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Relationship between GH-induced metabolic changes and changes in body composition: A dose and time course study in GH-deficient adults

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Abstract

Objective: Although growth hormone (GH)-induced changes in fat and protein metabolism are likely to underlie changes in body composition, the relationship has not been clearly defined. The aim was to study the effects of dose and time course on substrate metabolism and relate to body compositional changes during GH treatment.
Design: In an open randomised-controlled study, 16 GH-deficient adults were randomised to treatment with GH 3 μg/kg/d (low dose, $n = 6$) or 6 μg/kg/d (higher dose, $n = 10$) for 12 weeks. Changes in whole body protein metabolism, estimated using the leucine turnover technique, and resting energy expenditure (REE) were assessed after short-term GH (two weeks) and longer-term GH (12 weeks). Changes in lean body mass (LBM) and fat mass (FM) over 12 weeks were assessed by DXA.
Results: The maximal changes in leucine oxidation (Lox) (-3.9 ± 1.1 versus $+0.8 \pm 1.8$ μmol/min, $p = 0.03$) and REE ($+132 \pm 36$ versus -28 ± 41 kcal/d, $p = 0.01$) were significantly greater in the higher, than the low dose group. FM fell (-1.4 ± 0.4 kg, $p = 0.005$) and LBM increased ($+2.2 \pm 0.7$ kg, $p = 0.01$) significantly in the higher dose group only. The acute reduction in Lox at two weeks in the higher dose group was no longer significant after 12 weeks. The change in Lox after two ($r = -0.53$, $p = 0.035$), but not 12, weeks was significantly correlated with the change in LBM.
Conclusions: GH-induced changes in protein metabolism were influenced by the dose and duration of GH treatment. Suppression of protein oxidation occured soon after initiation of GH in the higher dose group and predicted a later gain in LBM. Early assessment of whole body protein metabolism may allow prediction of the anabolic potential of GH.

Keywords: Growth hormone; Whole body leucine turnover; Resting energy expenditure; Body composition

1. Introduction

Growth hormone (GH) status has a major impact on body composition. GH deficiency is associated with an increased fat mass (FM) and reduced lean body mass (LBM) [1], whereas the converse is found in acromegaly [2]. GH replacement in GH-deficient subjects reduces FM and increases LBM [3,4], however individual

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responsiveness to GH is variable [5]. Changes in fat and protein metabolism are likely to underlie GH-induced changes in FM and LBM. However the relationship between changes in metabolic indices and changes in body composition, and factors affecting the relationship, are poorly understood.

The whole body leucine turnover technique is a valuable tool for studying the dynamics of protein metabolism. In this model, which tracks the metabolic fate of isotopically labelled leucine, at steady state an equilibrium exists between protein breakdown and synthesis. Protein mass increases when the rate of protein synthesis is increased relative to breakdown, or conversely, if breakdown is reduced relative to synthesis. Both scenarios result in a reduction in irreversible protein loss by oxidation. GH replacement in GH-deficient subjects acutely increases whole body protein synthesis and reduces oxidation [6–11], changes that are assumed to increase LBM. However, the extent to which suppression of protein oxidation relates to a later gain in LBM has not been studied.

GH stimulates energy expenditure and fat metabolism [12]. While early studies reported no significant effect of GH deficiency on resting energy expenditure (REE) or fat oxidation (Fox) [1,12,13], in a larger study we recently reported both REE and Fox to be reduced in GH-deficient adults [14]. Changes in energy and fat metabolism may account for the reduction in FM with GH replacement, however data supporting the relationship between changes in metabolic indices and FM is lacking.

The aim of this study was to assess (i) whether the effect of GH on substrate metabolism and body compositional change is dose-dependent, (ii) the time course of stimulation of substrate metabolism changes and (iii) whether changes in substrate metabolism are correlated with changes in body composition.

2. Materials and methods

2.1. Study population

Sixteen GH-deficient subjects were recruited from amongst participants in a dose finding multi-centre trial of GH replacement in GH-deficient adults (GDED, Lilly) [15]. The subjects' treatment characteristics are shown in Table 1. All subjects had previously been confirmed to have severe GH deficiency based on a peak GH response to insulin-induced hypoglycemia (blood glucose < 2.2 mmol/L) of less than 3 ng/mL [16]. Subjects were receiving stable hormone replacement for adrenal ($n = 14$), thyroid ($n = 14$) and gonadal ($n = 15$) hormone deficiencies throughout the study. The study was approved by the Research Ethics Committee of St. Vincent's Hospital (Sydney, Australia) and all subjects provided written informed consent.

