Protein turnover in acid maltase deficiency before and after treatment with a high protein diet

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SUMMARY A patient with acid maltase deficiency was treated with a high protein diet for 7 months. Protein turnover expressed in terms of lean body mass was shown to be increased in this patient before the diet but was markedly reduced following the diet. The patient improved clinically whilst on the diet both subjectively and in terms of mobility, breathing and reduced peripheral cyanosis at rest.

The adult form of acid maltase (α 1-4 glucosidase) deficiency is characterised by progressive muscle weakness which can lead to acute respiratory failure. This weakness has been attributed to disruption of muscle fibres due to accumulation of glycogen. Glycogen accumulates in the lysosomes but also occurs in increased amounts in the sarcoplasm which suggests that some lysosomes may rupture as a result of over-distension.¹ Since disruption of lysosomes would also result in the release of autolytic enzymes the muscle weakness may result from increased tissue breakdown.

In a patient with the childhood form of the disease which is more severe than the adult form, Slonim *et al*² have demonstrated that the clearance of branched chain amino acids following protein ingestion is more rapid than in normal children, suggesting abnormal protein metabolism. Treatment of the patient with a high protein diet for 12 months reduced to normal the clearance of branched chain amino acids following protein ingestion and resulted in improved muscle function and strength. In the current study we have measured protein synthesis and degradation using 1-¹⁴C leucine in a patient with the adult form of acid maltase deficiency both before and after 7 months of a high protein diet. A full metabolic profile of the patient was also obtained and the response to an oral glucose tolerance test and 36 hour starvation was investigated.

Case history

A detailed clinicopathological account of this patient has been published (case 1, ref 3). At the time of study the patient was 33 years old. She was one of two sisters born to parents who were first cousins. Following normal early developmental milestones she noticed that her gait was abnormal at the age of 22 years and had symptoms suggestive of nocturnal respiratory insufficiency, aged 27. By the age of 30, she required nocturnal respiratory support on a rocking bed. On examination she was 183 cm tall and weighed 62 kg. She walked slowly, but unaided, with a marked pelvic waddle. There was severe truncal and pelvic girdle weakness, mild proximal limb weakness with normal cranial and bulbar function, preserved reflexes and flexor plantar responses: the respiratory muscles were weak and the diaphragms paralysed.

Biopsy of vastus lateralis showed the typical features of acid maltase deficiency with marked vacuolation of most fibres and glycogen accumulation (5.5% wet weight—normal up to 1%). The enzyme deficiency was confirmed biochemically in muscle and cultured skin fibroblasts (Professor A D Patrick, Enzyme Research Laboratory, Institute of Child Health).

Methods

The patients' usual daily caloric intake of approximately 2,000 calories (186 g carbohydrate, 104 g fat, 78 g protein) was maintained for the first part of the study. She was admitted for 14 days and the following tests were performed; leucine turnover study, oral glucose tolerance test and a 36 hour starvation study. Two to three days were allowed between each study. The patient was then placed on an isocaloric high protein diet (163 g carbohydrate, 104 g fat, 101 g protein) for 7 months, then readmitted for 7 days (being maintained on the high protein diet during admission) and the leucine turnover study repeated. During both admission periods the patient was placed on a meat-free diet. Twenty

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four hour urine samples were collected after 3 days on this diet, for measurement of 3 methyl histidine and creatinine. Daily blood samples were also taken to measure blood metabolites, plasma amino acids, insulin and creatinine.

The patient gave informed written consent. The study was approved by the Isotope Advisory Panel of the Medical Research Council and the Department of Health and Social Security.

Leucine turnover was studied the morning after an overnight fast. An antecubital vein in each arm was cannulated, one for the infusion of isotope and one for blood sampling. A priming dose infusion of 3.3 μCi 1^{14}C leucine was administered followed by a continuous infusion of 0.12 $\mu\text{Ci}/\text{minute}$ for 150 minutes. Blood samples for the measurement of leucine specific activity and collections of expired air for the measurement of $^{14}\text{CO}_2$ were taken at 40, 60, 80, 100, 120, 140, 150 minutes. Expired air was collected via a two-way valve into Douglas bags. Inspiratory volume was measured by a Wright Respirometer (British Oxygen Company, Harlow, Essex) attached to the inspiratory end of the two-way valve.

Leucine turnover was also measured in 10 control subjects (age, mean $\pm$ SEM; 36 ± 3) after an overnight fast. Following an intravenous injection of 25 μCi 1^{14}C leucine, blood samples and expired air collections were taken at frequent intervals for 2 hours.

The oral glucose test was performed the morning after an overnight fast. Following the ingestion of 100 g glucose blood samples were taken at 0, 15, 30, 45, 60, 90, 120, 150, 180, 240 and 300 minutes for the measurement of plasma glucose and insulin.

