

Effects of Growth Hormone on Leptin Metabolism and Energy Expenditure in Hemodialysis Patients with Protein-Calorie Malnutrition

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Abstract. The relationships among growth hormone (GH), leptin, and resting energy expenditure (REE) are not understood. It has been reported that in malnourished hemodialysis patients, GH increases muscle protein synthesis, a process that requires energy. The present study evaluated the arterial levels and the forearm exchange of leptin, as well as the REE of the same patients during their participation in the same study, in four sequential 6-wk periods: I, baseline; II, GH treatment; III, washout; and IV, GH + intradialytic parenteral nutrition. During periods II and IV, patients received GH (5 mg three times per week). REE rose by 5% in period II, declined during period III, and rose by 7% during period IV. Basal leptin levels were low (2.0 ± 0.19 ng/L). Insulin and leptin levels, as well as leptin release from the forearm, were unchanged during periods I through III but rose (+36%; $P < 0.05$) during period IV. Changes in arterial leptin were directly related to changes in

forearm leptin release ($P < 0.002$), indicating a role of leptin production by peripheral tissues on leptinemia. Changes in leptin release were directly related to insulin ($P < 0.001$) and, less consistently, to insulin-like growth factor-binding protein-1 levels ($P < 0.02$). Similarly, variations in leptin levels were directly related to insulin ($P < 0.01$). Variations in REE were not related to variations in leptin or insulin levels but to changes in muscle protein synthesis ($P < 0.025$). The data show that in malnourished hemodialysis patients, treatment with GH is not invariably associated with an increase in leptin production. An increase in leptin release by peripheral tissues and leptin levels occurs only in the setting of hyperinsulinemia. The increase in REE that is induced by treatment with GH is not dependent on changes in leptin but is largely accounted for by the energy cost of the stimulation of muscle protein synthesis.

Leptin, a hormone that is secreted by adipocytes, provides signals from the peripheral tissues to the central nervous system to inhibit appetite and increase energy expenditure (1). Leptin accumulates in uremia and is believed to be an important factor in the development of malnutrition in this condition (2–4). Serum leptin levels are highly correlated with body fat mass in normal adults, children, and newborns (5). In humans, there are differences in leptin gene expression in relation to the location of fat deposits; greater gene expression occurs in subcutaneous as opposed to omental fat, suggesting that subcutaneous fat is an important source of leptin (6). In this regard, it has been observed that subcutaneous fat is responsible for the

greater amount of leptin release, whereas intra-abdominal fat seems to play a minor role (7). We (8) recently observed that in the normal condition, in the postabsorptive state, leg peripheral tissues (which are predominantly composed of muscle but also contain a sizable amount of fat) exhibit a net leptin release into the circulation. In accordance with the net leptin release from the lower limb and with similar muscle and fat composition, we also observed the occurrence of a net leptin release across the upper limb in healthy volunteers (unpublished observations). Jensen *et al.* (9) showed that leptin is released from the lower limb in amounts that are proportional to the limb fat content and likely represent adiposity. It is of note, however, that leptin is also expressed in rat skeletal muscle, where it plays a role as a sensor for energy bioavailability (10). Therefore, the measure of leptin balance across limbs could provide information regarding the overall leptin export from peripheral fat and muscle, of which limbs are mainly composed.

One of the mechanisms for hyperleptinemia in uremic patients is decreased removal, given the role of the kidney in leptin clearance (2). However, leptin production can also be stimulated by several hormones that often increase in plasma in uremia, such as glucocorticoids (11), insulin (12), and growth hormone (GH) (5). There is no direct information regarding

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leptin production in uremic patients. Studies in animals have shown that fat metabolism, energy metabolism, and muscle protein turnover are closely connected in uremia (13,14). Li and Wassner (15) showed that in uremic rats, a decrease in fat mass is associated with the development of muscle loss. However, signals and metabolic pathways involved in muscle fat and protein metabolism are poorly explored in uremia. GH both causes lipolysis and increases energy expenditure (16). Fouque *et al.* (17) showed recently that GH but not insulin-like growth factor-1 (IGF-1) acutely increases leptin levels in hemodialysis (HD) patients. It is therefore possible that the GH-induced effects on energy expenditure are mediated by an increase in leptin arterial levels and production. There are several other observations that indicate that GH and leptin systems are physiologically connected, because leptin may be important in the regulation of GH secretion (18) as well as in bone growth (19).

