



Metabolic effects of biosynthetic growth hormone treatment in severely energy-restricted obese women

M Tagliaferri¹, M Scacchi², AI Pincelli², ME Berselli¹, P Silvestri¹, A Montesano¹, S Ortolani², A Dubini² and F Cavagnini²

¹IRCCS Ospedale San Giuseppe, Istituto Auxologico Italiano, Piancavallo (Verbania); ²Second Chair of Endocrinology, University of Milan, IRCCS Ospedale San Luca, Istituto Auxologico Italiano, Milan, Italy

OBJECTIVE: Severe energy restriction in the treatment of obesity is limited by catabolism of body protein stores and, consequently, loss of lean as well as fat tissue. Growth hormone (GH), whose secretion is markedly impaired in obesity, is endowed with both lipolytic and protein anabolic properties. The aim of this study was to verify the effects of GH administration on body composition, plasma leptin levels and energy metabolism in obese patients undergoing severe dietary restriction.

DESIGN: Single-blind placebo-controlled study. Twenty obese women were fed a diet of 41.86 kJ/kg ideal body weight (IBW) daily for 4 weeks: 10 of them were randomly assigned to a 4 week treatment with biosynthetic GH (rhGH, Saizen, Serono, Rome, Italy), 1 U/kg IBW/week in daily subcutaneous injections; the other 10 patients, matched for age and BMI, received vehicle only.

SUBJECTS: Twenty women with simple obesity (age: 25.4 ± 1.07 y, BMI: 35.9 ± 0.35 kg/m²).

MEASUREMENTS: Plasma IGF-I and leptin, serum markers of bone turnover (serum bone isoenzyme of alkaline phosphatase, osteocalcin and urinary hydroxyproline), nitrogen balance, body composition (by DEXA), and resting energy expenditure (REE, by indirect calorimetry) were evaluated at baseline and after 4 weeks.

RESULTS: Mean IGF-I plasma levels, not influenced by energy restriction in patients receiving placebo, displayed a significant increase in the group treated with rhGH. The mean weight reduction and fat mass loss were not significantly different in the two groups (6.0 ± 0.51 vs 7.2 ± 0.30 kg, NS, and 5.36 ± 0.460 vs 4.28 ± 0.572 kg, NS, with rhGH and placebo, respectively). Likewise, plasma leptin levels decreased significantly in weight-reduced subjects receiving either rhGH (from 16.2 ± 2.37 to 6.4 ± 0.39 ng/ml, $P < 0.05$) or placebo (from 14.3 ± 2.55 to 7.7 ± 3.77 ng/ml, $P < 0.05$). On the contrary, the mean decrease of lean body mass (LBM) was significantly lower in the GH-treated patients than in those receiving vehicle (1.52 ± 0.60 vs 3.79 ± 0.45 kg, $P < 0.05$). In keeping with these findings, the mean daily nitrogen balance was significantly less negative in the GH-treated subjects than in the vehicle-injected patients (mean of the 4 week daily urine collections -185.7 ± 40.33 vs -363.9 ± 55.47 mmol/d, $P < 0.05$, respectively). Further, a significant reduction of mean REE was recorded in the energy-restricted placebo-treated patients (from 8807 ± 498 to 7580 ± 321 kJ/24 h, $P < 0.05$), but not in the patients receiving rhGH (from 8367 ± 580 to 8903 ± 478 kJ/24 h, NS). Actually, when corrected for LBM, REE was even increased by GH administration (from 197.9 ± 11.76 to 219.3 ± 9.87 kJ/kg LBM/24 h, $P < 0.05$), whereas it was unchanged in the placebo group (from 201.7 ± 13.85 to 190.0 ± 9.87 kJ/kg LBM/24 h, NS). A tendency of serum markers of bone turnover to increase was observed in the patients treated with rhGH, however with no changes in bone mineral content and density.

CONCLUSION: rhGH treatment, though unable to enhance diet-induced weight and fat mass reduction, was effective in stimulating IGF-I production and conserving LBM and increasing its energy metabolism even in the presence of severe energy restriction.

