Differential Effects of Growth Hormone and Prednisolone on Energy Metabolism and Leptin Levels in Humans

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Short-term growth hormone (GH) exposure has been shown to stimulate energy expenditure (EE) without concomitant changes in body composition. To what extent this is related to thyroid function, sympathetic activity, hyperinsulinemia, or leptin secretion is unknown. It is also unknown whether the calorigenic effect of GH is influenced by glucocorticoids, which are known to antagonize the anabolic actions of GH. To pursue this, eight normal male subjects (aged 22 to 28 years; body mass index, 21.6 to 26.3 kg/m^2) were randomly studied during four 4-day treatment periods with (1) daily subcutaneous (SC) placebo injections and placebo tablets, (2) daily SC GH injections (0.1 IU/kg · d) and placebo tablets, (3) daily prednisolone administration (25 mg morning and evening) plus placebo injections, and (4) daily GH injections plus prednisolone administration. GH administration decreased plasma epinephrine significantly (mean ± SE, 34.7 ± 5.7 ng/L for control *v* 24.8 ± 5.8 for GH, $P < .05$), had no effect on plasma norepinephrine or serum leptin, and increased both free triiodothyronine (FT_3) levels (5.7 ± 0.3 pmol/L for control *v* 6.7 ± 0.3 for GH, $P < .05$) and resting EE ([REE] 1,861 ± 61 kcal/24 h for control *v* 1,996 ± 69 for GH, $P < .05$). Prednisolone administration did not affect epinephrine and REE, decreased norepinephrine (116 ± 13, $P < .05$) and FT_3 (4.7 ± 0.2, $P < .05$), and increased leptin (3.93 ± 0.71, $P < .05$). Concomitant GH and prednisolone administration increased REE (2,068 ± 85, $P \pm .05$) and leptin (4.82 ± 0.93, $P \pm .05$), had no effect on either epinephrine or norepinephrine, and decreased FT_3 (5.0 ± 0.2, $P < .05$). Resting heart rate (HR) increased only during GH, whereas sympathetic nerve activity was unchanged in all studies. Our data suggest that (1) the calorigenic effect of GH is not mediated by changes in sympathetic activity or leptin secretion, (2) rapid elevations in leptin induced by glucocorticoids do not affect EE in humans, and (3) the acute calorigenic effects of GH are probably related to increased cardiac workload.

SEVERAL LINES OF EVIDENCE support the notion that growth hormone (GH) stimulates energy expenditure (EE). Increased resting EE (REE) is reported in active acromegaly,[1,2] whereas GH deficiency is associated with subnormal REE.[3,4] Furthermore, GH administration in normal subjects,[5] obese women,[6,7] and GH-deficient adults[3,8] is accompanied by a significant increase in REE. It has been suggested that the calorigenic actions of GH are secondary to increments in lean body mass (LBM). On the other hand, stimulation of EE has been recorded after only 5 hours of intravenous GH infusion in normal subjects,[9] and a significant decline in REE has been noted after short-term (24-hour) discontinuation of GH substitution in GH-deficient adults,[8] both of which imply that GH may stimulate EE independent of body composition.

A recent study of the effect of GH administration following thermal injury in children reported that GH increased catecholamine levels.[10] Whether GH administration in the normal adult increases catecholamines and, if so, whether this increase in circulating catecholamines contributes to the concomitant increase in EE has not been experimentally addressed.

Several placebo-controlled trials have shown that GH administration stimulates peripheral conversion of thyroxine (T_4) to triiodothyronine (T_3) in both normal and obese adults, as well as GH-deficient adults.[5,7,8,11] Glucocorticoid therapy, by contrast, inhibits peripheral conversion of T_4 to T_3, causing a decline in serum T_3 of about 20% to 40%.[12,13] It is also previously reported that GH abolishes the protein-catabolic effects of prednisolone,[14] whereas coadministration of the two compounds causes insulin resistance in a synergistic manner.[14]

The interaction of GH and glucocorticoids on energy metabolism and thyroid function has not previously been examined. In this context, it is noteworthy that glucocorticoids and insulin are known to increase leptin levels,[15] whereas the effects of GH and iodothyronines on serum leptin levels are unknown.