During the starvation study in the patient and six control subjects (age, mean SEM = 41, ± 4 years; weight, mean, SEM = 67, ± 3 kg) blood samples were taken the morning after an overnight fast (12 hours) and then again the following morning (36 hour fast).

Plasma ^{14}C leucine and plasma amino acid concentrations were measured using 1.5 ml plasma samples deproteinised with sulphosalicylic acid (80%), freeze-dried, reconstituted in 250 μl lithium citrate buffer and analysed by split-stream analysis on a Chromaspek J180 Amino Acid Analyser (Rank Hilger, Margate, Kent). Six minute fractions of the divided column eluate were collected, 5 ml Pico-Fluor scintillant (Packard) was added to the fraction containing ^{14}C leucine and the samples counted in an Inter technique SL 4000 liquid scintillant counter. The remainder of the eluate was mixed with ninhydrin for measurement of the complete amino acid profile. Urinary 3 methyl histidine was measured using standard methods on the Chromaspek J180 Amino Acid Analyser. Plasma glucose was measured on a Clandon Scientific glucose analyser (Yellow Springs Instrument Company, USA). Blood ketones, pyruvate and lactate were determined enzymatically.^{4,5} Plasma free fatty acids were extracted by the method of Dole and Meinertz⁶ and concentration determined by the semi-automated method of Caruthers and Young.⁷ Plasma insulin was measured by radioimmunoassay.⁸

Expired $^{14}\text{CO}_2$ was measured by the method of Kaihara and Wagner⁹ and per cent CO_2 on an infra-red capnograph (Godart NV, Bilthoven, Holland).

In the acid maltase patient leucine turnover was calculated from: Infusion rate of ^{14}C leucine (dpm/min/kg body weight)/steady state level of leucine specific activity

(dpm/ μmol) and in the control subjects from: Dose of isotope injected/area under leucine specific activity curve. Leucine metabolism was calculated using the standard stochastic model¹⁰ in which leucine turnover (Q_L) = $S + O = B + I$ where S = rate of leucine incorporation into protein; O = rate of leucine oxidation; B = rate of leucine entering the plasma pool from protein breakdown and I = rate of dietary intake. Since the patient and control subjects were studied after an overnight fast I = zero. Estimates of protein synthesis and degradation were calculated from S and B respectively on the assumption that the leucine content of mixed body protein is 8%. Leucine oxidation rate (O) was calculated from the equation:

$$\text{Leucine oxidation rate } (\mu\text{mol}/\text{min}/\text{kg}) = \frac{\text{CO}_2 \text{ production rate } (\text{mmol}/\text{min}) \times ^{14}\text{CO}_2}{\text{Dose}} \times \text{Leucine turnover } (\mu\text{mol}/\text{min}/\text{kg})$$

where $^{14}\text{CO}_2$ = steady state level $^{14}\text{CO}_2$ (dpm/mmol) in the acid maltase patient or area under $^{14}\text{CO}_2$ curve (dpm/mmol $\times$ min) in the normal subjects and Dose = ^{14}C leucine infusion rate (dpm/min) in the patient or ^{14}C leucine injected (dpm) in the control subjects. Leucine metabolic clearance rate was calculated from:

$$\text{Leucine MCR } (\text{ml}/\text{min}/\text{kg}) = \frac{\text{Leucine turnover } (\mu\text{mol}/\text{min}/\text{kg})}{\text{Leucine concentration } (\mu\text{mol}/\text{ml})}$$

Muscle mass and lean body mass were calculated from the rate of creatinine excretion.^{11,12} In the control subjects, lean body mass was calculated from age, weight and height of the patients.¹³ The patient was assessed at intervals using a standard performance scale,¹⁴ including timed walking over 30 metres, muscle strength¹⁵ and supine/standing vital capacity measurements.

Results

The patient had normal postabsorptive levels of metabolites, amino acids and insulin as shown in table 1. The patient showed a normal response to an oral glucose tolerance test (fig 1) and to the 36 hour fast with the exception of alanine concentration which decreased markedly by 50% compared to 17% in normal subjects (table 1).

Urinary creatinine excretion was reduced compared to normal women (table 2), despite renal creatinine clearance being normal (97 ml/min). Excretion of 3 methyl-histidine was normal but when expressed in terms of creatinine excretion (that is, 3 methyl-histidine/creatinine $\times 10^3$) was twice normal.

Leucine turnover, metabolic clearance rate (MCR), oxidation rate and derived measurements of protein synthesis were normal when expressed in terms of body weight (table 3). However lean body mass in this patient calculated from the rate of creatinine excretion was considerably reduced. When expressed in terms of lean body mass leucine turnover, MCR, protein synthesis and protein degradation rates were above the limits of the range seen in control subjects.