Keywords: obesity; growth hormone treatment; body composition; plasma leptin; resting energy expenditure; metabolic and hormonal parameters

Introduction

A major drawback of energy restriction for the treatment of obesity is represented by catabolism of body proteins and negative nitrogen balance. As a consequence, weight-reducing diets result in loss of lean as

well as fat tissue.¹ Since protein-supplemented diets produce only a modest nitrogen sparing,² more effective tools are needed to preserve protein stores and lean body mass (LBM), which is the main determinant of resting energy expenditure (REE),³ during dietary restriction.

Growth hormone (GH) is endowed with both lipolytic and protein anabolic properties.^{4–7} Its action is mostly mediated by insulin-like growth factor I (IGF-I), whose synthesis is greatly influenced by the nutritional status.⁸ In obesity spontaneous as well as pharmacologically triggered GH secretion is greatly impaired while peripheral levels of IGF-I are only

Correspondence: Prof. Francesco Cavagnini, Istituto Scientifico Ospedale San Luca, Istituto Auxologico Italiano, via Spagnoletto 3, 20149 Milano, Italy.
Received 9 October 1997; revised 9 April 1998; accepted 20 April 1998

inconstantly blunted. In a few trials, treatment with high-dose GH appeared effective, when compared to placebo, in conserving LBM in energy-restricted obese subjects,^{9–12} and in reducing body fat in obese women during a weight-maintaining diet.¹³ In addition, GH administration to GH-deficient adults is associated with an increase in REE, likely through the enhancement of peripheral conversion of thyroxine (T4) to triiodothyronine (T3).^{14,15} GH treatment was also found capable of increasing serum markers of bone turnover in normal volunteers¹⁶ and GH-deficient adults¹⁷ and, in the latter, bone mineral density in the long term.¹⁷

A decrease in plasma leptin concentrations has been described in obese patients after diet-induced weight loss¹⁸ and in GH-deficient adults after GH substitution therapy.¹⁹ The impact of the combination of GH treatment and energy restriction on peripheral leptin levels in obese patients is unknown.

The present study was aimed at evaluating the effects of GH treatment on weight loss, body composition and plasma leptin levels in a group of obese subjects undergoing severe dietary restriction. Another objective was to establish the influence of severe hypocaloric diet, associated or not with GH administration, on circulating levels of IGF-I. Finally, we investigated the effects of GH administration on REE, glucose and lipid metabolism, bone turnover and bone mineral content (BMC) and density (BMD).

Methods

Subjects

Twenty obese women aged 25.4 ± 1.0 7 (mean \pm standard error) years with a body mass index (BMI, calculated as weight divided by height squared, kg/m²) of 35.9 ± 0.35 gave their informed consent to participate in this study, which was approved by the Ethical Committee of our Institution. The subjects were in good general health and a medical evaluation including history, physical examination and routine blood chemistry revealed no abnormalities. Thyroid function was normal. None of the patients was taking any medications or exercising regularly during the 3 months preceding the study. During the same period of time, their body weight had been stable.

The subjects were studied as inpatients in our department. They were fed a balanced diet of 41.86 kJ/kg ideal body weight (IBW) daily for 4 weeks. Energy was supplied in a ratio of 50% carbohydrate (73.3 g), 30% fat (18.2 g) and 20% protein (27.9 g).

Care was exercised to maintain, during hospitalisation, the usual daily energy expenditure; this was accomplished by individual diary records and programs of physical activity.

According to a single-blind design, 10 patients were randomly assigned to a 4 week treatment with biosynthetic GH (rhGH, Saizen, Serono, Rome, Italy) 1 U/kg IBW/week in daily subcutaneous injections given at 8 pm. The other 10 patients, matched for age and BMI, received vehicle only.

