Effects of Acipimox, an Antilipolytic Drug, on the Growth Hormone (GH) Response to GH-Releasing Hormone Alone or Combined With Arginine in Obesity

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Increased free fatty acid (FFA) levels of obese patients are likely involved in the pathogenesis of the growth hormone (GH) hyposecretion of obesity. To clarify their role, we studied the influence of inhibition of plasma FFA levels, induced by 500 mg oral acipimox (ACX), an antilipolytic drug, on the GH response to GH-releasing hormone (GHRH) alone or combined with arginine ([ARG] study A) in six normal women ([NS] aged 24 to 37 years; body mass index, 22.4 ± 0.9 kg/m^2) and six obese women ([OB] aged 21 to 40 years; body mass index, 39.5 ± 3.2 kg/m^2). In a group of seven OB patients (aged 18 to 58 years; body mass index, 35.8 ± 1.3 kg/m^2), the effect of ACX on either the GHRH- or GHRH+ARG–stimulated GH increase was also studied after a 4-day treatment with the same drug at 250 mg three times daily (study B). OB patients had baseline FFA levels higher than NS (0.77 ± 0.06 *v* 0.44 ± 0.09 mmol/L, $P < .05$). In study A, ACX reduced FFA levels to the same nadir in both groups (0.11 ± 0.02 and 0.12 ± 0.03 mmol/L, NS and OB subjects, respectively). In NS, ACX failed to significantly potentiate the GH response to either GHRH (1,371.9 ± 425.2 *v* 1,001.8 ± 229.0 μg/L · min) or GHRH+ARG (3,558.4 ± 1,513.7 *v* 3,045.9 ± 441.8 μg/L · min), while in OB patients it increased the GH response to GHRH (797.6 ± 277.3 *v* 353.8 ± 136.7 μg/L · min, $P < .01$) and did not modify the response to ARG+GHRH (1,010.5 ± 253.1 *v* 821.1 ± 222.0 μg/L · min). In study B, ACX reduced FFA levels in OB patients (nadir, 0.09 ± 0.04 mmol/L). This treatment strikingly increased the GH response to GHRH (1,734.0 ± 725.4 *v* 271.5 ± 112.8 μg/L · min, $P < .01$) and significantly potentiated that to ARG+GHRH (2,371.9 ± 571.3 *v* 1,020.0 ± 343.2 μg/L · min, $P < .05$). In conclusion, our present findings indicate that an acute reduction of plasma FFA levels in OB patients restores their somatotrope responsiveness, whereas it does not affect GH secretion in lean subjects. After prolonged treatment, ACX further improves GHRH-stimulated GH secretion in OB patients, suggesting that elevated FFA levels play a leading role in the GH hyposecretory state of obesity.

IT IS WELL KNOWN that human obesity is characterized by a reduction of both spontaneous and stimulated growth hormone (GH) secretion.[1-5] The mechanisms underlying these alterations are still unclear. Experiments in genetically obese animals pointed to a hypothalamic dysfunction,[6-8] but in human obesity it seems unlikely. In fact, drugs stimulating GH secretion via inhibition of hypothalamic somatostatin release, such as pyridostigmine, atenolol, and arginine (ARG), increased but did not normalize GH secretion[9-11]; moreover, a prolonged treatment with GH-releasing hormone (GHRH) did not modify GH hyposecretion in obese patients.[12] Even the new synthetic GH-releasing peptides elicited a reduced GH increase in obese patients.[13,14]

On the other hand, in obesity, the impaired GH secretion was reported to be restored by weight reduction induced by a very–low-calorie diet.[15] Metabolic alterations may be the cause for GH insufficiency in obese patients. Hyperinsulinism could have a role, considering the ability of insulin to inhibit GH synthesis and release.[16,17] Furthermore, in obesity, there are increased free fatty acid (FFA) levels,[18,19] which have a strong negative influence on GH secretion in humans.[20,21]

To clarify the role of elevated FFA levels in causing GH insufficiency in obesity, we studied the effect of acute and prolonged inhibition of plasma FFA levels induced by acipimox (ACX), an antilipolytic drug,[22] on the somatotrope responsiveness to GHRH alone or combined with ARG. This amino acid is known to enhance the GH response to GHRH, likely acting via inhibition of hypothalamic somatostatin release.[23]

SUBJECTS AND METHODS

Thirteen obese ([OB] body mass index, 37.5 ± 1.7 kg/m^2; waist to hip ratio, 0.96 ± 0.02) but otherwise normal women and six normal women ([NS] body mass index, 22.4 ± 0.9 kg/m^2) were studied after informed consent had been obtained. The study protocol had been approved by our Department's Ethics Committee.