2.2. Study design

Metabolic studies were performed in the Clinical Research Facility, Garvan Institute of Medical Research. Subjects underwent measurements of body composition by dual energy X-ray absorptiometry (DXA), whole body protein turnover using 1-[^{13}C] leucine tracer and assessment of resting energy expenditure and substrate oxidation using indirect calorimetry. Following a baseline visit, subjects were randomised to receive one of two GH doses: 3 μg/kg/d ($n = 6$) (low

Table 1
Characteristics of subjects with GH deficiency

Subject	Group	Age (years)	Gender (M, F)	Cause of GHD	Hormone deficiencies	Hormone replacement
1	LD	53	F	NFT	A, T, G	A, T, G
2	HD	28	M	NFT	A, T, G	A, T, G
3	LD	59	M	NFT	A, T, G, D	A, T, G, D
4	HD	27	M	IH	T, G	T, G
5	HD	44	F	NFT	A, T, G	A, T, G
6	LD	44	M	NFT	A, T, G	A, T, G
7	HD	50	F	LH	A, G	A, G
8	HD	24	M	NFT	A.T, G, D	A.T, G, D
9	HD	66	M	NFT	A, T, G	A, T, G
10	LD	68	F	NFT	A, T, G	A, T, G
11	HD	27	F	NFT	A, T, G, D	A, T, G, D
12	HD	32	F	LH	A, T, G, D	A, T, G, D
13	LD	53	F	CD	A, T, G, D	A, T, G, D
14	HD	48	M	NFT	A, T, G	A, T, G
15	HD	45	M	NFT	A, T, G	A, T, G
16	LD	38	M	NFT	Nil	Nil

LD = low dose group, HD = high dose group, NFT = non-functioning tumour, IH = idiopathic hypopituitarism, LH = lymphocytic hypophysitis, CD = surgery for Cushing's disease, A = adrenal hormone deficiency/replacement, T = thyroid deficiency/replacement, G = gonadal deficiency/replacement, D = diabetes insipidus.

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dose group) or 6 μg/kg/d ($n = 10$) (higher dose group). Randomisation, which was performed centrally [15], produced unequal group sizes at our site. Subjects were instructed in subcutaneous self-administration with GH using a pen device (Humatrope, Eli Lilly Australia). Whole body protein turnover studies and indirect calorimetry were then repeated after 2 and 12 weeks of GH replacement, with DXA repeated at 12 weeks.

2.3. Assessment of body composition

DXA was performed using a total body scanner (Lunar model DPX, software version 3.1, Lunar Corp., Madison, WI) to determine LBM, total FM and truncal fat [17]. At our institution, the coefficients of variation (CV) for LBM and FM are 1.5% and 2.9%, respectively [2].

2.4. Assessment of whole body protein turnover

Whole body protein turnover was assessed using a primed constant infusion of 1-[^{13}C] leucine as previously described [18]. $NaH^{13}CO_3$ and 99% 1-[^{13}C] leucine were obtained from Cambridge Isotope Laboratories (Woburn, MA). Solutions were prepared under sterile conditions using 0.9% saline.

Following an overnight fast, a 0.1 mg/kg priming dose of $NaH^{13}CO_3$ was immediately followed by a 3 h primed constant infusion of 1-[^{13}C] leucine (prime, 0.5 mg/kg; infusion, 0.5 mg/kg/h), based on previous studies demonstrating that steady state was achieved during this time period [18,19]. Blood and breath samples were collected before (−10, 0 min) and at the end of the infusion (140, 160 and 180 min). Blood was placed on ice and plasma was separated and stored at −80 °C until analysis. CO_2 production rates were measured with an open circuit ventilated hood system (Deltatrac metabolic monitor, Datex Instrumentation Corp., Helsinki, Finland), which was calibrated against standard gases before each study. Measurements of CO_2 production were collected during two 20 min periods and averaged.

2.5. Calculation of whole body protein turnover

The method is based on the principle of steady state kinetics in which the rates of appearance of substrate equals its rate of disposal. Rates of leucine appearance (leucine Ra, an index of protein breakdown), leucine oxidation (Lox, an index of oxidative loss of protein) and leucine incorporation into protein (LIP, an index of protein synthesis) were calculated using the reciprocal pool method as previously described [18,20,21]. As there are only two pathways of disposal for leucine, oxidation and re-incorporation into protein, during fasting leucine Ra = Lox + LIP. In the reciprocal pool method, α-keto-isocaproic acid (KIC), which is formed when leucine undergoes transamination, is used as a surrogate marker of leucine when calculating its rate of appearance, as intracellular KIC rapidly equilibrates with plasma and therefore plasma KIC more accurately reflects the intracellular environment [20]. Lox is calculated from the change in the isotopic enrichment of CO_2 with ^{13}C. LIP is derived as the difference between leucine Ra and Lox. As leucine represents 8% of total body protein, or 590 μmol leucine represents 1 g of protein, rates of protein turnover may be estimated using these constants [22]. The CVs for leucine Ra, Lox and LIP at our institution, based on 7 subjects studied on a second occasion after a median of 34 (range 15–101) days, were 3.5%, 6.1% and 3.5%, respectively.

2.6. Indirect calorimetry

Resting energy expenditure (REE) and substrate oxidation rates were calculated using the equations of Ferrannini [23]. VO_2 and VCO_2 were measured using the Deltatrac metabolic monitor. Protein oxidation was estimated from Lox based on the constants described above [22]. The mean day-to-day intra-subject CV in 20 subjects studied on 4 separate occasions at our institution was 4.3 ± 0.7% for REE [24] and 15.0 ± 2.4% for fat oxidation (Fox) (unpublished data).