Measurements

The subjects were weighed at the same time each morning before breakfast. Pulse rate and blood pressure were recorded daily. Circulating concentrations of glucose, insulin, free fatty acids (FFA), IGF-I, leptin, calcium, phosphate, bone isoenzyme of alkaline phosphatase (AP), osteocalcin, free thyroid hormones (fT4 and fT3), cholesterol and triglycerides were measured on blood samples collected after an overnight fast, under baseline conditions and at the end of the study. Twenty-four-hour urine samples were collected daily for nitrogen balance, and at baseline and after study completion for the estimation of calcium and hydroxyproline. Completeness of the collections was monitored by quantitating urinary creatinine excretion.²⁰ Urinary 24-h creatinine and urea nitrogen excretion were determined using autoanalyser modifications of published methods that measure both urea and ammonia nitrogen,^{20,21} 24-h nitrogen balance was calculated by subtracting the urinary urea nitrogen plus 4 g nitrogen from the total nitrogen ingested.^{22,23} Serum concentrations of glucose, calcium, phosphate, AP, cholesterol and triglyceride were measured by routine assays. Serum insulin concentrations were estimated by fluoro-enzymatic-immunoassay (FEIA) using a commercial kit (Tosoh, Tokyo, Japan); the normal range for insulin is 3.96–146.4 pmol/l. Plasma concentrations of IGF-I were measured, after extraction with acid-ethanol, by RIA using a commercial kit (Nichols Institute Diagnostic, San Juan de Capistrano, CA, USA); the normal range for IGF-I is, for females aged 16–26 years, 182–780 ng/ml. Plasma leptin was determined by RIA using a human leptin RIA kit (Linco Research, St. Charles, MO, USA). In our laboratory the mean value for plasma leptin obtained in 23 normal weight (BMI 23.6 ± 0.57) age and sex matched blood donors was 6.8 ± 0.93 ng/ml. Serum osteocalcin was measured by RIA using a commercial kit (Cis Biointernational, Gif sur Yvette, France); the normal range is 10.7–24.7 ng/ml. The assay of FFA was performed by an enzymatic method using reagents purchased from Boehringer Mannheim Yamanouchi (Tokyo, Japan); the FFA normal range is 250–270 mEq/l. The urinary concentrations of calcium, phosphate and hydroxyproline were measured by colorimetric assays, Boehringer Mannheim (Germany) for the first two parameters and by high-performance liquid chromatography (HPLC) for the third one. The normal ranges are 2.49–7.48 mmol/d for urinary calcium, 12.91–32.29 mmol/d for urinary phosphate and 76.26–305.0 μ mol/d/m² for urinary hydroxyproline. The analysis of body composition for

the evaluation of fat mass, LBM, BMC and BMD was performed at the beginning and at the end of the 4th week of the study by dual energy X-ray absorptiometry (DEXA, Hologic QDR-1000, Waltham, MA, USA). LBM comprises both muscle tissue and non muscle fat-free tissue (including the water space), with the exclusion of BMC. BMC and BMD are expressed as subtotal values, i.e. whole body minus head. REE was assessed by indirect calorimetry, after an overnight fast and a 30 min bed rest; patients were studied under basal conditions and weekly during the treatment. A computerized open circuit system was employed to measure gas exchange across a 251 canopy (Sensor Medics 2900, Yorba Linda, CA, USA). The values were expressed as kJ/24h and, when REE was corrected for LBM, as kJ/kg LBM/24h.

Statistical analysis

Data are expressed as mean \pm standard error, unless stated otherwise. Statistical analysis was performed using the Wilcoxon and Mann-Whitney non-parametric tests, as appropriate. A *P* value less than 0.05 was considered statistically significant.

Results

Mean IGF-I plasma concentrations were not influenced by energy restriction in patients receiving placebo (from 205.5 ± 40.6 to 239.6 ± 38.9 ng/ml, NS), whereas they displayed a significant increase in

the group tested with rhGH (from 240.7 ± 21.8 to 319.4 ± 30.0 ng/ml, *P* < 0.01).

The anthropometric parameters of all subjects are reported in Table 1. The mean weight reduction and fat mass loss were not significantly different in the two groups, although the GH-treated patients lost less weight and more fat mass than the placebo-treated patients (6.0 ± 0.51 vs 7.2 ± 0.30 kg, NS, and 5.36 ± 0.460 vs 4.28 ± 0.572 kg, NS, respectively). Plasma leptin concentrations were significantly decreased in weight-reduced subjects following either GH or placebo administration (Table 2). Like fat mass loss, leptin decrease was slightly greater in GH than in placebo-treated patients. On the contrary, the mean decrease of LBM was significantly lower in the GH-treated patients than in those receiving vehicle (1.52 ± 0.60 vs 3.79 ± 0.45 kg, *P* < 0.05). During the 41.86 kJ/kg IBW diet, mean nitrogen balance was less negative in the GH-treated patients compared to the vehicle-injected women (mean of the 4 week daily urine collections -185.7 ± 40.3 vs -363.9 ± 55.4 mmol/d, *P* < 0.05, respectively).