In the present double-blind, placebo-controlled study, we compared the effects of GH and prednisolone alone and in combination on REE, catecholamines, thyroid function, and leptin levels in a group of healthy young adults.

SUBJECTS AND METHODS

Subjects

Eight healthy male volunteer subjects (aged 22 to 28 years; body mass index, 21.6 to 26.3 kg/m^2) participated. Before participation, the nature, purpose, and potential risks of the study were explained to all subjects, and their written informed consent was obtained. The protocol was approved by the regional ethics committee.

Study Design

Each subject was studied in four different experimental situations: (1) after 4 days of placebo injections and placebo tablets, (2) after 4 days of GH injections and placebo tablets, (3) after 4 days of placebo injections and prednisolone tablets, and (4) after 4 days of GH injections and prednisolone tablets. At least 10 days separated each study. GH (Norditropin; Novo Nordisk, Gentofte, Denmark) or placebo were given by subcutaneous (SC) self-injections at 8:00 PM. The dose was 0.1 IU/kg/d or maximally 10 IU/d. Prednisolone (50 mg/d) or placebo tablets were given orally, 25 mg in the morning and 25 mg in the evening. The experiments were performed in random order. The patients were instructed to follow a weight-maintaining diet including a minimum daily intake of 200 g carbohydrate. At the end of each period, the subjects were hospitalized and kept fasted in the supine position

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from 10:00 PM. On the following day, REE was estimated by indirect calorimetry for 30 minutes.

Blood Sampling

Blood was sampled for measurement of catecholamine, thyroid hormone, GH, insulin, leptin, and insulin-like growth factor-I (IGF-I) levels before each study period (baseline) and on the day of calorimetry (day 4). Samples were immediately frozen after centrifugation at −20°C or −80°C until assayed.

Calorimetry

REE was assessed by indirect calorimetry. A computerized, open-circuit system was used to measure gas exchange across a 25-L canopy (Deltatrac; Datex Instrumentarium, Helsinki, Finland). The monitor determines carbon dioxide production and oxygen consumption by multiplying the dry air flow through the canopy by the alterations in gas concentrations over the canopy. The initial 5 minutes of calorimetry were used for acclimatization, and the calculations represent mean values for 25 measurements performed with 1-minute intervals.

LBM

LBM was estimated by bioelectrical impedance (Animeter; HTS Instruments, Odense, Denmark) at baseline and on day 4 of each study arm. The measurements were performed in the supine position immediately after the bladder had been emptied. LBM could be calculated according to the following algorithm[16,17]: LBM = total body weight − 41.3 × z × weight/height^2 + 30.03, where z is bioelectrical impedance.

Autonomic Nervous System Activity

Quantification of variations in spontaneous heart rate (HR) (RR intervals) can be used to measure autonomic function, reflecting both parasympathetic and sympathetic function.[18] RR intervals were measured using an online telemetrical transmitter (VariaPulse TF3; Sima Media Olomouc, Vienna, Austria). In the calculations, a fast Fourier transformation was used.[18,19] Low-frequency (0.05 to 0.15 Hz) and high-frequency (0.15 to 0.50 Hz) components were determined, and the coefficient of component variances (square root of power/mean RR) was calculated.[18,19] The tests also allowed measurement of resting.

Assays

Serum GH and IGF-I levels were measured using an immunofluorometric assay (DELFIA; Wallac, Turku, Finland). Serum insulin, total T_4 (TT_4), free T_4 (FT_4), TT_3, FT_3, and reverse T_3 (rT_3) levels were measured by radioimmunoassay.[20-22] The serum leptin concentration was measured by a commercial radioimmunoassay (Linco Research, St Charles, MO). Catecholamine levels were measured by electrochemical detection.[23]

Statistical Analysis

Data are presented as the mean ± SEM. For each of four studies, changes in the measured variables before treatment (baseline) and after treatment (day 4) were evaluated by a paired *t* test or Wilcoxon's signed-rank test, where appropriate. To compare treatment effects among the four studies, repeated-measures ANOVA was performed on delta values (baseline to day 4), except for data on REE, HR, and autonomic nervous system activity, which were analyzed by one-way ANOVA on the absolute values. The Student-Newman-Keuls method for pairwise multiple comparison was used post hoc in case of significant differences. *P* values less than .05 are considered significant.

