIDDM patients and matched controls, whereas the GH response to the same stimuli was lower in obese diabetics and obese controls.[2] The GH response to GHRH in poorly controlled NIDDM patients was not significantly impaired.[4] Cai et al[5] found a decreased GH peak after GHRH in NIDDM patients in comparison to a control group, concluding that the pituitary GH reserve is reduced due to a defect in central GH control in diabetics. Administration of pyridostigmine and acetylcholinesterase inhibitors significantly enhanced the GH response to GHRH in obese diabetics, obese controls, and non-obese controls, but not in non-obese type 2 diabetics.[6] Combined administration of arginine plus GHRH elicited a significant increase of GHRH-induced GH release in lean and obese NIDDM subjects.[3] A nonspecific GH response to other hypothalamic releasing hormones in diabetics was also reported, such as thyrotropin-releasing hormone[7] or luteinizing hormone–releasing hormone.[8] The effect of diabetes on the hypothalamic control of GH release appears to be determined by the quality of long-term glycemic control.[9]

GH-releasing peptides (GHRPs) were developed before the isolation and identification of GHRH in 1982, yet the clinical era of the GHRPs began in 1988. GHRPs and their analogs have no structural homology with GHRH and act via specific receptors present at either the pituitary or hypothalamic level. The GHRP receptor has recently been cloned, and it does not show sequence homology with other G-protein–coupled receptors known thus far.[10] An endogenous receptor implies the existence of an endogenous ligand, but its site of production,

From the Institute of Endocrinology, Diabetes and Diseases of Metabolism, Beograd, Yugoslavia, and the Departments of Physiology and Medicine, Faculty of Medicine and Complejo Hospitalario de Santiago, Santiago de Compostela University, Santiago de Compostela, Spain.

Submitted June 26, 1998; accepted September 29, 1998.

Supported by research grants from the Ministry of Science of Serbia and the Fondo de Investigacion Sanitaria, Spanish Ministry of Health and Conselleria de Education, Xunta de Galicia.

Address reprint requests to D. Micić, MD, PhD, Institute of Endocrinology, Diabetes and Diseases of Metabolism, Dr Subotića 13, 11000 Beograd, Yugoslavia.

0026-0495/99/4804-0020$10.00/0

relationship to xenobiotic pharmacological agents, and underlying physiological relevance remain unclear.[11] However, this evidence strongly suggests that these compounds could be the synthetic counterpart of an endogenous GH secretagogue involved in the neuroendocrine control of GH secretion, which has not yet been found.[12] Although the exact mechanism of the action of GHRPs has not been fully established, there is probably a dual site of action on both the pituitary and the hypothalamus, possibly involving regulatory factors in addition to GHRH and somatostatin. Several data favor the hypothesis that GHRPs could act by counteracting somatostatinergic activity at both the pituitary and hypothalamic level and/or, at least partially, via a GHRH-mediated mechanism.[13] Moreover, the possibility that GHRPs act via an unknown hypothalamic factor (U factor) is still open.[10] GHRP-6, hexarelin, and other nonclassic synthetic GHRPs appear to be promising new tools for exploring the GH secretory mechanism in patients with suspected GH deficiency.[14] Furthermore, the combined administration of saturating doses of GHRH + GHRP-6 is currently the most effective GH-releasing stimulus tested in a variety of settings related to altered somatotrophic function.[15] There is large variability in the stimulatory action of GHRH, in contrast to the reproducibility of the action of GHRPs. In different metabolic states, the GH response is impaired more after GHRH versus GHRP-6. On the other hand, in different neuroendocrine pathologies, the GH response is more impaired after GHRP-6 versus GHRH. Each secretagogue provides separate information on GH secretion, necessary not only for linear growth but also for general metabolism.[16]

Therefore, the aim of this study was to investigate the GH response to GHRP-6 alone, GHRH alone, and combined administration of GHRP-6 + GHRH in NIDDM patients and control subjects.