2.7. Analytical methods

KIC was extracted from plasma as described by Nissen et al. [25]. KIC enrichment with ^{13}C was measured as the butyldimethylsilyl derivative by gas chromatography (model 5890, Hewlett-Packard Co, Palo Alto, CA)-mass spectrometry (MSD 5971 A, Hewlett-Packard Co), with selective monitoring of ions 301 and 302 [26]. CO_2 enrichment with ^{13}C in breath samples was measured at St. Thomas' Hospital, London, United Kingdom on a SIRA Series II isotope ratio mass spectrometer (VG Isotech, Cheshire, UK). Insulin-like growth factor-1 (IGF-1) was measured using a two-site radioimmunoassay after acid ethanol extraction [15].

2.8. Statistical analysis

Statistical analysis was undertaken using the statistical software packages Statview 4.5 PPC (Abacus Concepts, Inc, Berkeley, USA) and SAS System (SAS Institute Inc, North Carolina, USA). A chi-square test was used to assess categorical variables (gender). Continuous variables are expressed as mean ± standard error unless otherwise stated. Data that were not normally distributed (changes in body composition and IGF-1 with GH treatment) were log-transformed for the purpose of statistical analysis, but means are presented in the text non-transformed.

Unpaired *t*-tests were used to compare differences between the groups. GH treatment effects in the entire

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group and within the low and higher dose groups was assessed using paired *t*-tests or ANOVA with repeated measures where appropriate. Post hoc paired *t*-tests with a Bonferroni correction were then used to ascertain whether changes in variables occurred during the first two weeks of treatment or over 12 weeks with data reported as the change from baseline. Simple linear and stepwise multiple regression analyses examined the statistical relationship between variables using combined data from the two groups. Correction of the change in leucine turnover data and REE over 12 weeks for changes in LBM was made using analysis of covariance (ANCOVA), as this avoids any statistical bias arising from changes in LBM [27–29].

3. Results

3.1. Subject characteristics

There were no baseline differences in gender distribution, weight, IGF-1, body composition, indices of whole body leucine turnover or energy metabolism between the group of subjects receiving the low ($n = 6$) and higher ($n = 10$) GH doses (Table 2). Subjects randomised to the low dose of GH were on average 13 years older than the higher dose group, although the difference did not reach statistical significance ($P = 0.06$). All patients completed the study protocol, and on the basis of returned cartridges, were compliant with GH administration. GH treatment was well-tolerated with no difference in the occurrence of side effects between the two groups.

3.2. IGF-1

IGF-1 significantly increased in both the low (75.6 ± 10.9 to 138.0 ± 20.0 ng/mL, $P = 0.004$) and higher dose groups (66.4 ± 6.5 to 193.6 ± 26.0 ng/mL, $P < 0.0001$). The higher dose group had a greater increase in IGF-1 than the low dose group (127.2 ± 22.4 versus 62.4 ± 17.0 ng/mL, $P = 0.037$).

3.3. Whole body leucine turnover

The effect of GH dose on whole body leucine turnover was first determined. In the low dose group, GH did not significantly change leucine Ra, Lox or LIP over the treatment period, although the increase in LIP approached significance ($P = 0.06$) (Table 3). In the higher dose group, GH significantly increased LIP ($P = 0.016$) and reduced Lox ($P = 0.019$), and induced an increase in leucine Ra that approached statistical significance ($P = 0.06$) (Table 3). When Lox was expressed as a percentage of leucine Ra (Lox/leucine Ra, %Lox), %Lox was not significantly changed in the low dose group but was reduced in the higher dose group ($P = 0.0008$). The reductions in Lox ($P = 0.034$, Fig. 1) and %Lox ($P = 0.03$, Fig. 2) after two weeks were greater in the higher, than the low dose group. Adjustment of changes in whole body leucine turnover over 12 weeks for the change in LBM did not significantly alter the above results (data not shown).

To ensure that the younger age and larger sample size did not explain the greater effect of GH in the higher dose group, the above analysis was repeated with only the 6 oldest subjects in the higher dose group included. The difference in mean age between the low and higher dose groups was now only 5 years (52.5 ± 4.3 versus 47.5 ± 4.5, $P = 0.44$). In this subset of subjects in the higher dose group, GH significantly reduced Lox ($P = 0.03$) and %Lox ($P = 0.004$), while leucine Ra and LIP were not significantly different. The reduction in Lox ($P = 0.02$) and %Lox ($P = 0.04$) after two weeks were significantly greater in this subset of subjects in the higher dose group than in the low dose group.