We recently reported the short-term effect of GH on muscle protein turnover in HD patients with protein-calorie malnutrition (20). In brief, our results showed that a 6-wk treatment with GH causes a significant increase in muscle protein synthesis (PS), an energy-requiring process; the observed anabolic changes were dependent on the increase in circulating “free” IGF-1 levels. In the present study, to evaluate the effects of GH administration on leptin arterial levels as well as production from peripheral tissues and their relations with energy expenditure, we report the results regarding the arterial levels and the forearm exchange of leptin, as well as the resting energy expenditure (REE) of the same patients (1) during their participation in the same study (20) and (2) during the administration of a sequential 6-wk cycle of therapy with GH associated with intradialytic nutrient supplementation.

Materials and Methods

Patient Characteristics

Six HD patients (five men and one woman) participated in the study. Their mean age was 60 ± 4 yr, and their mean dialytic age was 5.5 ± 2 yr. All patients showed evidence of chronic, stabilized malnutrition. They had lost weight (from 3.5 to 11 kg) more than 6 mo before the study, during intercurrent illnesses and/or reduction of dietary intake. They had been clinically stable, with no further loss or gain of weight, for at least 3 mo before the study and in the 6 wk of baseline follow-up. Their body mass index was 17.8 ± 0.8 (range, 15.9 to 21). Their body weight relative to ideal body weight was $79 \pm 3\%$ (range, 75 to 90%). According to the classification of protein-energy malnutrition based on weight for height (21), one patient could be classified with severe (weight for height < 70% relative to the median NCHS standards), three with moderate (weight for height < 70 to 79%), and two with mild (weight for height < 80 to 90%) protein-energy malnutrition. All of them were lymphocytopenic (lymphocyte count, 1334 ± 200 cells/mm³). Serum albumin was 4.1 ± 0.1 g/dl. In dialysis patients, serum albumin may be reduced because of chronic inflammation or the coexistence of acute catabolic illnesses. Patients with signs of inflammation were excluded from the present study. All patients studied here had CRP levels of less than 5 mg/L. Protein catabolic rate was 1.1 ± 0.1 g/kg, and calorie intake was approximately 28 to 31 kcal/kg, as estimated by nutritional interviews. Patients were on a thrice weekly dialysis schedule; their Kt/V was

1.3 ± 0.10 and was not changed during the study. The study was approved by the Ethical Committee of the Department of Internal Medicine of The University of Genoa. All subjects were informed about the nature, purposes, procedures, and possible risks of the study before their informed consent was obtained. The procedures were in accordance with the Helsinki declaration.

Protocol

The study consisted of a prospective crossover study in which patients were their own controls. The study was made up of four periods. The first three periods of the study design have been reported in detail previously (20). The periods of the study were as follows: period I, baseline; period II, GH treatment; period III, washout period; and period IV, GH + intradialytic parenteral nutrition (IDPN). During the GH treatment periods (periods II and IV), patients received GH (Genotropin; Pharmacia & Upjohn, Stockholm, Sweden) 5 mg subcutaneously at the end of each dialysis for 6 wk. During period IV, IDPN was combined with GH administration. The originally designed IDPN provided on average an additional 860 kcal (approximately 13 kcal/kg: 52% carbohydrate, 38% fat, and 10% amino acids; amino acids, 0.28 g/kg) for each dialysis session. The IDPN, starting with 50% of the total, was increased to the full strength in 2 wk. Four patients received the full IDPN for the full length of the study, whereas two patients developed symptoms (nausea, epigastric discomfort) during IDPN that impeded the complete administration. In these two patients, the amount of IDPN administered was 50% of the originally designed supplementation.

Methods

Patients were studied after approximately 72 to 74 h from the last dialysis, in the postabsorptive, overnight fasted state, to avoid the possible confounding effects of the dialytic treatment or of IDPN *per se* on measured variables. In the GH and the GH + IDPN periods, the last administration of GH (or of GH + IDPN) had been effected 72 to 74 h before the study. At approximately 8:00 a.m., a peripheral vein was cannulated with a Teflon catheter and used for isotope infusion. After 120 min, the procedures for catheter positioning were started. Catheters were introduced into a brachial artery and, in a retrograde fashion, into the ipsilateral deep forearm vein. After a 150-min tracer equilibration period, blood samples were taken simultaneously from the vein and the artery at 20-min intervals during a 60-min period.