SUBJECTS AND METHODS

Subjects

Twenty-one patients (12 men and nine women) with NIDDM diagnosed by established criteria[17] were studied. The NIDDM patients were divided into two groups according to the body mass index (BMI): group A, normal-weight (n = 8; four men and four women; age, 52.37 ± 2.91 years; BMI, 23.31 ± 0.62 kg/m^2); and group B, grade I overweight[18] (n = 13; eight men and five women; age, 49.15 ± 1.76 years; BMI, 27.62 ± 0.72 kg/m^2). The duration of diabetes was 87.25 ± 29.46 months for group A and 79.61 ± 27.31 months for group B. The mean hemoglobin A_{1c} (HbA_{1c}) level (HPLC Variant System; Bio-Rad Laboratories, Vienna, Austria) at the time of evaluation was 8.85 ± 0.99% for group A and 8.30% ± 0.33% for group B (normal HbA_{1c}, <6.0%). The patients had normal creatinine levels and no evidence of diabetic retinopathy and polyneuropathy. No medications other than oral hypoglycemics (glibenclamide 2.5 to 7.5 mg/d) were used by the patients before and during the study period.

The control group (group C) consisted of eight healthy subjects (three men and five women; mean age, 48.5 ± 2.51 years; mean BMI, 23.78 ± 0.70 kg/m^2).

Approval for the study was obtained from the Hospital Ethics Committee, and all subjects provided written informed consent.

Study Protocol

Each subject underwent three tests in random order separated by at least 3 days. The tests were performed after an overnight fast, and subjects remained recumbent throughout each test. The patients received no sulfonylurea on the morning of the study. At 8 AM, an indwelling catheter was placed in a forearm vein and kept patent by a slow 0.9% saline infusion. Thirty minutes later, the test was started. After three blood samples (−30, −15, and 0 minutes), each subject received GHRP-6 (Peninsula Laboratories, Madrid, Spain) at a dose of 1 μg/kg intravenously (IV), GHRH(1-29)NH2 (Geref; Serono, Madrid, Spain) at a dose of 100 μg IV, or a combination of 1 μg/kg GHRP-6 + 100 μg GHRH IV. Blood samples were taken for GH determinations at −30, −15, 0, 15, 30, 45, 60, 90, and 120 minutes.

Assays and Statistical Analysis

The serum GH concentration (milliunits per liter) was determined using a time-resolved fluoroimmunoassay (Delfia; Wallac, Turku, Finland) with a GH sensitivity of 0.052 mU/L and a coefficient of variation of 6.3% (1.04 mU/L), 5.3% (26.52 mU/L), and 4.2% (112.84 mU/L). Hormone levels are presented and analyzed as the absolute level, as the mean absolute GH peak, or as the maximal increase over the baseline GH concentration. The area under the secretory curve ([AUC] milliunits per liter per 120 minutes) was calculated by the trapezoidal method and compared between groups by the Wilcoxon rank test. Kruskal-Wallis one-way ANOVA was used for comparison of the fold-increase among the groups. The statistical level of significance was set at P less than .05.

RESULTS

The mean basal glucose value at the beginning of the tests was 8.67 ± 0.28 mmol/L for group A and 8.95 ± 0.43 for group B. There were no significant changes in glucose levels either during or at the end of the tests.

The GH response to GHRP-6 in NIDDM subjects and controls is presented in Fig 1. There was no significant difference between the GH peak in groups A, B, and C (50.95 ± 11.55, 51.96 ± 7.71, and 70.07 ± 15.59, $P > .05$; Table 1). The slight delay in the peak GH response in the control is not significant ($P > .05$). Also, no significant difference was found for the AUC after GHRP-6 in groups A, B, and C (2,340.06 ± 617.36, 2,684.54 ± 560.57, and 3,462.78 ± 1,223.53, $P > .05$; Fig 2).

The GH response to GHRH in NIDDM patients and controls is presented in Fig 3. There was a significantly decreased GH response to GHRH in group B in comparison to group A in terms of the peak GH response (8.25 ± 1.90 *v* 22.19 ± 8.81,

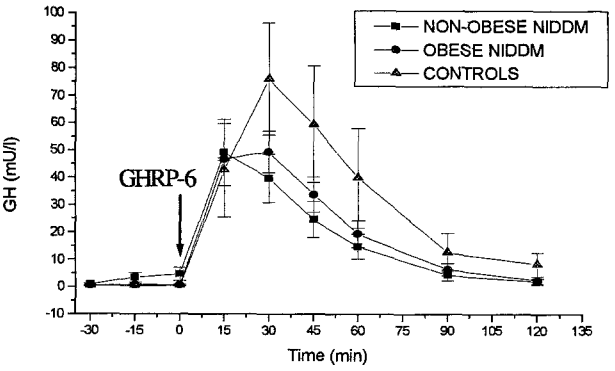

Fig 1. GH response to GHRP-6 in NIDDM patients and controls.