We next determined the effects of GH over 12 weeks of treatment. Lox ($P = 0.03$, Fig. 1) and %Lox

Table 2
Baseline characteristics of patients randomised to receive a low (3 μg/kg/d) and a higher (6 μg/kg/d) GH dose

	Low dose ($n = 6$)	Higher dose ($n = 10$)	*P*-value
Gender (M/F)	3/3	6/4	0.70
Age (years)	52.3 ± 4.3	39.1 ± 4.3	0.06
Weight (kg)	79.3 ± 4.8	76.6 ± 5.7	0.75
Body mass index (kg/m^2)	28.2 ± 1.6	25.9 ± 1.4	0.29
Insulin-like growth factor-1 (ng/mL)	75.6 ± 10.9	66.4 ± 6.5	0.27
Lean body mass (kg)	43.9 ± 2.9	43.0 ± 3.8	0.86
Fat mass (kg)	30.4 ± 4.6	28.0 ± 3.6	0.69
Leucine Ra (μmol/min)	121.7 ± 9.1	122.0 ± 11.1	0.98
Leucine oxidation (μmol/min)	23.7 ± 2.3	27.6 ± 3.3	0.42
LIP (μmol/min)	98.0 ± 7.5	94.5 ± 8.2	0.78
REE (kcal/d)	1307 ± 77	1275 ± 80	0.79
Fat oxidation (mg/min)	38.2 ± 6.9	30.7 ± 4.1	0.33
Carbohydrate oxidation (mg/min)	69.2 ± 11.2	84.9 ± 10.3	0.34

Values represent mean ± standard error.

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Table 3
Rates of leucine appearance (leucine Ra), leucine oxidation (Lox) and leucine incorporation into protein (LIP) in the entire group ($n = 16$) and in subjects treated with low dose GH (3 μg/kg/d, $n = 6$) and higher dose GH (6 μg/kg/d, $n = 10$) at baseline (0 W) and after two (2 W) and twelve (12 W) weeks

	Leucine Ra (μmol/min)				Lox (μmol/min)				LIP (μmol/min)			
	0 W	2 W	12 W	*P*-value	0 W	2 W	12 W	P value	0 W	2 W	12 W	*P*-value
Entire group	122 ± 8	134 ± 10	130 ± 9	0.015	26.1 ± 2.2	24.0 ± 2.0	23.7 ± 1.9	0.096	96 ± 6	110 ± 9	106 ± 7	0.003
Low dose GH	122 ± 9	129 ± 8	125 ± 10	0.21	23.7 ± 2.3	24.4 ± 2.5	22.9 ± 3.1	0.76	98 ± 7	105 ± 6	102 ± 7	0.06
Higher dose GH	122 ± 11	136 ± 16	133 ± 13	0.06	27.6 ± 3.3	23.7 ± 2.8	24.3 ± 2.6	0.019	94 ± 8	113 ± 13	109 ± 11	0.016

Values represent mean ± standard error. *P*-values were calculated using ANOVA with repeated measures.

($P = 0.002$, Fig. 2) were significantly reduced in the higher dose group after two weeks, whereas after 12 weeks the reductions in Lox and %Lox were not statistically significant. There was no significant change in leucine Ra and LIP between two and 12 weeks. The data suggest that the GH-induced fall in Lox is greatest early in treatment and wanes over time.

3.4. Indirect calorimetry

GH stimulated REE ($P = 0.04$) and Fox ($P = 0.03$) in the entire group (Table 4). The change in REE over 12 weeks was significantly greater in the higher, than in the low, dose group ($+132 \pm 36$ versus -28 ± 41 kcal/d, $P = 0.01$). The change in REE from baseline in the entire group was similar at 2 and 12 weeks, while that of Fox increased progressively to be significant at 12 weeks ($+13.5 \pm 4.9$ mg/min, $P = 0.047$), but not at two weeks ($+8.1 \pm 4.7$ mg/min, $P = 0.32$). Adjustment for the change in LBM did not significantly alter the findings (data not shown).

3.5. Body composition

In the low dose group there were no significant changes in LBM (-0.5 ± 1.1 kg, $P = 0.67$), FM (-0.1 ± 1.2 kg, $P = 0.96$) or truncal fat ($+0.4 \pm 1.3$ kg, $P = 0.74$) (Fig. 3). In subjects receiving the higher GH dose, LBM was significantly increased ($+2.2 \pm 0.7$ kg, $P = 0.01$) and FM (-1.4 ± 0.4 kg, $P = 0.005$) and truncal fat (-1.0 ± 0.1 kg, $P = 0.0001$) were significantly reduced (Fig. 3). The change in LBM in the higher dose group was significantly greater than that in the low dose group ($+2.2 \pm 0.7$ versus -0.5 ± 1.1 kg, $P = 0.04$). The changes in FM (-1.4 ± 0.4 versus -0.1 ± 1.2 kg, $P = 0.53$) and truncal fat (-1.0 ± 0.1 versus $+0.5 \pm 1.3$ kg, $P = 0.22$) in the two groups were not significantly different.

3.6. Determinants of changes in body composition

We first determined the relationship between IGF-1 and changes in substrate and energy metabolism. The increase in IGF-1 over 12 weeks was significantly positively correlated with the change in REE ($r = 0.57$, $P = 0.02$) after 12 weeks of GH treatment. The correlation between the change in Lox at two weeks and the change in IGF-1 during treatment almost reached statistical significance ($r = 0.48$, $P = 0.059$). The change in IGF-1 and the change in LBM were not significantly correlated (data not shown).

We next analysed the relationship between changes in metabolic indices and changes in body composition in the entire group. LBM and FM were log-transformed to achieve a normal distribution prior to statistical analysis. The change in Lox (Fig. 4a) and %Lox (Fig. 4b) after two, but not 12, weeks GH were significantly negatively correlated with the change in LBM at 12 weeks. The correlation between the changes in LIP over both two ($r = 0.07$, $P = 0.31$) and 12 ($r = 0.10$, $P = 0.22$) weeks and the change in LBM over 12 weeks were not statistically significant.