A significant reduction of mean REE was observed in the energy-restricted placebo-treated patients (from 8807 ± 498.9 to 7580 ± 321.9 kJ/24h, *P* < 0.05), whereas the same parameter was not modified in the patients receiving rhGH (from 8367 ± 580 to 8903 ± 478 kJ/24h, NS). Actually, when corrected for LBM, REE was even increased by GH administration (from 197.9 ± 11.76 to 219.3 ± 9.87 kJ/kg LBM/24h, *P* < 0.05), whereas it was unchanged in the placebo group (from 201.7 ± 13.85 to 190.0 ± 9.87 kJ/kg LBM/24h, NS).

Table 1 Effects of biosynthetic growth hormone treatment on anthropometric parameters and body composition determined by dual energy X-ray absorptiometry (DEXA)^a

| | Placebo | | GH | |
|--------------------------|------------------|-------------------|------------------|-------------------|
| | Baseline | After 4 weeks | Baseline | After 4 weeks |
| Body weight (kg) | 93.6 \pm 0.80 | 86.5 \pm 0.67* | 93.0 \pm 2.64 | 87.0 \pm 2.35* |
| BMI (kg/m ²) | 36.3 \pm 0.49 | 33.6 \pm 0.61* | 35.3 \pm 0.98 | 33.0 \pm 0.93* |
| Fat mass (kg) | 47.02 \pm 1.44 | 42.73 \pm 1.66* | 49.07 \pm 2.10 | 43.71 \pm 2.42* |
| % Fat mass loss | – | 9.1 \pm 1.34 | – | 10.9 \pm 1.40 |
| Lean mass (kg) | 43.83 \pm 1.15 | 40.04 \pm 1.14* | 42.14 \pm 1.42 | 40.61 \pm 1.29* |
| % Lean mass loss | – | 8.6 \pm 1.01 | – | 3.5 \pm 1.39** |

P* < 0.05 vs baseline. *P* < 0.05 between groups. ^aMean \pm standard error.

Table 2 Effects of biosynthetic growth hormone treatment on metabolic and hormonal parameters^a

| | Placebo | | GH | |
|----------------------------|------------------|--------------------|-------------------|-------------------|
| | Baseline | After 4 weeks | Baseline | After 4 weeks |
| Glucose (mmol/l) | 4.56 \pm 0.105 | 4.55 \pm 0.149 | 4.66 \pm 0.144 | 4.64 \pm 0.188 |
| Insulin (pmol/l) | 76 \pm 13.8 | 49 \pm 6.6** | 99 \pm 19.8 | 105 \pm 14.4** |
| Total cholesterol (mmol/l) | 5.07 \pm 0.212 | 3.83 \pm 0.173* | 4.52 \pm 0.338 | 3.68 \pm 0.201* |
| Triglycerides (mmol/l) | 1.19 \pm 0.230 | 1.04 \pm 0.073 | 1.34 \pm 0.163 | 1.33 \pm 0.099 |
| FFA (mEq/l) | 471.8 \pm 55.3 | 862.6 \pm 110.5* | 545.3 \pm 121.7 | 982 \pm 176.9* |
| FT4 (pmol/l) | 10.8 \pm 0.67 | 11.9 \pm 0.74 | 11.6 \pm 0.78 | 10.4 \pm 0.95 |
| FT3 (pmol/l) | 5.3 \pm 0.24 | 4.9 \pm 0.35 | 5.3 \pm 0.26 | 5.36 \pm 0.39 |
| Leptin (ng/ml) | 14.3 \pm 2.55 | 7.7 \pm 3.77* | 16.2 \pm 2.37 | 6.4 \pm 0.39* |

P* < 0.05 vs baseline. *P* < 0.05 between groups. ^aMean \pm standard error.