Table 1. GH Peak (mU/L) After GHRP-6, GHRH, and GHRP-6 + GHRH in Groups A, B, and C

	Group A			Group B			Group C		
Patient No.	GHRP-6	GHRH	GHRP-6 + GHRH	GHRP-6	GHRH	GHRP-6 + GHRH	GHRP-6	GHRH	GHRP-6 + GHRH
1	44.3	3.53	113.8	83.58	7.7	127.9	54.2	20.9	90.5
2	9.73	2.27	70.42	29.39	2.73	69.52	52.7	10.5	74.3
3	38.46	21.18	82.79	33.97	2.66	79.23	86.3	9.2	260.7
4	40.03	9.56	52.71	79.98	16.14	78.16	171.9	41.0	215.1
5	109.1	30.48	139.6	23.2	8.41	36.43	55.0	10.0	152.2
6	50.48	14.5	102.6	72.55	22.52	103.9	34.9	22.3	71.0
7	26.32	16.55	116.5	27.44	3.17	50.68	39.0	33.8	111.8
8	89.22	79.51	93.55	28.89	4.3	32.63	66.56	63.7	143.0
9				55.27	5.84	34.15			
10				105.9	19.98	59.58			
11				50.48	4.48	102.2			
12				66.78	1.92	63.0			
13				18.13	7.45	48.81			
Mean	50.95*	22.19‡§	96.49¶#	51.96†	8.25‖	68.38**	70.07	26.42	139.82
SEM	11.55	8.81	9.8	7.71	1.9	8.25	15.59	6.71	24.16

*$P > .05$, group A *v* group B and *v* group C after GHRP-6.
†$P > .05$, group B *v* group C after GHRP-6.
‡$P < .05$, group A *v* group B after GHRH.
§$P > .05$, group A *v* group C after GHRH.
‖$P < .05$, group B *v* group C after GHRH.
¶$P < .05$, group A *v* group B after GHRP-6 + GHRH.
#$P > .05$, group A *v* group C after GHRP-6 + GHRH.
**$P < .05$, group B *v* group C after GHRP-6 + GHRH.

$P < .05$) and the AUC for GH (479.62 ± 84.0 *v* 1,443.21 ± 743.76, $P < .05$; Table 1 and Fig 2). There was no difference between group A and group C for the peak GH response and AUC for GH after GHRH (peak, 22.19 ± 8.81 *v* 26.42 ± 6.71, $P > .05$; AUC, 1,443.21 ± 743.76 *v* 1,476.51 ± 386.56, $P > .05$). There was a significant difference in the same parameters in group B versus group C (peak, 8.25 ± 1.90 *v* 26.42 ± 6.71, $P < .05$; AUC, 479.62 ± 84.0 *v* 1,476.51 ± 386.56, $P < .05$) (Table 1 and Fig 2).

Simultaneous administration of GHRP-6 + GHRH elicited a synergistic GH response in both NIDDM patients and controls (Fig 4). There was a significant difference between group A and group B for the GH peak (96.49 ± 9.8 *v* 68.38 ± 8.25, $P < .05$), whereas there was no difference for the GH AUC (5,111.13 ± 703.77 *v* 3,425.95 ± 459.67, $P > .05$) (Table 1 and Fig 2). There was no difference in the peak GH after the combined test between group A and group C (96.49 ± 9.80 *v* 139.82 ± 24.16, $P > .05$), whereas the peak GH in the same test in group B versus group C was significantly different (68.38 ± 8.25 *v* 139.82 ± 24.16, $P < .05$; Table 1). The AUC for GH after combined GHRH + GHRP-6 for group A versus group C was not significantly different (5,111.13 ± 703.77 *v* 9,274.71 ± 1,541.46, $P > .05$), whereas there was a significant difference for the same test between group B and group C (3,425.95 ± 459.67 *v* 9,274.71 ± 1,541.46, $P < .05$; Fig 2). The fold-increase in the GH peak and AUC in all groups with GHRP-6 or GHRH and the combination of the two agents is presented in Figs 5 and 6. It is clear that a synergistic effect of