The change in LBM over 12 weeks was significantly negatively correlated with age (Fig. 4c). However, no significant correlation was found between age and the change in Lox or %Lox (data not shown). In stepwise multiple regression analysis, age and the two-week change in Lox were independent determinants of LBM accretion, whereas gender, GH dose and the change in IGF-1 were not.

The change in FM over 12 weeks treatment with GH was positively correlated with baseline BMI ($r = 0.58$, $P = 0.02$) and FM ($r = 0.51$, $P = 0.047$), therefore subjects with the lowest baseline FM had the greatest reduction in FM. Changes in REE or Fox at two or 12 weeks were not significantly correlated with the change in FM or truncal fat (data not shown).

4. Discussion

This open label study of GH replacement in GH-deficient adults demonstrates that changes in LBM are dose-dependent and related to early changes in protein oxidation. IGF-1 was increased to a greater extent in subjects receiving the higher GH dose, with the change

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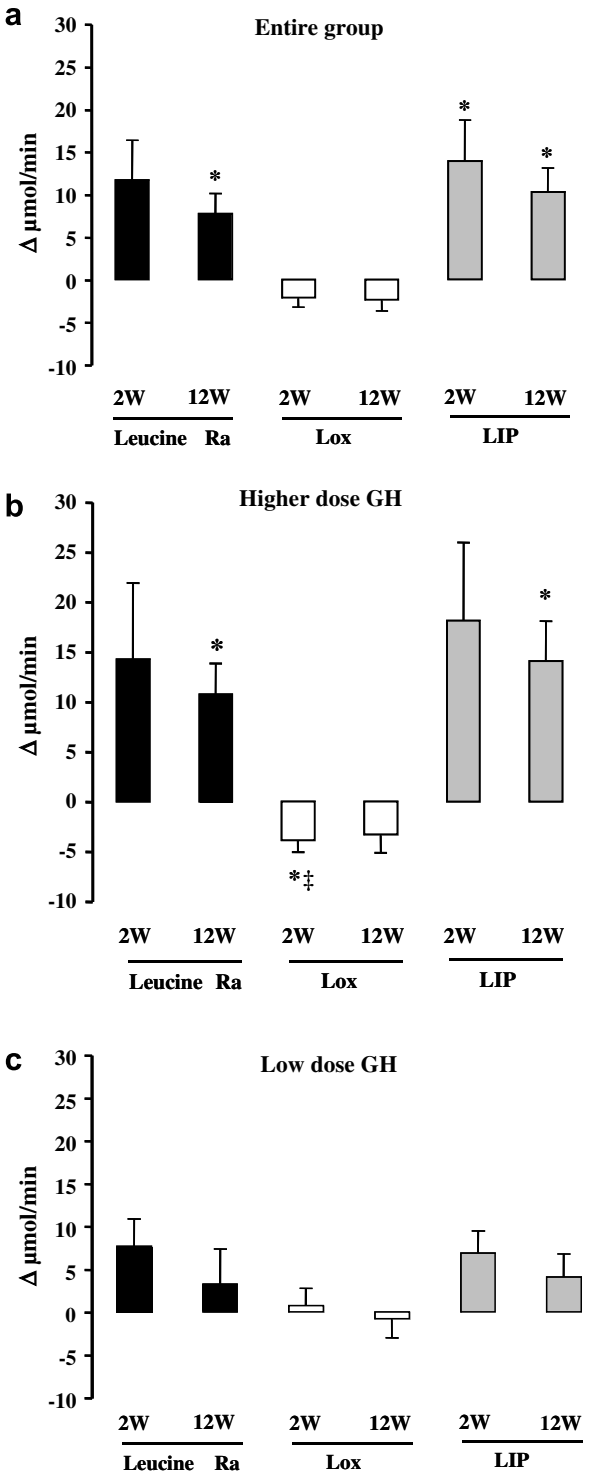

Fig. 1. Change in rates of leucine appearance (leucine Ra), leucine oxidation (Lox) and leucine incorporation into protein (LIP) in the entire group ($n = 16$) (a) and in 6 GH-deficient subjects receiving a low GH dose (3 μg/kg/d) (b) and 10 subjects receiving a higher GH dose (6 μg/kg/d) (c) over two (2 W) and 12 (12 W) weeks. Bars represent mean ± standard error. * $P < 0.05$ versus baseline, ‡ $P < 0.05$ versus low dose group.