All subjects were studied after an overnight fast, in a recumbent position, 30 minutes after cannulation of a cubital vein kept patent by infusion of isotonic saline. Two studies were planned.

Study A

Six OB (age, 29.0 ± 3.3 years; range, 20 to 40) and six NS (age, 30.7 ± 6.1; range, 24 to 37) underwent the following tests performed in the early follicular phase on different nonconsecutive days: (1) GHRH (GEREF, Serono, Milan, Italy; 1 μg/kg body weight as an intravenous bolus at 0 minutes), (2) GHRH + ARG (arginine hydrochloride, Pharmacia, Milan, Italy; 0.5 g/kg ideal body weight infused from 0 to 30 minutes), (3) GHRH + ACX (Olbetam, Farmitalia-Carlo Erba, Milan, Italy; 500 mg orally at −90 minutes), and (4) GHRH + ARG + ACX.

In each test, blood samples were taken at −90, −15, and 0 minutes and then every 15 minutes up to 90 minutes.

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Study B

Seven OB (age, 31.6 ± 5.8 years; range, 18 to 57) underwent GHRH and GHRH + ARG tests before and after 3 and 4 days of ACX treatment, respectively. ACX treatment was performed with 250 mg of the drug every 8 hours, with the last dose 500 mg (−90 minutes before GHRH). Serum GH was measured at each time point in duplicate by immunoradiometric assay (HGH-CTK IRMA; Sorin, Saluggia, Italy). Sensitivity of the assay was 0.1 μg/L. Interassay coefficients of variation were between 4.9% and 6.5% (calculated at 40.8 ± 2.0 and 2.8 ± 0.2 μg/L, respectively); intraassay coefficients were between 1.5% and 2.9% (calculated at 3.0 ± 0.1 and 31.8 ± 1.0 μg/L, respectively). Plasma FFA levels were measured at −90, 0, 60, and 90 minutes and were assayed by enzymatic analysis using the NEFA QUICK "BMY" kit (Boehringer-Mannheim, Yamanouchi KK, Tokyo, Japan). All samples from an individual subject were measured in the same assay. IGF-I levels were measured at −90 minutes by immunoradioassay (Nichols Institute Diagnostic, San Juan Capistrano, CA) with previous acid-ethanol extraction to avoid binding-protein interference. The actual sensitivity of the assay was 8.4 μg/L. Interassay coefficients of variation were 10.1% to 15.7% (calculated at 197.6 ± 20.0 and 96.7 ± 15.2 μg/L, respectively); intraassay coefficients were 7.6% to 15.5% (calculated at 89.5 ± 6.8 and 404.3 ± 62.8 μg/L, respectively).

Results (mean ± SEM) are expressed for GH as absolute values (micrograms per liter) and as areas under the curve (micrograms per liter per minute) calculated by trapezoidal integration, and results for FFA and IGF-I are absolute values (millimoles per liter and micrograms per liter, respectively). Statistical analysis of the data was performed with Wilcoxon's signed-rank test and nonparametric ANOVA (Kruskal-Wallis test) where appropriate.

RESULTS

Study A

Basal GH levels in OB were lower than in NS (0.5 ± 0.1 *v* 3.7 ± 1.1 μg/L, $P < .01$).

Basal FFA and insulin levels in OB were higher than in NS (0.77 ± 0.06 *v* 0.44 ± 0.09 mmol/L and 11.4 ± 1.6 *v* 6.8 ± 1.2 mU/L, respectively, both $P < .05$). After ACX, they were significantly reduced in OB to the same extent as in NS (0.12 ± 0.03 *v* 0.11 ± 0.02 mmol/L).