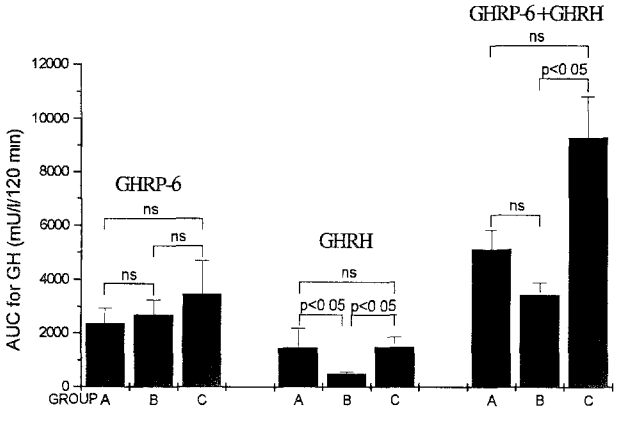

Fig 2. AUC for GH after GHRP-6, GHRH, and GHRP-6 + GHRH in non-obese NIDDM (group A), obese NIDDM (group B), and controls (group C). ns, not significant ($P > .05$).

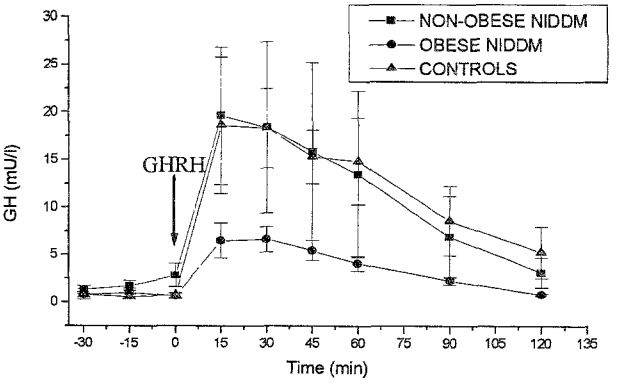

Fig 3. GH response to GHRH in NIDDM patients and controls.

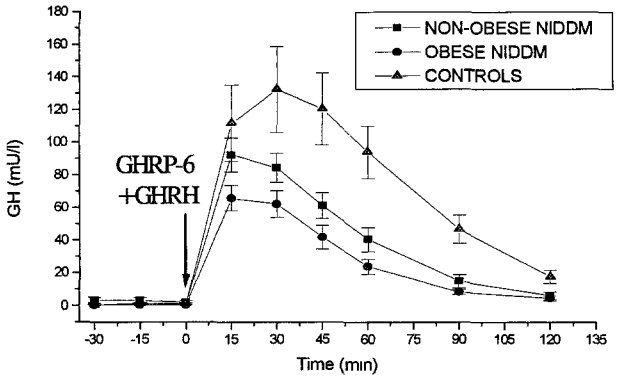

Fig 4. GH response to GHRP-6 + GHRH in NIDDM patients and controls.

both secretagogues was preserved in all tested groups, although it is much less pronounced in group B (Fig 6).

DISCUSSION

Investigations of GH secretion in patients with NIDDM have produced conflicting results. A specific increase in GH burst frequency was recently reported in NIDDM patients.[9] However, GH hypersecretion does not occur, because the GH burst mass is reduced in proportion to the degree of obesity. It was suggested that the effect of diabetes on the hypothalamic control of GH release appears to be determined by the quality of long-term glycemic control.[9] Our patients had a relatively long duration of NIDDM, and at the time of testing they had moderately elevated HbA_{1c}.