RESULTS

Indirect Calorimetry

Resting EE (kilocalories per 24 hours) was significantly increased following GH administration compared with both the control experiment and prednisolone treatment alone (1,996 ± 69 for GH *v* 1,861 ± 61 for control, $P < .05$, and 1,900 ± 85 for prednisolone, $P < .05$). Combined GH and prednisolone administration tended to increase EE even further compared with GH alone (1,996 ± 69 for GH *v* 2,068 ± 85 for GH + prednisolone, NS). Prednisolone alone did not alter EE compared with the control experiment (Fig 1). The respiratory quotient (RQ) was unchanged during prednisolone administration (0.84 ± 0.01 for control *v* 0.83 ± 0.01 for prednisolone, NS), while GH decreased the RQ, although not significantly (0.82 ± 0.01 for GH, $P = .09$). During GH + prednisolone, the RQ decreased significantly (0.79 ± 0.01 for GH + prednisolone, $P < .05$; Fig 1).

Catecholamines, Autonomic Nervous System Activity, GH, IGF-I, Insulin, and Leptin

Baseline plasma catecholamine levels (nanograms per liter) were similar in all experiments. Plasma epinephrine decreased significantly following GH administration (34.7 ± 5.7 for control *v* 24.8 ± 5.8 for GH, $P < .05$), but was unchanged in the other experiments (32.0 ± 4.6 for prednisolone and 31.2 ± 6.9 for GH + prednisolone, NS). Plasma norepinephrine decreased significantly following prednisolone (169 ± 19 for control *v* 116 ± 13 for prednisolone, $P < .05$), whereas norepinephrine was unchanged after GH alone and in combination with prednisolone (181 ± 34 for GH and 166 ± 26 for GH + prednisolone, NS). No changes in sympathetic nerve activity (percent) could be registered (2.33 ± 0.36 for control, 2.87 ± 0.26 for GH, 2.57 ± 0.32 for prednisolone, and 2.36 ± 0.39 for GH + prednisolone). HR (beats per minute) was significantly increased following GH administration compared with the control experiment (58 ± 2 for control *v* 65 ± 2 for GH, $P < .05$), and was significantly decreased following prednisolone (54 ± 2 for prednisolone, $P < .05$). GH + pred-

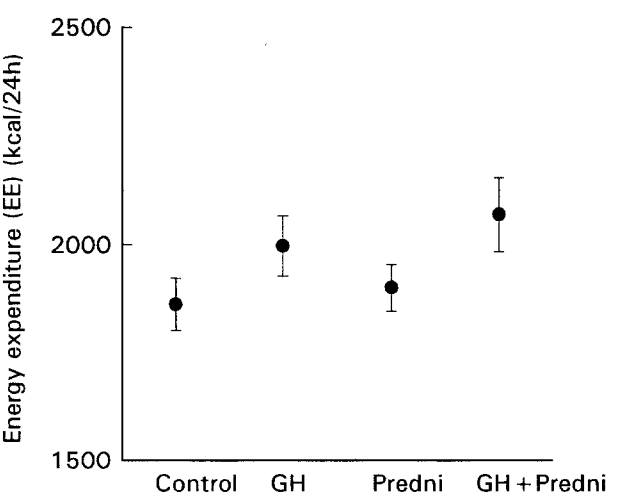

Fig 1. EE (kcal/24 h) following different modes of GH and T_3 exposure. Predni, prednisone.

nisolone administration normalized the HR (60 ± 4 for GH + prednisolone, NS).

Parasympathetic nerve activity (percent) was increased by prednisolone administration (2.91 ± 0.46 for control *v* 4.76 ± 0.89 for prednisolone, $P < .05$), whereas GH and GH + prednisolone had no recordable influence on parasympathetic nerve activity (2.77 ± 0.28 for GH and 3.31 ± 0.47 for GH + prednisolone, NS).

Baseline levels of serum GH and IGF-I (both in micrograms per liter) were similar in all experiments. Both serum GH and IGF-I were significantly elevated following GH administration (GH: 0.22 ± 0.09 for control *v* 2.73 ± 0.25 for GH *v* 3.44 ± 0.47 for GH + prednisolone, $P < .01$; IGF-I: 276 ± 9 for control *v* 677 ± 26 for GH *v* 542 ± 10 for GH + prednisone, $P < .01$). Prednisolone administration alone did not alter serum GH (0.56 ± 0.13, $P = .76$) or IGF-I (298 ± 9, $P = .60$).