The GHRH-induced GH increase was clearly lower in OB than in NS (area under the curve, 353.8 ± 136.7 *v* 1,001.8 ± 229.0 μg/L · min, $P < .01$). ARG significantly increased the GH response to GHRH in both groups ($P < .01$), but this response was less persistent in OB than in NS (821.1 ± 222.0 *v* 3,045.9 ± 441.8, $P < .01$) (Fig 1).

In NS, ACX did not significantly modify the GH response to either GHRH (1,371.9 ± 425.2 μg/L · min) or combined administration of ARG and GHRH (3,558.4 ± 1,513.7 μg/L · min). On the other hand, in OB, ACX increased the GHRH-induced GH increase (797.6 ± 277.3 μg/L · min, $P < .01$), but did not modify that induced by ARG+GHRH (1,010.5 ± 253.1 μg/L · min). After ACX, the GH response to GHRH in OB became similar to that in NS (Fig 1).

Study B

After long-term ACX administration, FFA levels were similar to those observed after acute administration (0.09 ± 0.04 mmol/L).

Long-term ACX treatment markedly enhanced the GH response to GHRH (1,734.0 ± 725.4 *v* 271.5 ± 112.8 μg/L · min, $P < .01$), so it was higher ($P < .05$) than that observed after acute ACX administration. Differently from short-term administration, long-term ACX even significantly increased the GH response to ARG+GHRH (2,371.9 ± 571.3 *v* 1,020.0 ± 343.2 μg/L · min, $P < .05$) (Fig 2).

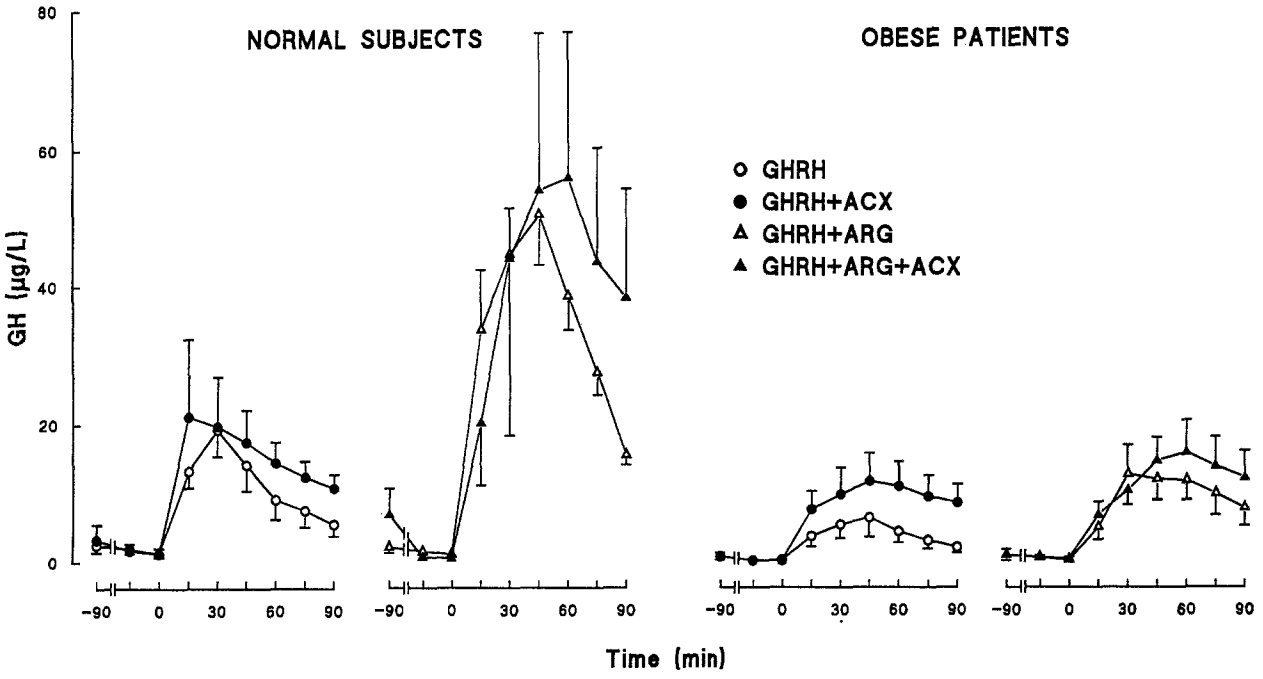

Fig 1. GH responses to GHRH alone and combined with ARG before and after short-term ACX administration in NS and OB (study A).