Table 3 Effects of biosynthetic growth hormone treatment on bone metabolism^a

| | Placebo | | GH | |
|---|-----------------|----------------|-----------------|----------------|
| | Baseline | After 4 weeks | Baseline | After 4 weeks |
| Serum calcium (mmol/l) | 2.2 ± 0.04 | 2.3 ± 0.07 | 2.1 ± 0.04 | 2.3 ± 0.02 |
| Serum phosphate (mmol/l) | 1.0 ± 0.06 | 1.2 ± 0.10 | 1.1 ± 0.06 | 1.3 ± 0.09 |
| Bone isoenzyme of alkaline phosphatase (% of total) | 22.8 ± 6.59 | 32 ± 5.4 | 26 ± 3.5 | 41.6 ± 5.42* |
| Osteocalcin (ng/ml) | 20.7 ± 1.24 | 18.4 ± 2.99 | 22.2 ± 4.45 | 34.7 ± 12.02 |
| Urinary calcium (mmol/d) | 2.8 ± 0.27 | 2.1 ± 0.41* | 2.0 ± 0.27 | 2.4 ± 0.57 |
| Urinary phosphate (mmol/d) | 16.1 ± 3.22 | 22.6 ± 2.58 | 19.3 ± 3.87 | 22.7 ± 2.76 |
| Urinary hydroxyproline (μmol/d/m ²) | 133.4 ± 33.70 | 152.5 ± 50.33 | 89.2 ± 20.20 | 199.8 ± 60.85 |
| BMD (g/cm ²) | 1.04 ± 0.013 | 1.06 ± 0.018 | 1.00 ± 0.027 | 1.00 ± 0.03 |
| BMC (g) | 2652.3 ± 177.66 | 2640.1 ± 142.0 | 2451.2 ± 111.05 | 2458.9 ± 110.3 |

**P* < 0.05 vs baseline. ^aMean ± standard error.

As reported in Table 2, a significant decrease in total cholesterol and increase in FFA, of comparable magnitude in the two groups of patients, were observed. Serum triglycerides were substantially unchanged in both groups, and the same held true for glucose and thyroid hormones. Diet tended to reduce plasma insulin, although not reaching statistical significance, in vehicle injected patients, but not in those receiving GH. Although no significant changes in insulin concentrations were detected within each treatment group even when considering delta values, i.e. differences between baseline and final concentrations (-27 ± 18.3 vs 7 ± 23.3 pmol/l, NS, in the placebo- and GH-treated patients, respectively), the absolute post-treatment insulin values were significantly different between the two groups (49 ± 6.6 vs 105 ± 14.4 pmol/l, *P* < 0.05, after placebo and after rhGH, respectively).

Serum AP bone isoenzyme increased significantly in GH-treated patients, who also displayed a moderate rise in serum osteocalcin and urinary hydroxyproline (Table 3). None of these parameters was modified in subjects receiving vehicle. While these latter showed a significant decrease in urinary calcium, the same parameter was unchanged in GH-treated patients. No significant changes in serum calcium and phosphate, as well as in subtotal BMC and BMD, were recorded in the two groups of obese patients along the study period.

Discussion

Nutritional status plays a central role in the regulation of IGF-I production and hence of most of the biological actions of GH. IGF-I plasma concentrations are low in malnourished patients⁸ while, in normal subjects, fasting lowers plasma IGF-I levels under basal conditions²⁴ and in response to GH administration.²⁵ In obese subjects, the IGF-I production induced by exogenous GH is gradually impaired by the progressive limitation of energy intake.^{9–11} The present study has shown that a number of biological actions of GH, i.e. stimulation of IGF-I synthesis, sparing of body proteins, enhancement of bone turnover and REE, are

maintained in obese patients in conditions of severe energy restriction. This finding is in line with the observation that in energy-restricted obese patients the administration of GH in increasing dosage results in a dose-dependent rise of plasma IGF-I levels. A significant IGF-I increase after prolonged GH administration has been reported, under experimental conditions similar to ours, by Drent *et al.*²⁶ These authors, however, failed to demonstrate changes in body composition by bioimpedance analysis (BIA). In our patients the IGF-I increase following GH treatment was accompanied by evident metabolic effects, chiefly by sparing of LBM estimated by DEXA. This finding is in keeping with the improvement of nitrogen balance observed in the present study and in other series of energy-restricted obese patients treated with GH,^{9,10,12} and demonstrates that GH anabolic properties are retained even when energy intake is markedly reduced. The lack of changes in IGF-I levels observed in our energy restricted placebo-treated patients fits in well with the resistance of obese patients to dietary restriction in terms of IGF-I reduction.⁸

