Whole-body leucine turnover in adults on conventional treatment for hypopituitarism

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This study has investigated protein metabolism in adults with hypopituitarism before and after growth hormone (GH) replacement and in matched controls. Whole-body leucine turnover was measured in 16 GH-deficient adult hypopituitary patients (nine females and seven males) on standard thyroid, adrenal and sex hormone replacement and in 20 normal controls using primed continuous infusion of $^{13}$C-leucine. In seven of the patients, leucine turnover was restudied following 6 months’ treatment with biosynthetic human GH (0.025–0.05 IU/kg body wt daily, with the final dose determined by patient tolerance). Compared with normal controls, hypopituitary patients had significantly reduced leucine flux (mean ± s.d: 97.8 ± 24.9 vs 131.0 ± 23.0 μmol·h$^{-1}$·kg$^{-1}$; p < 0.001), reduced leucine incorporation into protein (80.4 ± 20.9 vs 108.8 ± 19.6 μmol·h$^{-1}$·kg$^{-1}$; p < 0.001) and reduced leucine oxidation (17.4 ± 4.8 vs 22.2 ± 8.1 μmol·h$^{-1}$·kg$^{-1}$; p < 0.05). Leucine turnover was similar in male and female patients. In the patients, leucine flux correlated positively with body weight (p = 0.51, p < 0.05) and leucine incorporation in protein correlated positively with lean body mass (p = 0.55, p < 0.05) and in male patients leucine flux correlated positively with serum insulin-like growth factor I (IGF-I) levels (p = 0.71, p < 0.05). No significant relationship was observed with age or duration of hypopituitarism. Growth hormone replacement therapy did not produce a uniform effect on leucine metabolism. Mean values of leucine flux, oxidation and incorporation into protein increased, although the differences were not statistically significant. The patients on higher GH doses and with a higher serum IGF-I response were those who demonstrated an increase in leucine turnover. We conclude that leucine turnover in hypopituitary adults is reduced compared with normal controls. The effect of GH treatment for 6 months at conventional doses in hypopituitary adults is not uniform and may be dose-dependent.

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Hypopituitarism is associated with increased morbidity and mortality (1). Symptoms of poor general health are present frequently and there is objective evidence of muscle weakness and poor exercise performance. Hypopituitary adults are conventionally treated using thyroxine, adrenal steroid and sex hormone replacement where appropriate, but growth hormone (GH) is not usually replaced. Growth hormone deficiency is, however, common in pituitary disease at presentation or following treatment by surgery or radiotherapy. Growth hormone is a major anabolic hormone and its role in adults has received much attention in recent years. Discontinuation of GH therapy in young GH-deficient adults resulted in a significant decrease in both muscle bulk and strength after 6–12 months (2). Growth hormone replacement therapy in hypopituitary adults resulted in increased muscle mass, muscle strength and exercise tolerance (3, 4). Lean body mass also increased when measured by a variety of methods (3–5). This suggests a net protein anabolism. On the other hand, hypophysectomy causes a reduction in protein turnover in the rat (6). There is little information on protein metabolism and on the effect of GH therapy in adult hypopituitary man. Binnerts et al. (7), using $[^{15}$N]glycine as tracer, demonstrated a transient increase in the rate of protein synthesis and a smaller increase in protein degradation. The aim of the present study was to evaluate the effect of chronic GH deficiency on protein metabolism in adult hypopituitary patients treated with conventional hormone replacement and then to evaluate the effect of GH therapy.