Leptin was measured, in retrospective samples, when we observed that in the normal condition peripheral tissues are able to produce leptin (8). During the study, blood samples for hormone concentrations were collected in heparinized syringes and immediately kept in ice. Plasma was separated within 30 min by centrifugation at +4°C. Samples were stored at –80°C until assayed. Plasma leptin concentrations were assayed using a RIA method (DRG Instruments GmbH, Marburg, Germany). Serum concentrations of IGF-1, insulin-like growth factor-binding protein-1 (IGFBP-1), IGFBP-3, insulin, and cortisol were measured during the baseline and at the end of each study period as described previously (20). Serum FT3 and FT4 were measured by RIA.

IGFBP-2 levels were determined by double-antibody RIA using a nonequilibrium technique as described by Clemmons *et al.* (22). Specific IGFBP-2 antiserum was purchased from Upstate Biotechnology Incorporated (Lake Placid, NY), and the standard was a pure IGFBP-2 preparation obtained by DNA recombinant technology (ImmunoKontakt, Frankfurt, Germany). Radioiodination of IGFBP-2 was achieved by reacting 5 µg of protein with 1 mCi of Na¹²⁵I and 10 µg of chloramine T in a final volume of 100 µl of 0.5 M sodium

phosphate [pH 7.4]. After the reaction was terminated by the addition of 50 μg of sodium metabisulfite, unreacted iodide was removed by passage through a $0.7 \times 50\text{-cm}$ column of Sephadex G-100 in 0.01 M phosphate buffer 0.25% bovine serum albumin (pH 7.5). The specific activity was approximately 107 $\mu\text{Ci}/\mu\text{g}$ protein. Normal range for age-matched adult subjects is 150 to 350 ng/ml.

The methods for the measurement of muscle protein turnover are reported extensively elsewhere (20). Briefly, muscle protein metabolism was estimated by the forearm perfusion technique associated with ^3H -phenylalanine kinetics (23). By this technique, the rates of disposal of phenylalanine across the forearm in the steady state reflect the rates of incorporation of phenylalanine into protein, whereas tissue rates of appearance of phenylalanine reflect the release of phenylalanine from tissue protein breakdown.

The study of REE was performed simultaneously with the study of muscle protein turnover and leptin exchange. REE was assessed by indirect calorimetry (Beckman MMC-1 Calorimeter). The initial 30 min of calorimetry were used for acclimatization, and the calculations represent mean values of determinations obtained during 30 min thereafter.

Changes in body composition induced by treatment were estimated by anthropometry (24). Hematocrit was measured by a microcapillary procedure. All other serum chemical measurements were determined by routine clinical chemistry laboratory procedures.

Calculations

Forearm leptin balance was obtained by multiplying the arteriovenous difference for leptin by plasma flow. Forearm phenylalanine kinetics were calculated using the arteriovenous model (23) and are reported using arterial-specific activity as an expression of the phenylalanine precursor pool (20).

Statistical Analyses

All data are presented as the mean \pm SEM. Statistical analysis was performed using the two-tailed *t* test to compare arterial with venous data. When the arteriovenous difference was different from zero ($P < 0.05$) or when intragroup statistical significances were to be evaluated, a repeated measure ANOVA was used to compare the overall changes during the periods of the study. When ANOVA indicated statistical significance ($P < 0.05$), a *post hoc* F-based test was performed

between periods. Linear regression and correlation were used to evaluate the relationship between two variables. Statistical analysis was performed with the Statview Statistical Package (Abacus, Berkeley, CA).

Results

A summary of selected blood chemistries during different periods of the study is shown in Table 1. We have previously reported the effects of GH on some serum hormones, as well as IGFBP-1 and IGFBP-3 (20). In addition to what has been reported, IGFBP-2 levels declined markedly during GH treatment and tended to increase in the washout period (Table 1). Plasma insulin did not show changes during the first GH administration, whereas it increased significantly when GH was given together with IDPN (from 8 ± 0.9 to 14 ± 0.9 $\mu\text{U}/\text{ml}$ in the washout and GH + IDPN periods, respectively, $P < 0.02$). There was no overall change in serum thyroid hormones. Both treatments with GH and GH + IDPN were associated with a decline in BUN levels. There was no overall change in creatinine, glucose, and phosphate levels in response to both treatments.