In our patients, the combination of GH treatment and energy restriction induced a greater, though not statistically significant, fat mass loss compared to diet alone. Accordingly, leptin was reduced to a slightly greater extent by GH (60.5%) than by placebo administration (46.2%), in agreement with the data reported in GH deficient GH-treated adults.¹⁹ This is consistent with the failure of GH treatment to significantly enhance diet-induced fat loss in obese patients observed in other studies^{9,10,12} with the exception of a recent one²⁷ in which the patients received an energy intake (62.79 kJ/kg IBW daily) 50% higher than the one adopted in our protocol. A more striking decrease in body fat, especially at visceral level,²⁸ is well documented in obese patients receiving GH while on a normocaloric diet.^{13,28,29}

The observation of a smaller LBM loss in GH-treated women in spite of no significant differences in changes of body weight and fat mass between the two groups may not be in contradiction; in fact, statistically insignificant but not negligible variations in parameters of body composition may account for the apparent discrepancy of these results. The good correspondence between body weight loss estimated by

DEXA (sum of fat mass and LBM losses) and body weight estimated using a balance gives consistency to the data.

GH treatment has been found to increase REE in adult patients with GH deficiency.^{15,30,31} In one study¹⁵ the increase in REE was positively correlated with the increase in T3 serum levels, caused by the GH-induced peripheral conversion of T4 to T3 itself. A significant increase in REE, recognizable even when REE was corrected for LBM and not correlated with thyroid hormone concentrations, was also observed in obese women treated with rhGH for five weeks but not following a hypocaloric diet.²⁹ In the present experience, conducted in severely energy-restricted obese women, GH administration appeared able not only to prevent the diet-induced reduction of REE but also to significantly increase the value of REE corrected for unit of LBM. The latter finding suggests that in the GH-treated patients the maintenance of a REE similar to the pre-diet value was due not only to the sparing of LBM, but to the enhancement of energy metabolism of LBM itself. These effects did not appear to be correlated with changes in thyroid hormone concentrations.

Since DEXA is unable to distinguish between muscle tissue and water, the possibility that the fluid retentive action of GH has contributed to the smaller decrease in LBM observed in the patients treated with the hormone should be considered. However, the hypothesis of an actual preservation of muscle mass by GH treatment is supported by the less negative nitrogen balance observed in the treated patients and by the maintenance, in the same women, of baseline values of REE, whose main determinant is muscle tissue and not water. Furthermore, in experimental conditions similar to ours, the body water changes estimated by BIA were found to be not different between GH- and diet only-treated obese patients by Drent *et al.*²⁶

In our patients, the administration of rhGH did not affect the diet-induced modifications of serum lipid profile nor did it influence serum glucose levels. However, the decrease in insulin levels seen in energy-restricted vehicle-injected patients was completely prevented by GH treatment in patients undergoing the same diet regimen. These findings confirm the GH-induced worsening of carbohydrate metabolism usually seen in both normal man³² and GH deficient subjects.³³

The relevance of GH in the physiology of bone remodelling is well recognized with the GH/IGF-I system promoting bone turnover, with a prevalence of formation over reabsorption and a positive net balance at each remodelling site. In fact, long-term administration of rhGH has been shown to significantly improve bone mineralization in GH deficient adults,³⁴ who display reduced BMD and increased risk for fracture.³⁵ In the present study, a tendency of the serum markers of bone turnover (AP bone isoenzyme, serum osteocalcin and urinary hydroxyproline)

to increase was observed in obese patients treated with rhGH but not in energy-restricted placebo-treated patients. Moreover, rhGH treatment appeared to prevent, in our obese women, a diet-related reduction of urinary calcium. This might be due to the early activation of bone turnover induced by the hormone or to an enhanced intestinal calcium absorption secondary to increased vitamin D activation. In any case, no changes in subtotal BMC and BMD were recorded in the two groups of patients, possibly due to the short observation period.