Table 1 Metabolic profile of a patient with acid maltase deficiency and control subjects after a 12 hour and a 36 hour fast and in the patient after treatment with a high protein diet for 7 months

	Acid maltase patient			Control subjects (mean $\pm$ SEM)	
	12 hour fast (before diet)	36 hour fast	12 hour fast (after diet)	12 hour fast	36 hour fast
Glucose (mmol/l)	4.94	5.1	4.80	5.25 $\pm$ 0.15	4.33 $\pm$ 0.11
Pyruvate (mmol/l)	0.057	0.052	0.068	0.049 $\pm$ 0.008	0.055 $\pm$ 0.004
Lactate (mmol/l)	0.753	0.524	0.753	0.679 $\pm$ 0.098	0.886 $\pm$ 0.153
β -hydroxybutyrate (mmol/l)	0.122	1.085	0.237	0.105 $\pm$ 0.029	0.878 $\pm$ 0.107
Acetoacetate (mmol/l)	0.042	0.379	0.099	0.062 $\pm$ 0.012	0.259 $\pm$ 0.021
FFA (mmol/l)	0.650	1.070	0.635	0.650 $\pm$ 0.09	1.290 $\pm$ 0.16
Glycerol (mmol/l)	0.174	0.181	0.101	0.105 $\pm$ 0.17	0.104 $\pm$ 0.035
Leucine (μ mol/l)	96	147	104	98 $\pm$ 7	179 $\pm$ 14
Valine (μ mol/l)	211	227	224	187 $\pm$ 22	274 $\pm$ 22
Isoleucine (μ mol/l)	47	85	53	68 $\pm$ 17	96 $\pm$ 4
Alanine (μ mol/l)	267	134	177	349 $\pm$ 21	291 $\pm$ 22
Insulin (mU/l)	8.68	2.20	3.90	3.82 $\pm$ 1.11	3.15 $\pm$ 0.73

Fig 1 Plasma glucose and insulin response to a 100 g oral glucose tolerance test. The shaded area shows the mean $\pm$ 2 SD for 20 control females.

The rate of leucine oxidation expressed as lean body mass was similar to the rate in control subjects. After 7 months on the high protein diet the post-absorptive level of β hydroxybutyrate was elevated and alanine concentration was decreased. All the other metabolites, amino acids and insulin were normal (table 1). Urinary creatinine excretion although still lower than normal was greater than before treat-

ment suggesting an increase in muscle mass (table 2). There was a small decrease in 3 methyl-histidine excretion rate and the 3 methyl-histidine/creatinine ratio was reduced. Leucine turnover and rates of whole body protein synthesis and degradation were decreased below normal levels when expressed in terms of body weight. Leucine oxidation rate had not changed when expressed as a function of body weight or lean body mass. When looked at in terms of lean body mass leucine turnover and rates of whole body protein synthesis and degradation had fallen and were now similar to the rates found in control subjects. Leucine MCR was also reduced but was still greater than in control subjects.

The patient improved clinically whilst on the high protein diet both subjectively and in terms of general mobility, breathing (fig 2) and reduced peripheral cyanosis at rest. The mean time taken to walk 30 metres was 73 s (range 50–110) on the six occasions before treatment and 32 s (range 28–35) on the last six consecutive occasions 7–15 months after the start of the diet. There was no improvement in muscle strength in the eight groups measured (right and left shoulder abduction, right elbow flexion/extension, right hip flexion, right and left knee extension, neck flexion), but a small systematic increase in supine vital capacity from 0.45 to 0.81 was noted.

Discussion

The normal response to an oral glucose tolerance test and a 36 hour fast demonstrated that there was no apparent defect in glucose metabolism in this patient. Unlike control subjects who showed only a small decrease in alanine concentration after a 36 hour fast, the plasma alanine concentration of this patient fell markedly. This suggests that either hepatic uptake of alanine for gluconeogenesis was increased or that ala-

Table 2 Urinary creatinine, 3 methyl-histidine and 3 methyl-histidine/creatinine excretion and creatinine clearance in the acid maltase deficient patient and control subjects

	Acid maltase patient		Control female subjects (mean $\pm$ SEM) n = 5
	Before diet	After diet	
Urinary creatinine (mmol/24 h)	6.11	7.3	10.3 $\pm$ 0.4
3 methyl-histidine (μ mol/24 h)	176	146	177 $\pm$ 12
3 methyl-histidine/creatinine $\times 10^3$	28.9	20.1	17.4 $\pm$ 1.6

Table 3 Measurements of leucine and protein metabolism before and after a high protein diet in a patient with acid maltase deficiency and control subjects