Baseline serum insulin levels (picomolars) were similar in all experiments. Serum insulin concentrations increased during both GH and prednisolone (43.1 ± 2.8 for control *v* 86.1 ± 5.1 for GH and 74.9 ± 4.3 for prednisolone, $P < .05$), and GH + prednisolone increased insulin even further (134.9 ± 6.9, $P < .01$) (Fig 2).

Baseline serum leptin levels (micrograms per liter) were similar in all experiments. GH administration did not change leptin concentrations (3.15 ± 0.56 for control) *v* 2.94 ± 0.51 for GH, NS), whereas prednisolone administration increased leptin (3.93 ± 0.71, $P < .05$) and coadministration of GH and prednisolone augmented it even further as compared with the placebo and prednisolone experiment (4.82 ± 0.93, $P < .05$) (Fig 2).

Thyroid Function

For all parameters, baseline values were comparable. FT_3 concentrations were significantly higher after GH compared with the placebo experiment, while prednisolone and GH + prednisolone resulted in comparably decreased levels of FT_3. The same pattern was found for TT_3. FT_4 levels after placebo, GH, prednisolone, and GH + prednisolone were unaltered, and TT_4 levels were also unchanged except for a significant decrease following GH + prednisolone. The TT_3/TT_4 ratio was significantly elevated during GH and reduced during prednisolone. Thyrotropin decreased significantly after prednisolone and GH + prednisolone compared with both the control and the GH experiment. GH alone decreased rT_3 significantly compared with the control study. rT_3 was significantly increased following prednisolone and also during GH + prednisolone (Table 1).

Additional Parameters

No alteration in LBM (kilograms) was recorded (LBM difference, day 4 − day 1, −0.4 ± 0.9 for placebo, −0.7 ± 1.1 for GH, −0.7 ± 0.9 for prednisolone, and −1.0 ± 1.0 for GH + prednisolone, all comparisons NS).

Serum free fatty acids (nonesterified fatty acids [NEFAs]) increased significantly during GH administration (0.4 ± 0.1 for control *v* 0.6 ± 0.1 for GH, $P < .05$, and 0.7 ± 0.1 for GH + prednisolone, $P < .01$), whereas glucocorticoid had no influence on NEFA levels.

DISCUSSION

Several lines of evidence support the notion that GH stimulates EE.[1-8] The specific mechanisms by which GH exerts this effect have not been elucidated, but several suggestions have been made, ie, changes in LBM, increased cardiac workload, and increased levels of iodothyronines, insulin, or IGF-I. In a previous study, we have shown that the calorigenic effect of GH is not only attributable to changes in LBM or iodothyronines.[11]

The present study was undertaken to test whether the calorigenic effect of GH was associated with changes in catecholamines and/or autonomic nervous system activity. Inclusion of glucocorticoid therapy allowed us to study the effects of changes in iodothyronines on REE, the changes being reciprocal to those seen during GH alone. Further, an evaluation of the effect of hyperinsulinemia on REE with or without elevated GH/IGF-I concentrations was possible. We also included the measurement of serum leptin inasmuch as this peptide, which is regulated by insulin and cortisol, has been reported to stimulate EE in rodents.[24]

The study confirms the ability of GH administration to stimulate REE. We did not find evidence of increased sympathetic activity following GH administration. Moreover, the ability of GH to increase resting HR, which was reproduced in the present study, does not seem to be mediated by sympathetic