The estimates of fat-free mass showed an increase of approximately 1 kg after a 6-wk GH course, with no further significant change in the following periods (Table 1). Fat mass tended to decrease, although not significantly.

Changes in REE that occurred during the study are shown in Table 2. After GH administration, REE rose significantly, by approximately 5%. REE declined subsequently after a 6-wk washout and rose by approximately 7% during GH + IDPN. Respiratory quotient exhibited a tendency toward a decline, although not statistically significant, in both periods of GH administration. Table 3 shows the effects of treatment on leptin. Basal leptin levels were markedly low and were not significantly modified by the first GH treatment or washout. During GH + IDPN, leptin levels rose by approximately 50%, even in the presence of a tendency toward a decrease in fat mass. At the baseline, leptin levels in the deep forearm vein

Table 1. Biochemical measurements and estimates of body composition using anthropometry, at the baseline, during GH, washout, and GH + IDPN periods^a

	Baseline	GH	Washout	GH + IDPN
BUN (mg/dl)	75 \pm 7	59 \pm 7 ^b	72 \pm 7	57 \pm 7 ^{c,e}
Glucose (mg/dl)	76 \pm 5	78 \pm 5	77 \pm 6	85 \pm 6
Phosphate (mg/dl)	5.0 \pm 0.0	5.7 \pm 0.3	6.0 \pm 0.5	5.0 \pm 0.5
Calcium (mg/dl)	10 \pm 0.4	10.1 \pm 0.4	9.7 \pm 0.4	9.7 \pm 0.3
IGFBP-2 (ng/ml)	1655 \pm 102	1053 \pm 64 ^{b,e}	1323 \pm 48	NA
FT3 (pg/ml)	1.9 \pm 0.3	1.8 \pm 0.2	2.3 \pm 0.2	1.8 \pm 0.2
FT4 (ng/ml)	11 \pm 1.1	13 \pm 0.3	11 \pm 1.4	12 \pm 0.4
Fat-free mass (kg)	47.6 \pm 2.8	48.6 \pm 2.8 ^c	48.2 \pm 2.8	48.8 \pm 2 ^c
Fat mass (kg)	4.6 \pm 1.0	4.2 \pm 0.8	4.0 \pm 0.79	4.0 \pm 0.8

^a GH, growth hormone; IDPN, intradialytic parenteral nutrition; IGFBP, insulin-like growth factor-binding protein; NA, not available.

^b Significance of difference from the corresponding value at baseline: $P < 0.02$ or less.

^c Significance of difference from the corresponding value at baseline: $P < 0.05$.

^d Significance of difference from the corresponding value at washout: $P < 0.05$.

^e Significance of difference from the corresponding value at washout: $P < 0.02$ or less.

Table 2. Effects of treatment with GH on REE and respiratory quotient^a

	Baseline	GH Treatment	Washout	GH + IDPN
REE (kcal/day)	1256 ± 46	1321 ± 41 ^b	1278 ± 50	1349 ± 51 ^c
Respiratory quotient	0.80 ± 0.02	0.76 ± 0.02	0.80 ± 0.03	0.76 ± 0.02

^a REE, resting energy expenditure.

^b *P* < 0.01 versus baseline.

^c *P* < 0.05 versus baseline.

Table 3. Leptin arterial levels and forearm leptin exchange during different periods of the study

	Baseline	GH	Washout	GH + IDPN
Leptin arterial levels (ng/l)	2.0 ± 0.19	2.2 ± 0.26	2.2 ± 0.28	3.0 ± 0.72 ^a
Leptin exchange (ng/min 100 ml)	-0.42 ± 0.08	-0.2 ± 0.05	-0.52 ± 0.15	-1.16 ± 0.26 ^b

^a *P* < 0.05 versus baseline.

^b *P* < 0.01 versus baseline.

were consistently greater (+11%; *P* < 0.001) than the corresponding arterial values. A similar arteriovenous gradient for leptin has been shown to occur across the leg in the healthy condition (8,9). During the first GH treatment, the release of leptin from the forearm tended to decrease. Conversely, treatment with GH + IDPN caused an increase in leptin release from peripheral tissues. It is of note that GH + IDPN caused a significant increase in insulin levels. In all periods of the study, changes in arterial leptin were directly related to changes in leptin release from the forearm (Figure 1).