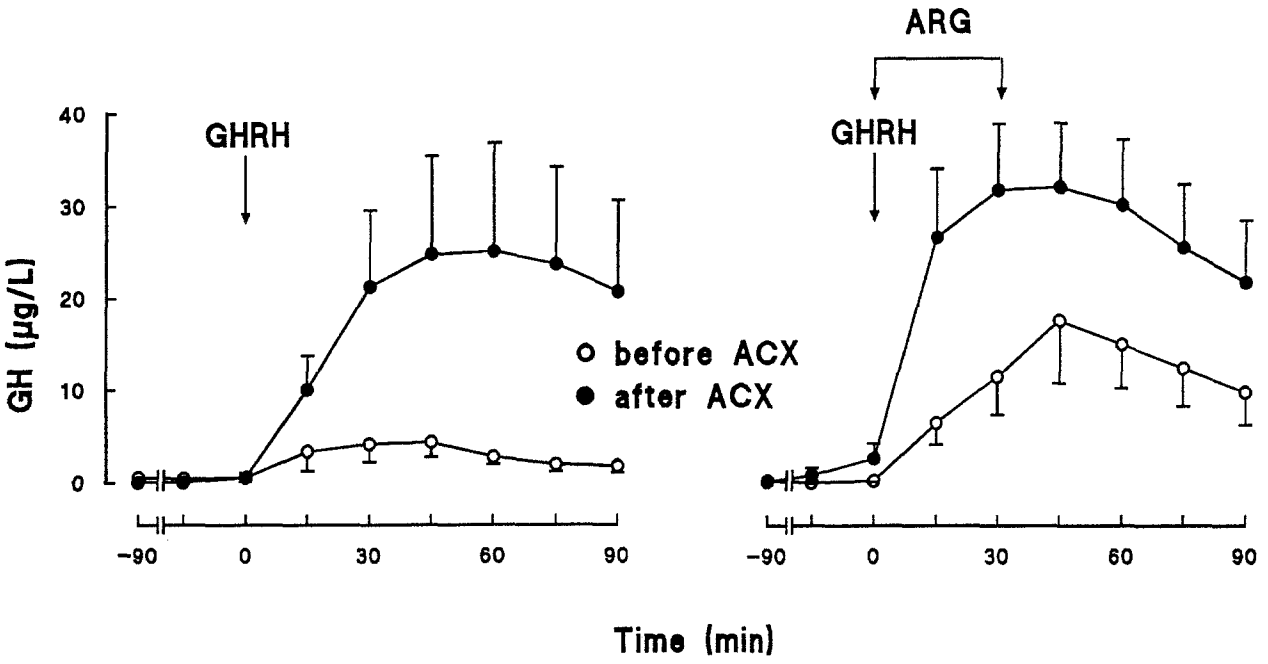

Fig 2. GH responses to GHRH alone or combined with ARG before or after prolonged ACX administration in OB (study B).

Side Effects

Most subjects complained of transient facial flushing after GHRH. No side effects were observed after ARG administration. All subjects experienced facial flushing lasting 1 to 2 hours after ACX administration.

DISCUSSION

Our present findings demonstrate that, differently from NS, in OB acute administration of ACX increases somatotrope responsiveness to GHRH. This effect is likely mediated by the reduction of high FFA levels. Moreover, prolonged ACX treatment more markedly increases the GHRH-stimulated GH increase and even enhances the effect of ARG on the GH response to GHRH, so that the somatotrope responsiveness to these stimuli become similar in OB and NS.