	Acid maltase patient		Control subjects (mean $\pm$ SEM) Subjects (n = 10)
	Before diet	After diet	
Weight (kg)	60.8	61.0	73 $\pm$ 3
Lean body mass (kg)	27.5	31.3	55 $\pm$ 3
Muscle mass (kg) (percent of bodyweight)	13.82 (23)	16.47 (27)	—
Leucine production rate: (μ mol/min/kg BW) (μ mol/min/kg LBM)	1.70 3.76	1.20 2.33	1.78 $\pm$ 0.14 2.34 $\pm$ 0.02
Leucine oxidation rate: (μ mol/min/kg BW) (μ mol/min/kg LBM)	0.12 0.27	0.13 0.25	0.19 $\pm$ 0.02 0.25 $\pm$ 0.02
Leucine MCR: (ml/min/kg BW) (ml/min/kg LBM)	16.43 36.33	13.11 25.55	14.04 $\pm$ 1.36 18.38 $\pm$ 1.38
Whole body protein: synthesis (g/day/kg BW) (g/day/kg LBM)	3.88 8.58	2.62 5.11	3.88 $\pm$ 0.30 5.10 $\pm$ 0.28
Whole body protein: Degradation (g/day/kg BW) (g/day/kg LBM)	4.15 9.18	2.93 5.71	4.35 $\pm$ 0.33 5.71 $\pm$ 0.32

BW = body weight.
LBM = Lean body mass.

Fig 2 Supine vital capacity (litres), maximum voluntary isometric knee extension force (Newtons) and time to walk 30 metres (seconds) before and after the start of a high protein diet.

nine production (that is, from glycolysis or proteolysis) was reduced compared to normal. When expressed in terms of body weight this patient had a normal leucine production rate and normal rates of whole body protein synthesis and degradation. However the comparison of protein turnover, expressed as body weight, with protein turnover measured in control subjects is of dubious significance in a patient with a major muscle wasting disease. The patient's lean body mass judged by clinical examination and the rate of creatinine excretion was considerably reduced. By expressing protein turnover per kilogram lean body mass, a more meaningful comparison can be made with protein turnover rates measured in control subjects. When expressed as lean body mass, protein turnover (synthesis and degradation) was increased in this patient compared with normal. This discrepancy between protein turnover measurements when expressed as body weight and as lean body mass was found by Nair *et al*¹⁶ in obese patients who have increased fat and reduced lean body mass for their weight. In this patient since

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tissues other than muscle are not clinically affected by the disorder, the increase in whole body protein degradation/kg lean body mass is probably due to increased muscle proteolysis. This may result from the accumulation of muscle glycogen causing the disruption of lysosomes and the release of autolytic enzymes.¹ Alternatively because lean body mass is reduced, protein degradation may be increased to maintain a normal supply of amino acids to other tissues. The increased protein synthesis could be a compensatory increase.

An alternative measure of muscle protein breakdown which has been widely used is urinary excretion of 3 methyl-histidine.^{17 18} This amino acid, formed by the methylation of peptide-bound histidine in actin and myosin, is excreted when these proteins are broken down and is not re-used for protein synthesis. As a major fraction of creatinine excretion is proportional to muscle mass¹¹ the 3 methyl-histidine/creatinine ratio in the urine describes the relationship between muscle catabolism/muscle mass. In this patient the 3 methyl-histidine/creatinine ratio was nearly twice normal. This also suggests the increased whole body protein degradation (expressed as lean body mass) was due to an increased muscle proteolysis. It has, however, been suggested that 3 methyl-histidine is not a good measure of muscle protein breakdown since actin and myosin are also found in skin and gastrointestinal tract.

Although muscle mass and lean body mass were still lower than normal after treatment with a high protein diet for 7 months, whole body protein synthesis and degradation rate (expressed as lean body mass) was reduced to normal and 3 methyl histidine/creatinine ratio was decreased. It is not clear how the high protein diet may have reduced whole body protein degradation. Possibly the supply of an alternative amino acid source spares muscle protein. The decrease in synthesis is probably a compensatory decrease, secondary to the fall in degradation.

Patients with acid maltase deficiency may be functionally limited by respiratory muscle insufficiency as in this case. The improvement in mobility following treatment with a high protein diet was probably secondary to enhanced respiratory function which was critically reduced. It seems plausible that respiratory muscles (those most selectively and severely affected by the disease) might, through only a small gain in contractile strength, improve ventilation sufficiently to increase wellbeing and mobility. More precise measurements utilising transdiaphragmatic measurements and inspiratory/expiratory pressure measurements may clarify this. If such were the case only a small shift in muscle protein turnover in favour of net synthesis might be required for significant clinical movement.

A high protein diet has been shown to be beneficial

in a patient with the childhood form of acid maltase deficiency.² It is interesting that a high protein diet has also been found to improve a patient with McArdle's syndrome¹⁹, another disease which results in muscle wasting.

The response of the patient with acid maltase deficiency in the present study to the high protein diet endorses and extends the observations of Slonim *et al*² and indicates the need for more extensive trials of high protein diet in this previously untreatable condition. These are currently being undertaken.

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