Patients and methods

Study population

Sixteen adult hypopituitary patients (seven males and nine females) and 20 normal controls (10 males and 10 females) were studied. Patients were recruited from the endocrine clinic at St Mary’s Hospital and adjacent centres, and controls were normal volunteers from hospital staff and students or their acquaintances. The
weights of patients and controls (mean (range)) were 73 (49–85) and 70 (52–88) kg, respectively. None of the patients had acromegaly, Cushing’s disease or diabetes (Table 1). They had been on stable therapy for at least 1 year and were taking thyroid, adrenal and sex hormone replacement therapy and desmopressin as appropriate. They had severe GH deficiency as defined by a GH response to insulin-induced hypoglycaemia of less than 6 mIU/l. Their mean (±SD) serum insulin-like growth factor 1 (IGF-1) concentration was 82 ± 47 μg/l (normal control value 171 ± 88 μg/l). The study was approved by the Parkside Health Authority Ethical Committee and all patients and controls gave informed written consent.

Materials
L-[1-13C]Leucine (99 at. %) and NaH13CO3 (99 at. %) were obtained from Cambridge Isotope Laboratories, Woburn, MA, USA. Solutions of each labelled species were prepared in 0.9% saline and were shown to be sterile and pyrogen-free. Freeze-dried biosynthetic human growth hormone (Norditropin®, Novo Nordisk A/S, Denmark) was supplied in vials containing 12 IU, and was reconstituted by patients in 3 ml of 0.9% benzyl alcohol.

Experimental protocol
Patients and controls attended the Metabolic Day Ward at St Mary’s Hospital at 8.00 a.m. after a 10–12 h overnight fast. No alcohol or strenuous exertion had been taken in the preceding 24 h. A vein in the dorsum of one hand was cannulated for sampling and the catheter was kept patent by a normal saline infusion. The hand was kept warmed by placing it in a hot box (ambient temperature 50–60°C) to arterialisate the blood (8). A second cannula was positioned in a superficial vein in the contralateral arm for the infusion of L-[1-13C]leucine. Baseline blood and expired air samples were collected to determine basal 13C-enrichment. Prime bolus doses of NaH13CO3 (0.09 mg/kg) and L-[1-13C]leucine (0.5 mg/kg) were administered at time 0, after which a continuous infusion of L-[1-13C]leucine (0.5 mg·h−1·kg−1) in 40 ml of saline was started using a volumetric pump (Perfusor® Secora, B. Braun Melsungen AG, Germany). The infusion was continued for 4 h and arterialized venous blood and expired air samples were collected every 15 min over the final 2 h. The total CO2 excretion rate was measured for 1 h during the study by means of a microcomputer-controlled ventilated hood system (Deltatrac MBM-100, Datex Instrumentarium Corp., Helsinki, Finland).

Growth hormone therapy
Seven patients were restudied after 6 months’ treatment with GH. Growth hormone was given in a daily dose of 0.05 IU/kg body wt injected subcutaneously at bed time. In four patients the dose was reduced to 75% (0.0375 IU·kg−1·body wt·day−1) or 50% (0.025 IU·kg−1·body wt·day−1) of the original dose owing to the development of symptoms of fluid retention. The experiments were performed 10–12 h after the last dose of GH.
Analytical methods

Arterialized plasma samples were collected into lithium heparin tubes on ice, separated at 2500 rpm at 4°C and stored at −20°C until analysis. Plasma KIC (α-ketoisocaproate, a deamination metabolite of leucine) enrichment was measured using the quinoloxinol trimethylsilyl derivative (9). Ions at m/e 232 and 233 were monitored following electron impact ionization using selected-ion-monitoring gas chromatography–mass spectrometry with a Finnigan 4500 mass spectrometer (Finnigan MAT Ltd., Hemel Hempstead, UK). Ketovaleric acid was employed as the internal standard. Expired breath 13CO2 was determined by radioisotope mass spectrometry (10). The reciprocal pool model was used to calculate whole-body leucine flux and oxidation rates (11). This model utilizes plasma [13C]KIC rather than leucine enrichment. [13C]KIC enrichment reflects more accurately the intracellular leucine enrichment and it may provide a more precise index of whole-body leucine kinetics (12). Plateau enrichments for both plasma KIC and expired CO2 were obtained after 2 h of infusion.