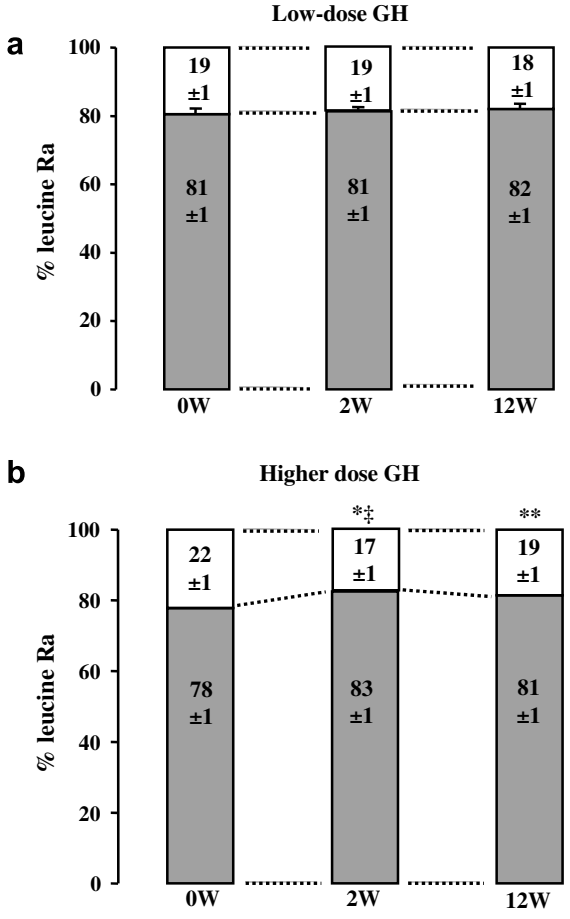

Fig. 2. Leucine oxidation (white bars) and leucine incorporation into protein (grey bars) expressed as a percentage of rate of leucine appearance (leucine Ra) in 6 GH-deficient subjects receiving a low GH dose (3 μg/kg/d) (a) and 10 subjects receiving a higher GH dose (6 μg/kg/d) (b) at baseline and after two (2 W) and 12 (12 W) weeks. Bars represent mean ± standard error. * $P < 0.005$ versus baseline, ** $P < 0.10$ versus baseline, ‡ $P < 0.05$ versus low dose group.

in IGF-1 significantly correlated with the change in REE and Lox. The higher, but not the low GH dose significantly reduced Lox and increased LIP and LBM. GH induced changes in Lox that were not constant throughout the 12 weeks of treatment. The reduction in Lox induced by GH treatment after two weeks was significantly correlated with an increase in LBM at 12 weeks.

While the changes in LBM and protein metabolism induced by GH have been previously reported, this study demonstrates for the first time that the GH-induced reduction in Lox is significantly correlated with a later gain in LBM. While LBM accrual is multifactorial and affected by dietary intake and physical activity levels, the early suppression of protein oxidation explained up to 30% of the gain in LBM over 12 weeks. The importance of a reduction in protein oxidation was underscored by the finding that Lox was significantly reduced only in the higher GH dose group, in whom LBM was also increased. The relationship between a reduction in Lox and an increase in LBM was independent of age, gender, GH dose and the change in IGF-1.

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Table 4
Resting energy expenditure (REE), fat oxidation (Fox) and carbohydrate oxidation (CHOox) in the entire group ($n = 16$) and in subjects treated with low dose GH (3 μg/kg/d, $n = 6$) and higher dose GH (6 μg/kg/d, $n = 10$) at baseline (0 W) and after two (2 W) and twelve (12 W) weeks

	REE (kcal/d)				Fox (mg/min)				CHOox (mg/min)			
	0 W	2 W	12 W	*P*-value	0 W	2 W	12 W	*P*-value	0 W	2 W	12 W	*P*-value
Entire group	1287 ± 56	1360 ± 65	1359 ± 70	0.04	33.5 ± 3.6	41.6 ± 5.0	47.0 ± 4.6	0.03	79.0 ± 7.7	72.0 ± 7.6	58.2 ± 7.5	0.11
Low dose GH	1307 ± 77	1347 ± 84	1279 ± 65	0.27	38.2 ± 6.9	45.6 ± 6.9	44.4 ± 4.7	0.26	69.2 ± 11.2	57.8 ± 10.6	48.2 ± 4.3	0.18
Higher dose GH	1275 ± 80	1368 ± 95	1408 ± 104	0.01	30.7 ± 4.1	39.2 ± 7.0	48.5 ± 6.9	0.075	84.9 ± 10.3	80.5 ± 9.8	64.1 ± 11.5	0.36

Values represent mean ± standard error. *P*-values were calculated using ANOVA with repeated measures.

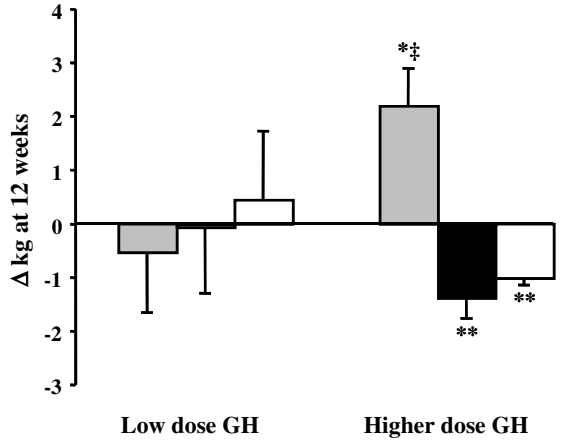

Fig. 3. Changes in lean body mass (grey bars) and fat mass (black bars) and truncal fat (white bars) in 6 GH-deficient subjects receiving a low GH dose (3 μg/kg/d) and 10 subjects receiving a higher GH dose (6 μg/kg/d) over 12 weeks. Bars represent mean ± standard error. * $P < 0.05$ versus baseline, ** $P < 0.01$ versus baseline, ‡ $P < 0.05$ versus low dose group.