The somatotrope responsiveness to GHRH alone or combined with arginine, which is able to enhance the GH response to GHRH, probably through inhibition of somatostatin release from the hypothalamus, was previously investigated in normal-weight and obese patients with NIDDM.[3] The GH response to GHRH in normal-weight diabetics and obese diabetics was similar to the response in obese control subjects, and all responses were lower than in normal-weight control subjects. Arginine caused a significant increase of GHRH-induced GH release in all groups. Our overweight NIDDM patients have a similarly blunted GH response to GHRH. On the other hand, assessments of the GH response to GHRH in lean NIDDM subjects have produced conflicting data.[4,19] Similar to our study protocol, the GH response to GHRH was investigated in five age-matched groups: normal-weight controls, obese controls, insulin-dependent diabetics, normal-weight NIDDM patients, and obese NIDDM patients.[2] The peak GH response to GHRH was similar in the controls, insulin-dependent diabetics, and normal-weight NIDDM patients, but was significantly reduced in the two obese groups. These previous results do not confirm the previous reports of abnormal GH secretion in diabetes, but do demonstrate a markedly impaired GH response to GHRH as a feature of obesity. These findings are in agreement with the fact that GH secretion in response to all provocative stimuli is decreased in patients with obesity.[20]

An alteration in hypothalamic somatostatinergic tone was postulated in NIDDM, since pyridostigmine did not increase the GH response to GHRH in lean NIDDM patients.[6] On the other hand, increased somatostatinergic tone was suggested in obesity, since pyridostigmine administration has a stimulatory effect on GHRP-6–induced GH secretion in obese subjects.[20] Although arginine, in addition to GH-releasing substances, presumably acts by inhibiting hypothalamic somatostatinergic tone, it was shown that patients with NIDDM, irrespective of body weight, had an impairment of the GH response to GHRH alone or combined with arginine.[3] It was also suggested that

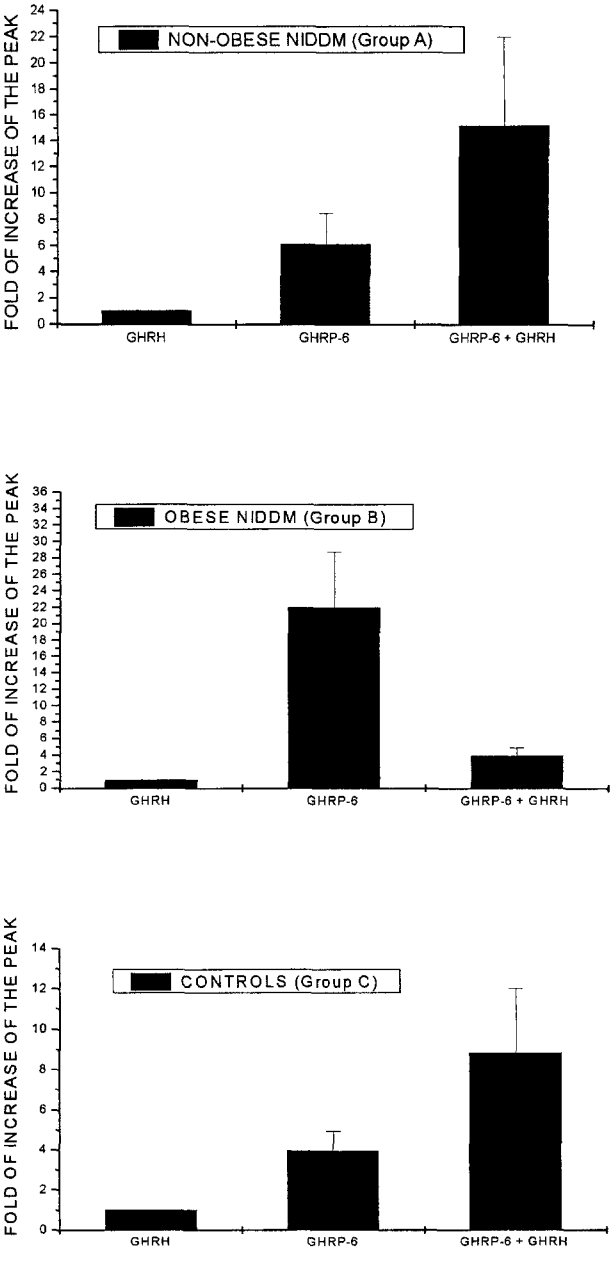

Fig 5. Fold-increase of the GH peak value in groups A, B, and C for GHRP-6, GHRH, and GHRP-6 + GHRH. GHRP-6 and GHRH *v* combined administration: group A, $P < .05$; group B, $P > .05$; group C, $P < .05$.