Calculation of whole-body protein turnover

Protein turnover was determined using a two-pool stochastic model as described previously (12, 13). In the fasting steady state, the amino acid flux (Q, μmol·kg−1·h−1) was calculated from the principles of isotopic dilution

\[ Q = \frac{E_i}{E_p} - 1 \]

where i is the rate of infusion of the tracer (μmol·kg−1·h−1) and Ei and Ep are the respective plasma enrichments of the infused [13C]leucine and [13C]KIC at the plateau. The oxidation of leucine (O) was determined from the rate of appearance of 13CO2 in expired air (12, 13).

In the steady post-absorptive state: Q=S+O=C and S=Q−O, where S is the exit of leucine from the free amino acid pool for protein synthesis (μmol·kg−1·h−1), O is the irreversible loss of leucine by oxidation (μmol·kg−1·h−1) and C is the rate of entry of leucine into the free amino acid pool from protein catabolism (μmol·kg−1·h−1).

Metabolic assessments and body composition measurements

Serum IGF-I levels were measured by polyethylene glycol (PEG)-assisted second antibody radioimmunoassay (14) using a rabbit antiserum developed by Underwood and Van Wyk and distributed by the National Hormone and Pituitary Program of University of Maryland School of Medicine, USA. Iodine-labelled IGF-I was provided by Dr Teale, St Luke’s Hospital, Guildford, Surrey, UK. The sensitivity of the assay was 0.5 μg/l. The within-assay and between-assay coefficients of variation were 1.2% and 3%, respectively. Insulin was measured by PEG-assisted second antibody radioimmunoassay using a polyclonal antiserum (MF/GP/9) fromGuildhay Ltd, Guildford, Surrey. Lean body mass (LBM) was calculated from body mass index (weight/height2) using published regression formulae (15) in controls and in all patients. Lean body mass was also calculated in hypopituitary patients by measurement of total body potassium (TBK) (16). Body fat distribution was measured by waist-to-hip ratio (WHR), calculated as the ratio of the narrowest waist circumference to the widest hip circumference. Glucose tolerance in patients was assessed before GH treatment by a 75-g oral glucose tolerance test with measurements of glucose and insulin at 30-min intervals. Fasting insulin levels were measured after GH treatment.

Statistics

Unless indicated otherwise, all values are expressed as mean ± sd. Comparisons between patients and controls and before and after GH treatment were made using the Mann–Whitney U test and Wilcoxon signed rank test respectively. Relations between various parameters were explored using Spearman’s rank correlation coefficient (ρ).