Table 4 gives the results of linear regression and correlations between leptin release from the forearm and other variables. In all four periods of the study, changes in net leptin release were directly related to changes in arterial insulin (the greater the increase in insulin, the greater the increase in leptin production from peripheral tissues). In the basal, GH treatment, and washout periods, changes in net leptin release were also related to IGFBP-1 and, less consistently, to IGFBP-2 concentrations. Leptin release showed no significant relationships with changes in cortisol, total IGF-1, or IGFBP-3 levels over basal values. Similarly, during all periods of the study, the arterial leptin levels were directly related to arterial insulin (Table 5).

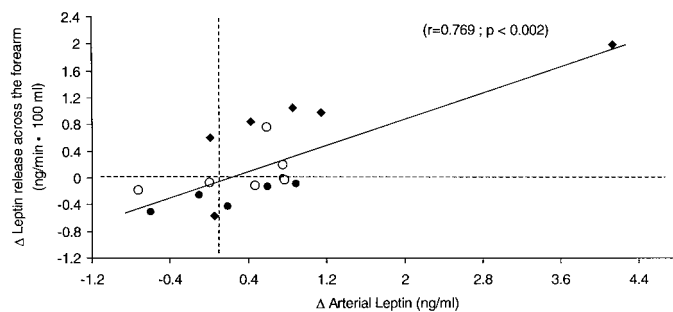


Figure 1. Relationship between changes in arterial leptin and leptin output from the forearm during treatment with growth hormone (GH) or GH + intradialytic parenteral nutrition (IDPN) in malnourished hemodialysis (HD) patients. ●, GH; ○, washout; ◆, GH + IDPN.

Table 4. Correlations between changes in leptin release from the forearm and other variables during treatment with GH

	<i>r</i>	<i>P</i> ^a
Δ insulin	0.703	0.001
Δ cortisol	0.336	NS
Δ IGF-I	-0.223	NS
Δ IGFBP-1	0.619	0.02
Δ IGFBP-2	0.394	0.1
Δ IGFBP-3	0.234	NS

^a NS, not significant.

Table 5. Correlations between changes in arterial leptin levels and other variables during treatment with GH

	<i>r</i>	<i>P</i>
Δ insulin	0.569	0.01
Δ cortisol	0.151	NS
Δ IGF-I	-0.170	NS
Δ IGFBP-1	0.316	NS
Δ IGFBP-2	0.03	NS
Δ IGFBP-3	0.281	NS

During all periods of the study, there was no relationship between changes in leptin release and changes in phenylalanine Rd (*r* = 0.25; *P* = NS) or net balance (*r* = -0.260; *P* = NS) across the forearm.

Variations in REE over basal values were not related to changes in arterial leptin levels during all periods of the study. However, variations in REE were positively related to changes in phenylalanine Rd across the forearm (Figure 2).

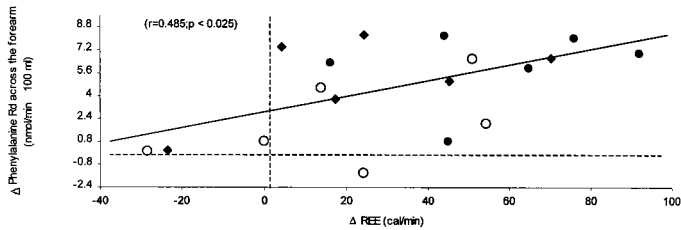


Figure 2. Relationship between changes in resting energy expenditure (REE) and muscle protein synthesis (as expressed by changes in the phenylalanine rate of disposal across the forearm) in malnourished HD patients during treatment with GH or GH + IDPN.

Discussion

Recent observations suggest that REE is increased in HD patients (25) and that it could in part account for the marked alterations in body composition that often are found in these patients, including a loss of both fat and fat-free mass, most of which is composed of skeletal muscle protein. It has been observed in cross-sectional studies that serum leptin levels are a strong positive determinant of REE (26) and food ingestion (27) in normal human. Moreover, leptin accumulates in patients with end-stage renal disease, and its levels are inversely correlated with protein catabolic rate, an index of protein intake, as well as with albumin concentrations (28), suggesting that leptin is important for determining nutritional status in dialysis patients.