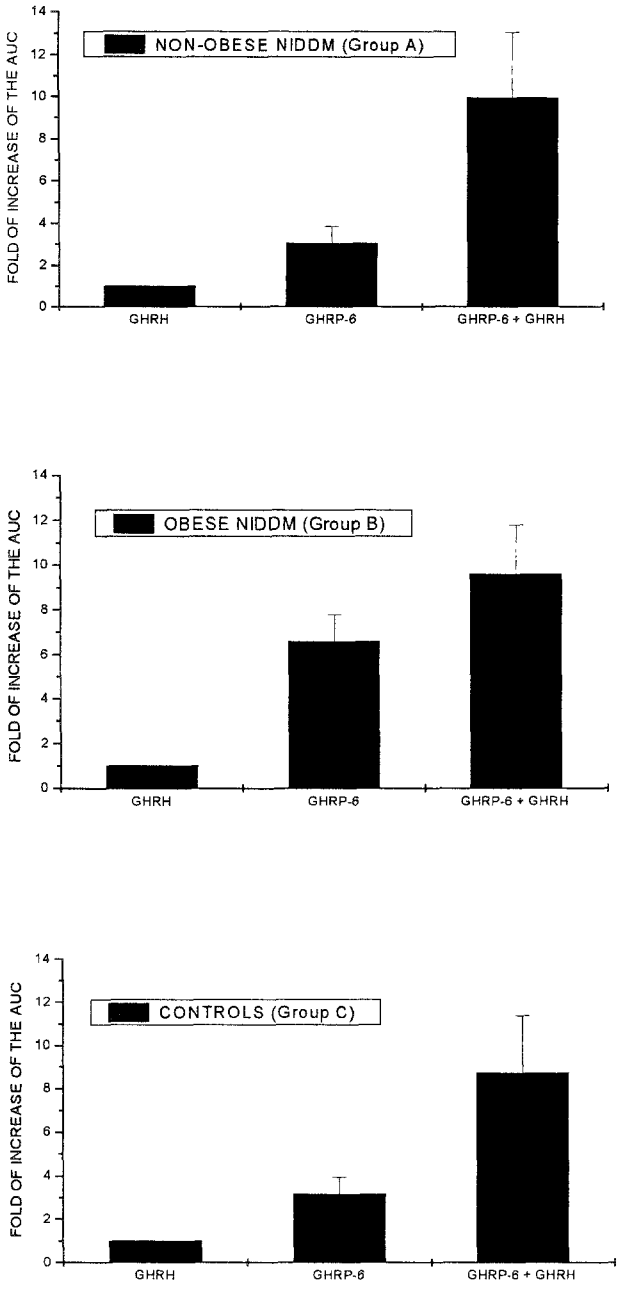

Fig 6. Fold-increase of the AUC for GH in groups A, B, and C for GHRP-6, GHRH, and GHRP-6 + GHRH. GHRP-6 and GHRH *v* combined administration: group A, $P < .05$; group B, $P > .05$; group C, $P < .05$.