Results

Leucine kinetics in hypopituitary and control subjects

All measures of leucine kinetics (flux, oxidation, and incorporation into body protein) expressed per kilogram body weight were reduced in the hypopituitary patients compared with controls (Table 2). The difference in leucine oxidation between hypopituitary patients and controls was no longer significant when the data were expressed in terms of lean body mass. The rates of leucine flux and incorporation into protein continued to be significant (Table 2). There was no significant difference between male and female hypopituitary patients in leucine flux, incorporation into protein or oxidation (expressed either way), although the values tended to be higher in men when they were expressed in terms of kilogram body weight. Leucine flux and leucine incorporation into protein were both decreased in the patients when men and women were considered separately, although statistical significance was lost for leucine oxidation (Table 3). The patients were divided for further analysis into obese (BMI > 25 kg/m2) and lean (BMI < 25 kg/m2). For the whole group (male and female), rates of flux (89.6 ± 30.9 vs 102.7 ± 18.0 μmol·kg−1·h−1), oxidation (17.6 ± 5.9 vs 17.3 ± 3.8 μmol·kg−1·h−1) and incorporation into protein (83.8 ± 21.3 vs 78.3 ± 20.3 μmol·kg−1·h−1) were similar in lean and obese patients, respectively. The percentage of leucine oxidized (100 × oxidation/flux) was sig-
Table 2. Leucine kinetics in hypopituitary patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>Hypopituiary</th>
<th>Controls</th>
<th>Significance level (Mann-Witney test)</th>
</tr>
</thead>
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<tr>
<td>Number</td>
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<td>20</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44 (26–64)</td>
<td>34 (22–62)</td>
<td>-</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73 (49–85)</td>
<td>70 (52–88)</td>
<td>-</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.4 (19.4–32.7)</td>
<td>23.9 (18.5–33.6)</td>
<td>-</td>
</tr>
<tr>
<td>Flux per kg BWt</td>
<td>97.8 ± 24.9</td>
<td>131.0 ± 23.0</td>
<td>0.0005</td>
</tr>
<tr>
<td>Flux per kg LBM</td>
<td>137.4 ± 37.3</td>
<td>169.5 ± 25.0</td>
<td>0.007</td>
</tr>
<tr>
<td>Synthesis per kg BWt</td>
<td>80.4 ± 20.9</td>
<td>108.8 ± 19.6</td>
<td>0.005</td>
</tr>
<tr>
<td>Synthesis per kg LBM</td>
<td>112.0 ± 28.4</td>
<td>140.6 ± 20.8</td>
<td>0.004</td>
</tr>
<tr>
<td>Oxidation per kg BWt</td>
<td>17.4 ± 4.8</td>
<td>22.2 ± 8.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Oxidation per kg LBM</td>
<td>24.4 ± 6.7</td>
<td>28.7 ± 10.0</td>
<td>0.1</td>
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</tbody>
</table>

* Data of males and females are described together and expressed in terms of µmol·h⁻¹·kg body wt. (BWt) and BMI-derived lean body mass (LBM). Values are means ± sd or mean (range).

Table 3. Clinical characteristics and data of leucine kinetics in hypopituitary patients (HP) and controls subjects (C).*

<table>
<thead>
<tr>
<th></th>
<th>HP</th>
<th>C</th>
<th>Females</th>
<th>C</th>
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<td>10</td>
<td>9</td>
<td>10</td>
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<tr>
<td>Age (years)</td>
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<td>34 (22–62)</td>
<td>40 (24–57)</td>
<td>34 (22–39)</td>
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<td>Weight (kg)</td>
<td>79 (63–95)</td>
<td>75 (64–88)</td>
<td>69 (49–85)</td>
<td>66 (52–88)</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>25.1 (19.4–30.0)</td>
<td>24.5 (21.9–28.1)</td>
<td>27.5 (21.5–32.7)</td>
<td>23.2 (18.5–33.6)</td>
</tr>
<tr>
<td>Flux per kg BWt</td>
<td>108.6 ± 23.3*</td>
<td>137.2 ± 20.7</td>
<td>89.4 ± 23.9*</td>
<td>124.2 ± 24.9</td>
</tr>
<tr>
<td>Flux per kg LBM</td>
<td>137.0 ± 30.6*</td>
<td>170.5 ± 23.1</td>
<td>137.6 ± 43.7</td>
<td>168.5 ± 28.0</td>
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<tr>
<td>Synthesis per kg BWt</td>
<td>89.4 ± 18.6*</td>
<td>114.7 ± 17.9</td>
<td>73.4 ± 20.8*</td>
<td>102.2 ± 20.2</td>
</tr>
<tr>
<td>Synthesis per kg LBM</td>
<td>112.8 ± 24.5*</td>
<td>142.6 ± 20.2</td>
<td>111.3 ± 32.5</td>
<td>138.0 ± 22.4</td>
</tr>
<tr>
<td>Oxidation per kg BWt</td>
<td>19.2 ± 4.9</td>
<td>22.5 ± 3.5</td>
<td>16.0 ± 4.4</td>
<td>21.9 ± 10.7</td>
</tr>
<tr>
<td>Oxidation per kg LBM</td>
<td>24.3 ± 6.4</td>
<td>27.9 ± 6.4</td>
<td>24.4 ± 7.4</td>
<td>29.5 ± 13.0</td>
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</table>

* Data of males and females are described separately. Leucine kinetics are expressed in terms of µmol·h⁻¹·kg⁻¹ body wt (BWt) and BMI-derived lean body mass (LBM). Values are means ± sd or mean (range).

nificantly higher in the lean compared with obese patients (19.4 ± 1.7 vs 16.8 ± 2.4%, p < 0.05). Leucine kinetics were not influenced significantly by age in either the patients or controls.