In conclusion, rhGH treatment, if performed at adequate doses, appears to be effective in obese patients when associated with severely hypocaloric diets. The major benefits are represented by reduction of the LBM loss which follows energy restriction and improvement of LBM metabolic efficiency. These results encourage additional studies aimed at evaluating whether the administration of less expensive compounds capable of stimulating endogenous GH release is equally effective in improving the outcome of severe dietary restriction in obesity.

References

- 1 Bray GA. Obesity: a serious symptom. *Ann Intern Med* 1972; **77**: 779–795.
- 2 Bistran BR, Winterer J, Blackburn GL, Young V, Sherman M. Effect of a protein sparing diet and a brief fast on nitrogen metabolism in mildly obese subjects. *J Lab Clin Med* 1977; **89**: 1030–1035.
- 3 Ravussin E, Lillioja S, Anderson TE, Christin L, Bogardus C. Determinants of 24 hour energy expenditure in man. Methods and results using a respiratory chamber. *J Clin Invest* 1986; **78**: 1568–1578.
- 4 Raben MS, Hollenberg CH. Effect of growth hormone on plasma fatty acids. *J Clin Invest* 1959; **38**: 484–488.
- 5 Manson JM, Wilmore DW. Positive nitrogen balance with human growth hormone and hypocaloric intravenous feeding. *Surgery* 1986; **100**: 188–197.
- 6 Mautalen C, Smith RW Jr. Lipolytic effect of human growth hormone in resistant obesity. *J Clin Endocrinol Metab* 1965; **25**: 495–498.
- 7 Bray GA, Raben MS, Londono J, Gallagher TF Jr. Effects of triiodothyronine, growth hormone, and anabolic steroids on nitrogen excretion and oxygen consumption in obese patients. *J Clin Endocrinol Metab* 1971; **33**: 293–300.
- 8 Thissen JP, Ketelslegers JM, Underwood LE. Nutritional regulation of the insulin-like growth factors. *Endocr Rev* 1994; **15**: 80–101.
- 9 Clemmons DR, Snyder DK, Williams R, Underwood LE. Growth hormone administration conserves lean body mass during dietary restriction in obese subjects. *J Clin Endocrinol Metab* 1987; **64**: 878–883.
- 10 Snyder DK, Clemmons DR, Underwood LE. Treatment of obese, diet-restricted subjects with growth hormone for 11 weeks: effects on anabolism, lipolysis, and body composition. *J Clin Endocrinol Metab* 1988; **67**: 54–61.
- 11 Snyder DK, Clemmons DR, Underwood LE. Dietary carbohydrate content determines responsiveness to growth hormone in energy restricted humans. *J Clin Endocrinol Metab* 1989; **69**: 745–752.
- 12 Snyder DK, Underwood LE, Clemmons DR. Anabolic effects of growth hormone in obese diet-restricted subjects are dose dependent. *Am J Clin Nutr* 1990; **52**: 431–437.
- 13 Skaggs SR, Crist DM. Exogenous human growth hormone reduces body fat in obese women. *Horm Res* 1991; **35**: 19–24.