NIDDM patients have decreased endogenous GHRH tone, since they exhibited an impaired GH response to L-dopa, an agent that presumably acts by increasing hypothalamic GHRH release.[21] Our data showing a normal GH response to GHRP-6 in NIDDM patients contradict this hypothesis, since it is known that GHRP-6 requires the presence of GHRH in order to elicit GH secretion.[22] The GHRP acts at two anatomical sites—the hypothalamus and the pituitary—to release GH. Using a coupled in vitro–in vivo approach, it has been possible to demonstrate that the scope of the relation between GHRP and GHRH in eliciting GH release can be independent and dependent, additive and synergistic, as well as permissive.[23] The GH-releasing activity of GHRPs is synergistic with that of GHRH and is not affected by opioid receptor antagonist, while it is only blunted by inhibitory influences that are known to nearly abolish the effect of GHRH, such as neurotransmitters, glucose, free fatty acids, glucocorticoids, recombinant human GH, and even exogenous somatostatin.[10] It is possible that the GHRP-6 + GHRH synergistic release of GH in humans is the primary result of the hypothalamic action of GHRP-6, with pituitary action being secondary.[23] Furthermore, our data showing a marked increase in plasma GH levels following GHRP-6 + GHRH administration in all tested groups suggest that the somatrope secretory capacity is not severely compromised in patients with NIDDM as suggested by some investigators.[5] However, the decreased synergistic effect of GHRP-6 + GHRH in our obese NIDDM patients could theoretically be due to increased somatostatin tone in obesity. A similar conclusion was reported in normal individuals by testing the GH response with a maximal dose of GHRH + 0.1 μg/kg GHRP-2, suggesting that if somatostatin had been increased, the low dose of GHRP-2 would not have induced synergism.[23] It was concluded by the same investigator that GHRP does not induce synergism by releasing endogenous GHRH, except at high dose, or by inhibiting somatostatin release. However, pyridostigmine pretreatment did not significantly change the GH response to combined administration of GHRP-6 + GHRH in obese subjects, leading to the conclusion that GHRP-6 can behave as a functional somatostatin antagonist, and that somatotrope responsiveness to the combined administration of GHRP-6 + GHRH is largely independent of somatostatinergic tone.[24] The presence of GHRP receptors in hypothalamic structures and the evidence that GHRP-elicited GH secretion is not mediated by changes in endogenous GHRH or somatostatin, but requires an operational hypothalamus, suggest that exogenous GHRP may induce the release of another hypothalamic factor with GH-releasing capabilities (U factor, or unknown factor).[25] The synergistic release of GH, in turn, is mediated by the dual complementary pituitary action of U factor plus GHRH. To what degree U factor may play a physiological role versus only a pharmacological role is presently unknown.[23] In any case, the most interesting quality of GHRP-6 is that its GH-releasing capabilities are not altered by metabolic signals. This property may well explain the preserved effectivity of GHRP-6 in the diabetic patients described in this report, and may be a tool to further understand the contribution of the somatotrope axis to the complications of diabetes mellitus.

In conclusion, our results demonstrate that normal-weight NIDDM patients have a preserved response of GH to GHRP-6, GHRH, and GHRP-6 + GHRH. Overweight NIDDM patients have a blunted response to GHRH and GHRP-6 + GHRH. The preserved GH response to GHRP-6 in both diabetic groups suggests that the secretory potential of somatotropes is preserved in NIDDM patients. The impairment of the GH response to GHRH in overweight NIDDM patients could be a functional defect possibly due to the obesity, since it could be overridden with administration of GHRP-6.

REFERENCES

1. Villas-Boas Weffort RF, Ramos-Dias JC, Chipoch C, et al: Growth hormone (GH) response to GH-releasing peptide-6 in patients with insulin-dependent diabetes mellitus. Metabolism 46:706-710, 1997

2. Kopelman PG, Mason AC, Noonan K, et al: Growth hormone response to growth hormone releasing factor in diabetic men. Clin Endocrinol (Oxf) 28:33-38, 1988

3. Martina V, Bruno G, Tagliabue M, et al: Blunted GH response to growth hormone–releasing hormone (GHRH) alone or combined with arginine in non–insulin-dependent diabetes mellitus. Horm Metab Res 27:26-30, 1995

4. Koev D, Zacharieva S, Popova J, et al: Effect of growth hormone releasing hormone on growth hormone, prostaglandin E2 and insulin in non–insulin-dependent diabetic patients. Methods Find Exp Clin Pharmacol 18:137-140, 1996

5. Cai HM, Jin JF, Lu YR: Blunted growth hormone response to hGRF 1-29 NH2 in patients with non–insulin-dependent diabetes mellitus. Chung Hua Nei Ko Tsa Chih 31:758-760, 1992

6. Giustina A, Bresciani E, Tassi C, et al: Effect of pyridostigmine on the growth hormone response to growth hormone–releasing hormone in lean and obese type II diabetic patients. Metabolism 43:993-998, 1994