There are several lines of evidence to suggest that GH can modulate energy expenditure and influence the leptin system. However, the relationships among leptin, GH, and energy expenditure are complex and not completely understood. GH is a determinant of REE in the young age (26); in several settings, the administration of GH increases REE independent of changes in body composition (26). However, leptin increases GH gene transcription and secretion in animals (29). Moreover, Fouque *et al.* (17) observed recently that the administration of GH in addition to IGF-1—but not IGF-1 alone—was able to increase leptin levels. Because GH + IGF-1—but not IGF-1 alone—increased energy expenditure (30), it is possible that leptin may be a mediator for these changes. The effects of GH on leptin levels may be variable, however (31). The present study was aimed at investigating the effect of GH administration on leptin levels and metabolism by forearm peripheral tissues, as well the relationships between REE and leptin metabolism in HD patients with protein-calorie malnutrition. Our data show that in HD patients with protein-calorie malnutrition, short-term treatment of GH is able to increase REE significantly. These observations are in accordance with results provided by several studies in healthy volunteers and in GH-deficient (16) and HD patients (31). However, our data do not support the hypothesis for the role of leptin in GH-induced thermogenesis. Patients studied here presented very low leptin levels, in keeping with that observed by Merabet *et al.* (3) in underweight uremic patients. However, in response to treatment with GH, leptin levels exhibited no significant change, and leptin increased only when GH was associated with IDPN.

Moreover, in all periods of the study, we observed no significant relationship between changes in arterial leptin and changes in REE.

It is of note that a significant release of leptin was found across the forearm in the baseline period, even in the presence of markedly low leptin levels and indices of body fat composition. Venous leptin concentrations were approximately 11% greater than in the arterial blood, demonstrating that leptin increases substantially in a single pass across peripheral tissues in malnourished patients. A similar (+14%) arteriovenous gradient for leptin has been shown to occur across the leg in the healthy condition (8). An estimate of leptin production by fat contained in forearm tissues can be obtained by the rate of leptin release. In the basal period, leptin release by peripheral tissues was approximately $0.4 \text{ ng/min} \times 100 \text{ ml}$ of forearm. From upper-arm-based anthropometric values, the percentage of fat of patients studied here was only 8 to 9%, a figure that is less than 50% of what is observed in normal nutritional conditions (32). Therefore, one could estimate their forearm fat content to be approximately 50% decreased from the average normal 20% forearm composition (33), *i.e.*, approximately 10 g. An estimate of the amount of leptin provided by forearm fat yields a figure of $4 \text{ ng/min} \times 100 \text{ g}$ of fat; such a figure is higher than the estimate provided by Jensen *et al.* (9) across the leg of healthy subjects (lean men, 2.4 to $3.1 \text{ ng/min} \times 100 \text{ g}$ of fat/min) and by Klein *et al.* (34) (3.2 ng/100 g of fat/min) across adipose tissue. This observation suggests that in malnourished HD patients, it is not the absolute rate of production by adipocytes but the decrease in overall fat mass that causes low leptin levels.

We observed that leptin release from the forearm declined slightly when GH alone was given but markedly increased when GH was given in association with nutritional supplements. Changes in arterial leptin were closely related to changes in forearm leptin release, suggesting an important role of peripheral fat in influencing leptin levels in uremia. Our data show, however, that a correlation between leptin levels and GH does not exist under all conditions. From our data, the major determinant of leptin is insulin concentration. Although during the first GH administration we found no significant changes in insulin levels, during the second period, in which GH was given in association with IDPN, insulin levels increased. In this regard, it is interesting that changes in both the forearm release of leptin and arterial leptin were related to changes in insulin levels. This is in keeping with previous studies indicating an important role of insulin in stimulating leptin production and with what was observed by Fouque *et al.* (17) during acute GH administration in dialysis patients. In the latter study, an increase in leptin was found in the presence of a significant increase in insulin levels. It is also of note that dialysis patients may have normal or high leptin levels, depending on insulin concentrations, and that fasting insulin levels of more than $14 \mu\text{U/ml}$ are associated with significantly greater plasma leptin concentrations, which are independent of body fat content (35).

In our study, we found no relationship between arterial leptin or peripheral tissue release and total IGF-1 levels. It is of note that in previous studies, no correlation between IGF-1 and

leptin levels has been observed either in the basal state or after IGF-1 administration in uremic patients (36). However, other authors have observed an inverse relationship between plasma leptin and IGF-1/IGFBP-3 ratio (37). Because the latter reflects the concentration of free IGF-1, it is conceivable that leptin may be related inversely to free IGF-1. In our study, leptin release from peripheral tissues was also inversely related to changes in IGFBP-1 and, less significantly, in IGFBP-2: the greater the decrease of the latter IGFBP (therefore, the greater the amount of free IGF-1), the lower the release of leptin from peripheral tissues. Thus, it seems that peripheral leptin production is modulated in an opposite fashion by insulin and the amount of circulating free IGF-1. It is interesting that, owing to the decline in kidney metabolic activity, patients with chronic renal failure may have slight hyperinsulinemia and raised levels of low molecular weight IGFBP, thus being “predisposed” to a stimulation of leptin production.