For the patient group as a whole, leucine flux (expressed per kilogram TBK-derived LBM) correlated positively with body weight (r = 0.51, p < 0.05) and leucine incorporation into protein (expressed per kilogram body weight) correlated with TBK-derived LBM (r = 0.55). In the male patients, leucine flux (expressed per kilogram body weight) correlated also with serum IGF-I (r = 0.71, p < 0.05). No significant relationships were observed with percentage body fat, WHR ratio, fasting glucose and insulin levels nor with the glucose and insulin areas under the curve following oral glucose.

The effect of GH replacement on leucine metabolism in hypopituitary adults

Leucine flux, incorporation into protein and oxidation did not change significantly following GH therapy (Table 4). Leucine turnover increased in four out of seven patients, did not change in one and was reduced in two...
patients. The direction of change for both turnover and oxidation was the same in six subjects. The response to GH treatment was not related to the age, duration of hypopituitarism or the pretreatment leucine flux. The patients who showed an increase in leucine turnover tended to be those who tolerated the higher GH treatment doses. Similarly, the serum IGF-I response was higher in patients who showed an increase in leucine turnover than in those who did not (mean range: 287 (159–401) vs 107 (33–149) μg/l). This was not the case with fasting insulin levels (7.1(2.7–15.3) vs 10.8 (7.3–14.2) mU/l).

Discussion

Growth hormone is an important anabolic agent. Lean body mass, of which skeletal muscle and visceral organs are the main components, is the target tissue for this anabolic action. It is thought to be mediated mainly by IGF-I. Insulin modulates the effect by having permissive role (17). Adults with hypopituitarism have decreased LBM and muscle mass and an increase in adipose tissue mass, probably as a result of GH deficiency.

The present study was designed to investigate the kinetics of protein metabolism in hypopituitary adults on routine replacement therapy before and after GH replacement. All hypopituitary patients were severely deficient in GH, as illustrated by the GH response to insulin-induced hypoglycaemia and by a low serum IGF-I concentration. The technique of leucine turnover employed in the study is well documented and the results from the controls in the present study are similar to other published figures (13, 18). When comparing hypopituitary patients and controls and when seeking relationships with clinical and metabolic parameters, the males and females were analysed both separately and together.

We have demonstrated that whole-body protein turnover, as measured by leucine kinetics, is reduced significantly in adult hypopituitarism. Both the rate of protein synthesis and the rate of protein breakdown were reduced. The reduced LBM observed in hypopituitary patients could not account for the reduced leucine flux and leucine incorporation into protein because these differences persisted even when the leucine kinetic data were expressed in terms of LBM. The reduced leucine flux could not be attributed to obesity because the effect of obesity per se is to increase protein turnover and synthetic rates (19). Leucine incorporation into protein was related positively to LBM, suggesting that the reduced LBM is a result of reduced protein synthesis. There was no significant difference in leucine kinetics between lean and obese hypopituitary patients, suggesting that factors other than abnormalities in body composition were responsible. Age is unlikely to have been a factor, in that previous workers have demonstrated whole-body protein turnover rates remaining fairly constant between the third and sixth decades of life (20). A role for GH deficiency is suggested by the positive correlation between leucine flux and serum IGF-I level in the male patient group.