7. Pan CY, Lu JM, Chang XM, et al: Non-specific response of serum growth hormone to thyrotropin-releasing hormone in diabetics. Chin Med J (Engl) 102:41-44, 1989

8. Giampietro O, Miccoli R, Ferdeghini M, et al: Inappropriate growth hormone response to luteinizing hormone–releasing hormone in diabetes mellitus. Metabolism 36:1149-1153, 1987

9. Franek E, Schaefer F, Bergis K, et al: Abnormal pulsatile secretion of growth hormone in non–insulin-dependent diabetes mellitus. Clin Endocrinol (Oxf) 47:471-478, 1997

10. Camanni F, Ghigo E, Arvat E: Growth hormone–releasing peptides and their analogs. Front Neuroendocrinol 19:47-72, 1998

11. Robinson IC: Hypothalamic targets for growth hormone secretagogues. Acta Paediatr Suppl 423:88-91, 1997

12. Muccioli G, Ghe C, Ghigo MC, et al: Specific receptors for synthetic GH secretagogues in the human brain and pituitary gland J Endocrinol 157:99-106, 1998

13. Ghigo E, Arvat E, Muccioli G, et al: Growth hormone–releasing peptides. Eur J Endocrinol 136:445-460, 1997

14. Carro E, Senaris R, Peino R, et al: Neuropharmacological assessment of growth hormone secretion. Acta Paediatr Suppl 423:12-16, 1997

15. Micić D, Mallo F, Peino R, et al: Regulation of growth hormone secretion by the growth hormone releasing hexapeptide (GHRP-6). J Pediatr Endocrinol 6:283-289, 1993

16. Popović V, Micić D, Damjanović S, et al: Evaluation of pituitary GH reserve with GHRP-6. J Pediatr Endocrinol Metab 9:289-298, 1996 (suppl 3)

17. Keen H, Barnes D: The diagnosis and classification of diabetes mellitus and impaired glucose tolerance, in Pickup J, Williams G (eds): Textbook of Diabetes, vol 1. Oxford, UK, Blackwell Science, 1997, pp 2.1-2.10

18. World Health Organization Expert Committee: Physical status: The use and interpretation of anthropometry: Overweight adults. World Health Organ Tech Rep Ser 854:312-344, 1995

19. Richards NT, Wood SM, Christofides ND, et al: Impaired growth hormone response to human pancreatic growth hormone releasing factor [GRF(1-44)] in type 2 (non–insulin-dependent) diabetes. Diabetologia 27:529-534, 1984

20. Cordido F, Penalva A, Dieguez C, et al: Massive growth hormone (GH) discharge in obese subjects after the combined administration of GH-releasing hormone and GHRP-6: Evidence for a marked somatotroph secretory capability in obesity. J Clin Endocrinol Metab 76:819-823, 1993

21. Mitsuhashi S, Yamasaki R, Miyazaki S, et al: Effect of oral administration of L-dopa on the plasma levels of growth hormone–releasing hormone (GHRH) in normal subjects and patients with various endocrine and metabolic diseases. Nippon Naibunpi Gakkai Zasshi 63:934-946, 1987

22. Popović V, Damjanović S, Micić D, et al: Blocked growth hormone–releasing peptide (GHRP-6)-induced GH secretion and absence of the synergic action of GHRP-6 plus GH-releasing hormone in patients with hypothalamopituitary disconnection: Evidence that GHRP-6 main action is exerted at the hypothalamic level. J Clin Endocrinol Metab 80:942-947, 1995

23. Bowers CY: Synergistic release of growth hormone by GHRP and GHRH: Scope and implication, in Bercu BB, Walker RF (eds): Growth Hormone Secretagogues in Clinical Practice. New York, NY, Dekker, 1998, pp 1-25

24. Cordido F, Penalva A, Peino R, et al: Effect of combined administration of growth hormone (GH)-releasing hormone, GH-releasing peptide-6, and pyridostigmine in normal and obese subjects. Metabolism 44:745-748, 1995

25. Bowers CY, Sartor AA, Reynolds DG, et al: On the actions of the growth hormone–releasing hexapeptide, GHRP-6. Endocrinology 128: 2027-2035, 1991