The results indicate that metabolic measures of the anabolic effect of GH are a predictor of later gain in LBM.

There has been considerable interest in exploring the potential of GH to induce protein anabolism in other catabolic states, including post-operative patients, patients with burns, sepsis, trauma, pulmonary disease and chronic renal failure and patients receiving long-term glucocorticoids [30]. However, the dose of GH required to achieve an anabolic effect is not known and may vary between these conditions. The choice of dose is usually empirical and often markedly supraphysiologic, which while ensuring an anabolic response, may contribute to detrimental outcomes [31]. Our finding that a reduction in Lox predicts an increase in LBM suggests that measurement of acute changes in protein metabolism provide a useful guide in the investigation of a minimally effective dose of GH.

One limitation of this study is that we did not account for changes in ECW. The LBM is a heterogeneous compartment comprising extracellular water (ECW) and protein or body cell mass (BCM), both of which are increased by GH [32]. Therefore part of the increase in LBM is water, not protein mass, and thus unrelated to changes in protein metabolism. However, GH-induced changes in ECW are dose-dependent and the mean GH doses in the low and higher dose groups of ~0.24 and 0.45 mg/d, respectively, were lower than those used in earlier clinical studies. Therefore, any increase in ECW is likely to have represented a relatively small component of the overall gain in LBM. Previous studies have reported that equivalent GH doses to that used in the higher dose group increase ECW by approximately 1 kg [32,33]. The average increase in LBM in the higher dose group was 2.2 kg,

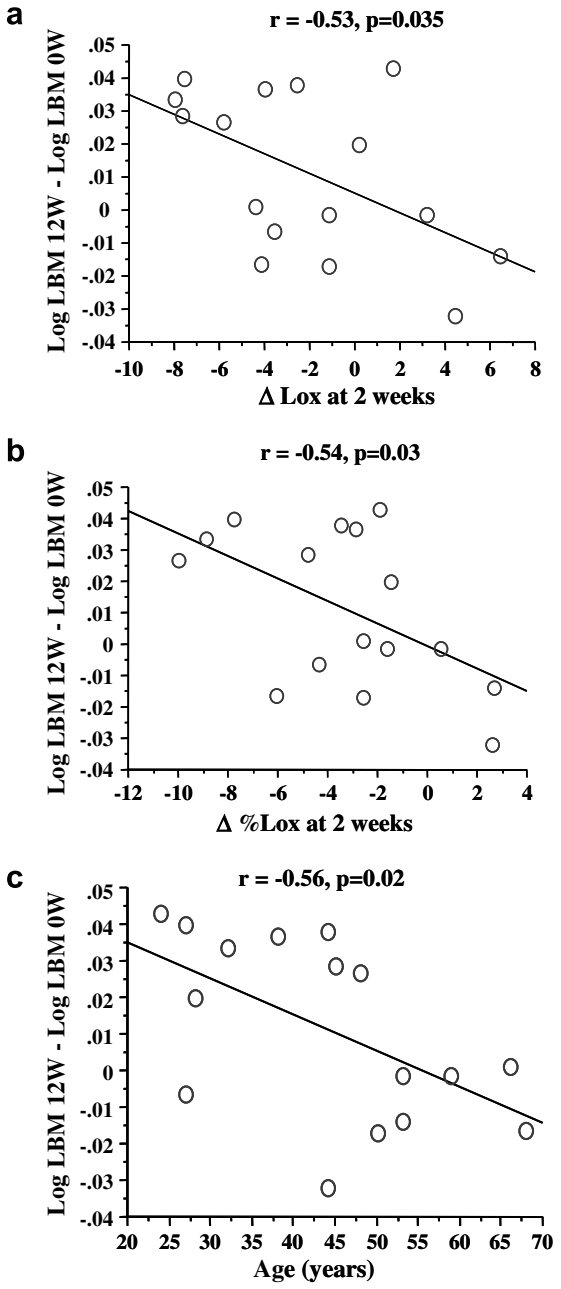

Fig. 4. Correlation in 16 growth hormone (GH) deficient adults between the change in lean body mass (log-transformed) over 12 weeks GH (log LBM 12 W – log LBM 0 W) and the change in leucine oxidation after two weeks GH replacement (Δ Lox at 2 weeks, μmol/min) (a), the change in the proportion of rate of leucine appearance oxidised after two weeks GH replacement (Δ %Lox at 2 weeks) (b) and age (c).

suggesting approximately 50% of the increase in LBM may have been ECW and 50% protein mass. Based on the constants described by Matthews et al [22], which assume leucine represents 8% of total body protein, the predicted gain in protein mass in the higher dose group from the observed reduction in Lox is about 0.8 kg, i.e., approximating what is predicted indirectly after accounting for ECW.