The reduction of both spontaneous[1,2] and stimulated[1,3-5] GH secretion in obesity is well known, although its pathogenesis is still unclear. Based on many data in animals, a hypothalamic impairment has been hypothesized. Particularly, alterations in the activity of GHRH and/or somatostatinergic neurons have been suggested.[6-8] However, studies in genetically obese animals[24] or in man[9-11] make somatostatinergic hyperactivity unlikely. On the other hand, the finding that repetitive GHRH administration fails to increase the response to GHRH[12] makes it also unlikely that hypoactivity of GHRH-secreting neurons plays a key role in causing GH insufficiency in obesity.

Alternatively, GH insufficiency in OB could be due to metabolic alterations. Among them, there is evidence that OB have high levels of FFA,[18,19] which are known to strongly inhibit GH secretion in humans.[20,21] Our present data showing that the ACX-induced inhibition of FFA levels increases the somatotrope responsiveness to GHRH in OB but not in NS strengthen the hypothesis that elevated FFA levels play an important role in the pathogenesis of GH insufficiency in obesity. The ineffectiveness of acute ACX in increasing the GH response to GHRH in NS is in contrast with a previous report by Pontiroli et al.[25] The different timing of GHRH administration in this study or the well-known variability of GH response to the neurohormone[26] could account for this discrepancy. Moreover, in a more recent study,[27] ACX was found to increase nocturnal spontaneous GH secretion in OB but not in NS. Thus, the reduction of FFA levels seems to allow an increase of somatotrope secretion only when FFA levels are elevated.

The mechanisms by which elevated FFAs inhibit GH secretion are still unclear. Although a somatostatin-mediated action has been proposed,[28] there is strong evidence for a direct inhibitory action on somatotrope cells. In fact, (1) oleic and caprylic acids inhibit both basal and GHRH-stimulated GH secretion from rat anterior pituitary[29,30]; (2) the inhibitory effect of FFAs persists even in hypophysectomized rats bearing the pituitary gland implanted under the renal capsule,[31] and it is not modified by immunization against somatostatin[31]; and (3) the increase of FFAs induced by lipid-heparin infusion in man overrides the GH-potentiating effect of substances acting via inhibition of hypothalamic somatostatin.[32,33] At the pituitary level, FFAs would act by counteracting the membrane depolarization of somatotrope cells.[30,34] Thus, chronic exposure of somatotrope cells to increased FFA levels could account for the reduction of GH secretion in obesity, while the inhibition of lipolysis by ACX would allow an improvement of membrane depolarization and GH release. The evidence that prolonged treatment with ACX has an effect

more clear than with acute administration, increasing GH response to GHRH even when combined with ARG, ie, when somatostatin tone is decreased, would indicate that long-lasting inhibition of FFAs allows a progressive improvement of somatotrope secretory activity and perhaps synthesis. In fact, there are data indicating that the somatotrope responsiveness to GHRH combined with ARG explores the maximal GH-releasable pool of somatotrope cells.[35]

As alluded to before, it should also be considered that a somatostatin-mediated action had been hypothesized to explain the inhibitory effect of FFAs on GH secretion, and we cannot definitively rule out this possibility. However, more recent data are strongly against this hypothesis, showing that FFAs do inhibit somatostatin release from the hypothalamus.[36] A somatostatin-mediated action for FFAs is unlikely also, based on our present results showing the additive effect of ACX and ARG on GHRH-stimulated GH release, at least after prolonged treatment.

Finally, the possibility has to be considered that the ACX-induced inhibition of high FFA levels in OB favors an increase of somatotrope secretion via other indirect mechanisms. In obesity, increased insulin levels[37,38] (and the present report) could have an inhibitory effect on GH synthesis and secretion,[16,17] and ACX is reported to strongly reduce the elevated insulin levels in OB with[39] and without[27] diabetes.

In conclusion, our present findings about the effect of acute and prolonged treatment with ACX in OB indicate that elevated FFA levels play an important role in causing GH insufficiency in obesity, and suggest that treatment with inhibitors of lipolysis could be useful to restore the somatotrope function.

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