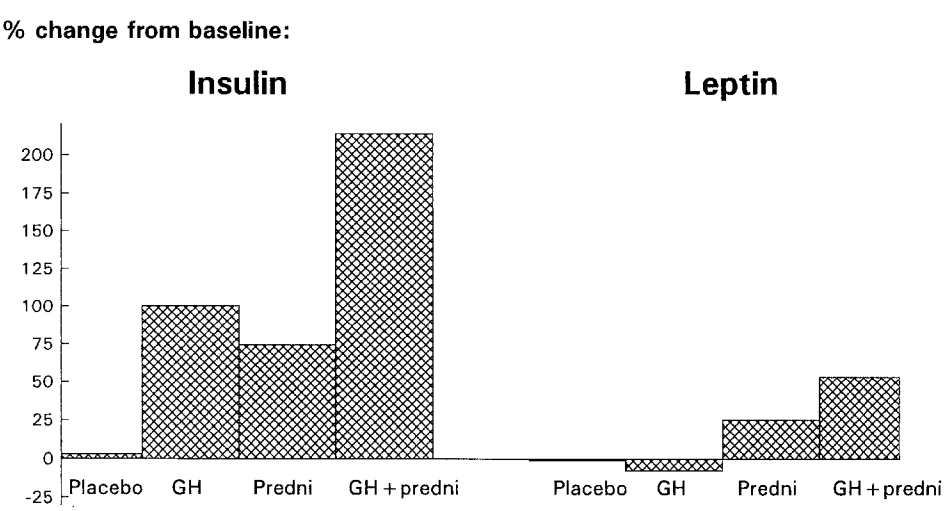

Fig 2. Changes in insulin and leptin levels (% change from baseline) following 4 different conditions.

Table 1. Thyroid Status Before and After 4-Day Therapy

	Placebo		GH		Prednisolone		GH + Prednisolone	
	Baseline	Day 4	Baseline	Day 4	Baseline	Day 4	Baseline	Day 4
TT_3 (nmol/L)	1.89 ± 0.1	1.70 ± 0.1	1.78 ± 0.1	1.93 ± 0.1*	1.83 ± 0.1	1.41 ± 0.1*†	1.86 ± 0.1	1.59 ± 0.1*†
TT_4 (nmol/L)	157 ± 9	147 ± 6	147 ± 5	138 ± 5	149 ± 6	137 ± 9	150 ± 7	128 ± 7*
TT_3/TT_4	0.012 ± 0.001	0.012 ± 0.001	0.012 ± 0.001	0.014 ± 0.001*	0.013 ± 0.001	0.011 ± 0.001*†	0.012 ± 0.001	0.012 ± 0.001†
FT_3 (pmol/L)	5.6 ± 0.2	5.7 ± 0.3	5.2 ± 0.3	6.7 ± 0.3*	5.5 ± 0.2	4.7 ± 0.2*†	5.5 ± 0.2	5.0 ± 0.2*†
FT_4 (pmol/L)	18.4 ± 1.5	17.3 ± 1.6	18.1 ± 1.5	18.0 ± 1.3	16.9 ± 0.8	16.1 ± 1.4	19.0 ± 1.9	15.8 ± 0.8
rT_3 (nmol/L)	0.31 ± 0.03	0.27 ± 0.03	0.26 ± 0.03	0.19 ± 0.01*	0.30 ± 0.03	0.52 ± 0.07*†	0.28 ± 0.02	0.39 ± 0.04*†
Thyrotropin (mU/L)	1.8 ± 0.3	1.6 ± 0.3	1.4 ± 0.2	1.4 ± 0.2	1.4 ± 0.3	0.82 ± 0.1*†	1.6 ± 0.2	0.5 ± 0.1*†

NOTE. All baseline values were comparable. For comparisons of prednisolone *v* GH + prednisolone, all NS except rT_3 ($P < .05$).

*$P < .05$, placebo *v* GH, prednisolone, and GH + prednisolone.

†$P < .05$, GH *v* prednisolone and GH + prednisolone.

pathways. The results on catecholamines are in conflict with results from a previous study showing increased levels of catecholamines following GH administration.[10] That trial was performed in severely burned children who had undergone an autograft operation and an optional reoperation. It is obviously difficult to compare results from healthy adults and severely catabolic children.

We monitored autonomic nerve activity with an online telemetrical transmitter measuring RR intervals, a method that has been validated in several studies.[18] No influence on sympathetic activity could be registered in either of the clinical experiments compared with the control experiment. Parasympathetic activity increased during glucocorticoid administration, which is compatible with the concomitant decrease in HR.