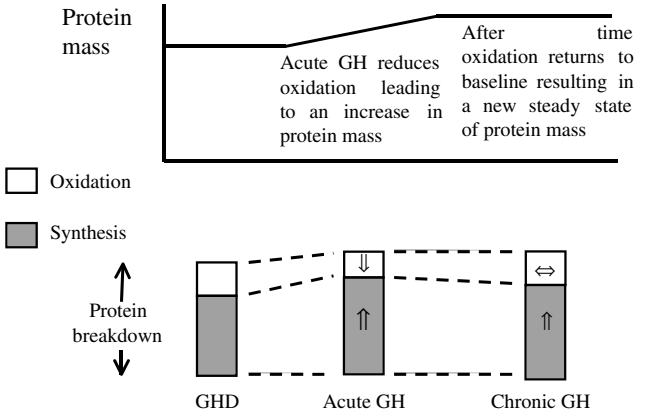

Fig. 5. Proposed model demonstrating the time course of GH-induced changes in protein metabolism and lean body mass (LBM).

In contrast to the significant relationship between changes in protein oxidation and changes in LBM, we did not find a significant correlation between GH-induced changes in REE or Fox and changes in FM. However the higher GH dose had a greater effect on energy and substrate metabolism, and it was only this group in whom a significant reduction in FM was found. The day-to-day reproducibility in the indirect calorimetric assessment of Fox is poor [34,35], and may have contributed to the lack of a significant correlation. Baseline measures of adiposity were the only significant predictor of the change in FM during GH treatment, with subjects with greater adiposity less responsive to GH-induced fat loss, as reported in a previous study [5].

The time course of GH-induced changes in protein metabolism provides further insight into the relationship between changes in protein metabolism and protein mass. GH acutely stimulates protein synthesis and breakdown and reduces oxidation. Our data suggest the effect of GH treatment on protein metabolism may change with time. Lox, the major predictor of LBM accretion, was significantly reduced at two but not at 12 weeks in the higher dose group, suggesting that the effect was waning with time. While this cannot be stated categorically, as there was no significant differences in the GH-induced change in Lox over two and 12 weeks, the findings are consistent with those of Shi et al., who reported that the GH-induced reduction in oxidation was attenuated during prolonged use [8]. From these observations, we propose a model relating GH-induced changes in protein metabolism and LBM (Fig. 5). During the first few months of GH treatment protein synthesis is increased to a greater extent than breakdown, therefore oxidation is reduced and LBM accrues. During prolonged GH treatment, LBM plateaus at a new steady state [36]. In this time period, as oxidation returns to baseline, GH-induced changes in breakdown and synthesis are matched and there is no net loss of protein. Normalisation of protein oxidation may repre-

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sent a metabolic adaptation to achieve a new steady state of protein mass. A similar, but opposite, mechanism may explain the proportionate reduction in breakdown and synthesis but no difference in oxidation in GH-deficient subjects, ensuring protein mass does not waste away completely [18,37].

Randomisation resulted in the assignment of a lesser number of subjects to the low dose group, which limits the ability of this study to assess the efficacy of the low GH dose. Despite this limitation, the higher GH dose had a greater effect on body composition, protein oxidation and REE. Previous studies using similar GH doses (2–6 μg/kg/d) have reported a dose-dependent effect of GH on protein metabolism [6] and LBM [15], unlike studies employing higher GH doses (10–25 μg/kg/d) where a dose-dependent increase in LBM has not been reported [4,38]. It is possible that above a certain GH dose a threshold effect on protein metabolism and gain of LBM is reached. A reduction in FM and truncal fat was only found in the higher dose group, consistent with previous reports that the influence of GH on FM is dose-dependent [4,15,38].

The older age of the low dose group may be another factor contributing to the lack of change in LBM in the low dose group, as we, and others, have found the change in LBM with GH to be attenuated in older GH-deficient patients [5,39]. Short et al. reported that rates of all 3 indices of whole body leucine turnover decline by 3–4% per decade, and suggested that ageing results in a reduced ability to remodel lean tissue [27]. However, the GH-induced reduction in Lox was statistically significant in the higher dose group when only the 6 oldest subjects were included in the analysis. Furthermore, there was not a significant correlation between GH-induced changes in protein metabolism and age. The impact of age on the GH-induced change in LBM was independent of changes in protein oxidation and GH dose and remains to be fully explained. Another factor that should be considered when interpreting changes in protein metabolism during GH treatment is that they may in part be mediated by a GH-induced reduction in bioavailable cortisol [40] or increase in conversion of thyroxine to triiodothyronine [41]. However, the effect of a small reduction in bioavailable cortisol on protein metabolism is unknown, while a slight increase in triiodothyronine concentration may exert an opposite effect to that of GH.

In summary, this study confirms previous findings that GH reduces protein oxidation and increases synthesis in a dose-dependent manner. However, this is the first demonstration that GH-induced changes in whole body protein metabolism are correlated to a later change in LBM, with the acute reduction in Lox predictive of subsequent gain in LBM. The effect of GH on protein oxidation diminishes over time and may explain the stabilization of LBM during GH treatment after an initial increase. Early changes in indices of protein oxidation may predict the potential of GH to induce protein anabolism in other catabolic states.

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