We have already reported the effects of GH on muscle PS (20). During treatment with both GH and GH + IDPN, the negative net phenylalanine balance across the forearm, *i.e.*, the result of difference of PS and protein degradation, declined significantly (by 36 to 46% *versus* the baseline or washout period). This was due to a similar increase in PS in both study periods, whereas no change in phenylalanine rate of appearance was observed. Changes in leptin release from peripheral tissues were not related to changes in muscle protein dynamics. Such an observation suggests that these processes, *i.e.*, muscle protein turnover and leptin export, are not interrelated.

What process may account for the GH-induced thermogenesis? Previous studies have shown that both short-term GH administration (30) and GH discontinuation in GH-deficient subjects (38,39) induce acute changes in REE independent of changes in body composition. In the short-term study presented here, patients underwent a slight increase in fat-free mass, which is unlikely to affect REE significantly. Moreover, changes in REE were not related to changes in fat-free mass. As an alternative possibility, we have to consider the GH-induced changes in muscle protein dynamics. PS is a process that requires energy (40). However, the relations between muscle PS and REE are poorly defined. There is only a limited number of reports in which both PS and oxygen consumption have been measured at the same time in humans (40). By this approach, the energy requirements of whole-body PS in humans have been estimated to account for approximately 20% of REE (41). Because muscle protein turnover accounts for approximately 40% of whole-body protein turnover in humans (42), one could estimate the cost of muscle PS to be approximately 8 to 10% of REE. Malnutrition reduces both protein turnover and REE (43), suggesting that the reduced muscle mass is responsible for the decline in energy expenditure. In the present study, the variations in REE and forearm phenylalanine rate of disposal in the same patients during different periods were significantly correlated (Figure 2), suggesting that GH-induced variations in muscle PS account for a significant fraction of REE changes. On the grounds that 60% of forearm volume is muscle, that muscle mass corresponds to 35 to 40% of body weight, and that phenylalanine is approxi-

mately 4% of actin composition, one could estimate that in these patients the overall daily muscle PS was 20 to 27 g. Considering that the energy cost of PS is approximately 5 kcal/g (43), one can estimate that the 25% increase in muscle PS induced by GH accounts for a rise in energy expenditure of approximately 25 to 38 kcal/d, as much as 38 to 58% of the increase in REE observed in the present study. It has to be underlined, however, that even if GH increases PS but has no effects on protein degradation, additional energy may be used not directly in PS but in other processes that are necessarily increased during GH administration.

It is interesting that an increase in basal insulin levels was not seen in the first part of the study, when GH was given alone, but was observed in the second part of the study, when patients received nutrient supplementation. These data suggest that GH antagonism regarding glucose disposal, with its attendant hyperinsulinemia, may be revealed by caloric supplementation (44). The insulin resistance caused by GH might exert an additional anabolic effect in muscle (45) with an acceleration of recovery of lean body mass, because in humans, even a small increase in insulin levels can cause a decrease in muscle protein degradation (46). In our study, however, the increase in systemic insulin observed during the administration of GH + IDPN was not associated with any significant change in muscle protein degradation. In this regard, it has also been observed that during the acute, combined administration of GH plus insulin, GH blunts the action of insulin to suppress muscle proteolysis (47). Therefore, our observation suggests that a compensatory increase in insulin levels during GH administration does not increase protein anabolism in addition to those caused by GH.

It is noteworthy that together with an increase in fat-free mass, a decrease in fat mass was observed in elderly HD patients who were treated with GH for 6 mo (48). In the protein-energy malnourished patients studied here, we observed a tendency toward a further decrease of a fat mass already diminished. The increased REE stimulated by GH may be a matter of concern in patients who are already energy depleted.

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GH and Leptin Metabolism and Energy Expenditure

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Dialysis

Effects of Growth Hormone on Leptin Metabolism and Energy Expenditure in Hemodialysis Patients with Protein-Calorie Malnutrition

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