The present study also indirectly addressed the possible influence of peripheral T_4 to T_3 conversion on REE. In accordance with previous studies, GH administration increased peripheral T_4 to T_3 conversion,[5,7,8,11] whereas prednisolone decreased it. The combination of GH + prednisolone resulted in a moderate reduction in T_4 to T_3 conversion together with a significant increase in REE. Taken together, these observations suggest that the calorigenic effects of GH are not mediated by the associated mild elevation in T_3, which is in agreement with a previous study from our group.[11]

Variations in dietary habits may also influence REE. The subjects were instructed to follow a weight-maintaining diet including a minimum daily intake of 200 g carbohydrate to avoid glycogen depletion. Changes in dietary habits are more likely to affect 24-hour EE, whereas postabsorptive EE as measured in our study should be less dependent on dietary intake. Further, no changes were recorded in body weight.

It is firmly established that LBM is a positive determinant of REE.[25,26] In the present short-term study, no change in LBM was found during either GH or glucocorticoid administration. To extrapolate LBM from bioimpedance analysis requires a constant hydration of lean tissues. It is well known that GH may increase extracellular volume, which will lead to an overestimation of the change in LBM. Thus, values for LBM should be interpreted with some caution.[27-29] Still, the fact that LBM is overestimated, if anything, strengthens our observation that the observed increase in REE was independent of LBM.

Changes in cardiac workload and skeletal blood flow following GH may potentially affect REE. We observed a significantly increased HR following GH administration, whereas HR returned to normal during GH + prednisolone despite mildly increased REE.

Prolonged GH excess during nonfasting conditions causes hyperinsulinemia,[30] which is known to increase REE in normal adults.[31] In the present experiment, we found equally elevated insulin levels during GH and glucocorticoid administration, respectively. Furthermore, the additional increase in insulin during coadministration of GH and glucocorticoid did not alter REE from the level seen during GH alone.

The role of IGF-I, which mediates several GH-induced metabolic changes, is also unclear. Conditions of GH hypersecretion and low IGF-I, which is a common denominator of catabolic states, are associated with low REE.[32] Other experiments with concomitant high GH and IGF-I showed elevated REE.[5] These results suggest that IGF-I could mediate the effects on REE observed during GH therapy. On the other hand, studies with biosynthetic IGF-I in GH-deficient patients showed that REE did not increase to the same extent following IGF-I compared with GH administration.[33] In the present study, high IGF-I during GH and GH + glucocorticoid was followed by increased REE. Conversely, coadministration of GH and glucocorticoid tended to decrease IGF-I ($P = .09$) and increase REE, respectively. By and large, IGF-I may mediate some but not all calorigenic effects of GH.

Leptin, the product of the obese gene, is a peptide secreted by the adipocytes and is suggested to signal the amount of adipose tissue, thereby taking part in the hypothalamic regulation of appetite.[34] Further, leptin administration in the obese mouse increases EE.[24] We observed no effect of 4-day GH administration on leptin concentrations, indicating that leptin is not involved in the calorigenic effect of GH. To our knowledge, the present study is the first to evaluate leptin concentrations during controlled glucocorticoid administration in humans. The observation of a 25% increase in leptin following short-term prednisolone clearly suggests that glucocorticoids in humans also regulate leptin secretion independent of changes in total body fat. The greater than 50% elevation in leptin levels after GH + prednisolone was surprising, considering that GH alone had no effect. It is likely that this further increase in leptin is related to the associated pronounced elevation in insulin.

Taken together, our data suggest that the increase in REE following short-term GH administration is not mediated by changes in body composition, thyroid function, sympathetic activity, or leptin secretion. A putative mechanism could be the

elevation in resting HR. It has previously been shown that this GH-induced increase in resting HR is accompanied by unaltered systolic and diastolic blood pressure, and therefore, the cardiac workload (rate-pressure product) is increased.[35] Moreover, echocardiographic studies have also shown that GH increases cardiac output, which implies a reduction in peripheral resistance.[35] In this regard, it is interesting that both GH and IGF-I have been shown to directly increase forearm blood flow.[36,37] Since skeletal muscle metabolism is a major determinant of REE,[38] it can be speculated that GH increases REE through a flow-induced increase in skeletal oxygen uptake. Clearly, substantiation of this hypothesis will require additional studies.

Our study also confirmed that GH stimulates lipid oxidation. At present, it is unknown whether this is a cause or an effect of the increased REE. To scrutinize this relationship will also require new experiments involving, eg, experimental inhibition of lipolysis.

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