- 14 Jorgensen JO, Pedersen SA, Laurberg P, Weeke J, Skakkebaek NE. Effects of growth hormone therapy on thyroid function of growth hormone-deficient adults with and without concomitant thyroxine-substituted central hypothyroidism. *J Clin Endocrinol Metab* 1989; **69**: 1127–1132.
- 15 Jorgensen JOL, Moller J, Laursen T, Orskov H, Christiansen JS, Weeke J. Growth hormone administration stimulates energy expenditure and extrathyroidal conversion of thyroxine to triiodothyronine in a dose-dependent manner and suppresses circadian thyrotrophin levels: studies in GH-deficient adults. *Clin Endocrinol* 1994; **41**: 609–614.
- 16 Brixen K, Nielsen HK, Mosekilde L, Flyvbjerg A. A short course of recombinant human growth hormone treatment stimulates osteoblasts and activates bone remodeling in normal human volunteers. *J Bone Miner Res* 1990; **5**: 609–618.
- 17 O'Halloran DJ, Tsatsoulis A, Whitehouse RW, Holmes SJ, Adams JE, Shalet SM. Increased bone density after recombinant human growth hormone (GH) therapy in adults with isolated GH deficiency. *J Clin Endocrinol Metab* 1993; **76**: 1344–1348.
- 18 Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kern PA, Friedman JM. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nature Med* 1995; **1**: 1155–1161.
- 19 Fisker S, Vahl N, Hansen TB, Jorgensen JOL, Hagen C, Orskov H, Christiansen JS. Serum leptin is increased in growth hormone-deficient adults: relationship to body composition and effects of placebo-controlled growth hormone therapy for 1 year. *Metabolism* 1997; **46**: 812–817.
- 20 Fabiny DL, Ertingshausen G. Automated reaction-rate method for determination of serum creatinine with the CentrifChem. *Clin Chem* 1971; **17**: 696–700.
- 21 Marsh WH, Fingerhut B, Miller H. Automated and manual direct methods for determination of blood urea. *Clin Chem* 1965; **11**: 624–627.
- 22 Wright RA. Nutritional assessment. *JAMA* 1980; **244**: 559–560.
- 23 Benotti P, Blackburn GL. Protein and caloric or macronutrient metabolic management of the critically ill patient. *Crit Care Med* 1979; **7**: 520–525.
- 24 Clemmons DR, Klibanski A, Underwood LE, McArthur JW, Ridgway EC, Beitins IZ, Van Wyk JJ. Reduction of plasma immunoreactive somatomedin-C during fasting in humans. *J Clin Endocrinol Metab* 1981; **53**: 1247–1250.
- 25 Merimee TJ, Zapf J, Froesch ER. Insulin-like growth factors in the fed and fasted states. *J Clin Endocrinol Metab* 1982; **55**: 999–1002.
- 26 Drent ML, Wever LDV, Ader HJ, van der Veen EA. Growth hormone administration in addition to a very low calorie diet and an exercise program in obese subjects. *Eur J Endocrinol* 1995; **132**: 565–572.
- 27 Snyder DK, Underwood LE, Clemmons DR. Persistent lipolytic effect of exogenous growth hormone during caloric restriction. *Am J Med* 1995; **98**: 129–134.
- 28 Richelsen B, Pedersen SB, Borglum JD, Moller Pedersen T, Jorgensen J, Jorgensen JO. Growth hormone treatment of obese women for 5 wk: effect on body composition and adipose tissue LPL activity. *Am J Physiol* 1994; **266**: E211–E216.
- 29 Jorgensen JO, Pedersen SB, Borglum J, Moller N, Schmitz O, Christiansen JS, Richelsen B. Fuel metabolism, energy expenditure, and thyroid function in growth hormone-treated obese women: a double-blind placebo controlled study. *Metabolism* 1994; **43**: 872–877.
- 30 Chong PK, Jung RT, Scrimgeour CM, Rennie MJ, Paterson CR. Energy expenditure and body composition in growth hormone deficient adults on exogenous growth hormone. *Clin Endocrinol* 1994; **40**: 103–110.
- 31 Snel YEM, Doerga ME, Brummer RJM, Zelissen PMJ, Zonderland ML, Koppeschaar HPF. Resting metabolic rate, body composition and related hormonal parameters in growth hormone-deficient adults before and after growth hormone replacement therapy. *Eur J Endocrinol* 1995; **133**: 445–450.
- 32 Bratusch-Marrain PR, Smith D, DeFronzo RA. The effect of growth hormone on glucose metabolism and insulin secretion in man. *J Clin Endocrinol Metab* 1982; **55**: 973–982.
- 33 Salomon F, Cuneo RC, Hesp R, Sonksen PH. The effects of treatment with recombinant human growth hormone on body composition and metabolism in adults with growth hormone deficiency. *N Engl J Med* 1989; **321**: 1797–1803.
- 34 Baum HBA, Biller BMK, Finkelstein JS, Cannistraro Baker K, Oppenheim DS, Schoenfeld DA, Michel Hoskins T, Wittink H, Klibanski A. Effects of physiologic growth hormone therapy on bone density and body composition in patients with adult-onset growth hormone deficiency. *Ann Intern Med* 1996; **125**: 883–890.
- 35 Degerblad M, Bengtsson BA, Bramnert M, Johnell O, Manhem P, Rosen T, Thoren M. Reduced bone mineral density in adults with growth hormone (GH) deficiency: increased bone turnover during 12 months of GH substitution therapy. *Eur J Endocrinol* 1995; **133**: 180–188.