All patients were treated with conventional hormone replacement. Serum thyroxine was within the normal range, suggesting that thyroid hormone excess or deficiency did not contribute to the findings observed. The effects of glucocorticoid therapy are unlikely to be a major factor but are difficult to exclude. Simons et al. (21) demonstrated that a short-term increase in plasma cortisol, within the physiological range, increased the rate of leucine appearance and Beaufre et al. (22) observed that short-term high-dose glucocorticoid treatment increased the rate of leucine oxidation. In the present study the patients on cortisol replacement had circulating cortisol levels checked as part of their routine care and these were within the normal range, although standard replacement regimens do not reproduce normal physiology. The known directions of change in leucine flux with glucocorticoids do not suggest an influence of glucocorticoid replacement in the present findings. Male patients, where appropriate, were on testosterone replacement producing levels of plasma

Table 4. Details of growth hormone dosage, serum IGF-I responses, lean body mass (LBM) and fasting insulin levels and the effect of GH treatment for 6 months on leucine turnover in seven hypopituitarism patients.

<table>
<thead>
<tr>
<th>No.</th>
<th>GH dose (IU/kg)</th>
<th>Serum IGF-I (μg/l)</th>
<th>LBM (kg)</th>
<th>Fasting insulin (mU/l)</th>
<th>Flux (μmol·h⁻¹·kg⁻¹)</th>
<th>Synthesis (μmol·h⁻¹·kg⁻¹)</th>
<th>Oxidation (μmol·h⁻¹·kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td>Post</td>
<td>Pre</td>
<td>Post</td>
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<td>Post</td>
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<tr>
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<tr>
<td>2</td>
<td>0.0375</td>
<td>95</td>
<td>405</td>
<td>48.9</td>
<td>49.5</td>
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</tr>
<tr>
<td>5</td>
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<td>70</td>
<td>229</td>
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<tr>
<td>10</td>
<td>0.0375</td>
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<td>174</td>
<td>37.5</td>
<td>40.3</td>
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</tr>
<tr>
<td>13</td>
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<td>239</td>
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<td>10.9</td>
</tr>
<tr>
<td>16</td>
<td>0.025</td>
<td>68</td>
<td>101</td>
<td>47.8</td>
<td>46.8</td>
<td>1.0</td>
<td>14.2</td>
</tr>
</tbody>
</table>

Mean 0.04 87 297 46.2 47.2 1.8 8.7 139.8 149.5 114.4 122.4 25.4 27.1

* Measures of leucine metabolism are expressed per kilogram of total body potassium-derived LBM. The numbering of patients is the same as in Table 1.
Anyaoku, optimal Acknowledgments. causal related conventional demonstrated tracer, the latter assessing the study absence crease nitrogen blood, situations, rats in lowing plasma GH-deficient seven months, may the evident Association in GH-treated upper patients were by computed tomography scanning (3). Muscle strength, exercise tolerance and total LBM measured by TBK have all been demonstrated to increase following 6 months of GH treatment (5). Both of these studies in GH-deficient adults used higher doses of GH than were employed in the present study. The absence of an effect on leucine kinetics in the present study may reflect our lower dose, although in five out of the seven GH-treated patients the serum IGF-I response was in the upper normal or supraphysiological range, or the fact that 6 months may not be the optimal time for assessing the actions of GH on leucine metabolism. The latter is suggested by other studies using [15N]glycine as tracer, in which an increase in protein synthesis was demonstrated after 1 month of GH therapy but was no longer evident at 6 months (7).

In conclusion, we have demonstrated in this study reduced whole-body leucine turnover in adults on conventional replacement treatment for hypopituitarism compared with healthy controls. Leucine flux was related positively to circulating IGF-I levels, suggesting a causal relation to GH deficiency. Despite this, GH replacement did not uniformly restore leucine flux to normal levels, suggesting either that the replacement regimen was not optimal or that 6 months is not the optimal time to demonstrate the effect. Alternatively, factors other than, or in addition to, GH deficiency are operative in the decreased leucine turnover of adults with hypopituitarism.

References

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