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The effect of a high protein diet on leucine and alanine turnover in acid maltase deficiency

A M UMPLEBY,* P StJ TREND,† D CHUBB,* J V CONAGLEN,* C D WILLIAMS,* R HESP,§ I N SCOBIE,* C M WILES,† G SPENCER,‡ P H SÖNKSEN*

From the Departments of Medicine, Neurology† and the Respiratory Unit,‡ St Thomas' Hospital, and the Radioisotopes Division,§ MRC Clinical Research Centre, Harrow, London, UK*

SUMMARY Leucine and alanine production rate was measured in 5 patients with acid maltase deficiency in the postabsorptive state, following 6 months on a normal diet with placebo and 6 months on an isocaloric high protein diet (16–22% protein). Whole body leucine production rate, a measure of protein degradation, expressed in terms of lean body mass was significantly greater than in five control subjects. Following the high protein diet, leucine production rate was decreased in four of the five patients but this was not statistically significant. Alanine production rate expressed in terms of lean body mass was significantly greater than in control subjects. After the high protein diet, alanine production rate and concentration were significantly decreased ($p < 0.05$). There were no significant improvements in any of the clinically relevant variables measured in these patients. It is possible that a larger increase in protein intake over a longer time period may have a clinical effect.

Type II glycogenosis (acid alpha-1,4-glucosidase deficiency) presents either in infancy, or in juvenile or adult forms.[1] Infants are floppy and develop gross cardiomegaly and hepatomegaly before perishing in the first few years of life.[2] Adults tend to present in their late 'teens or twenties with marked truncal and pelvic girdle weakness and impressive paraspinal muscle wasting. However, the key clinical feature is the presence of respiratory insufficiency which is chiefly caused by diaphragmatic weakness or paralysis.[3,4] The diagnosis can be confirmed by finding absent or low levels of acid maltase in muscle,[5] cultured skin fibroblasts[6] and leucocytes.[7,8]

The muscle wasting and weakness in the adult-onset disease has been attributed to the disruption of muscle fibres caused by accumulation of glycogen in the sarcoplasm.[9] Measurement of protein turnover in a patient with this disorder has demonstrated an increase in whole body protein degradation which suggests that the myopathy may be due to increased muscle breakdown.[10] Treatment of the patient with a high protein diet for 7 months resulted in a decrease in whole body protein breakdown and a general improvement in the patient's condition.[10] The beneficial effects of a high protein diet have also been reported in a child with this disorder[11] and in another adult.[12]

The response of these patients to a high protein diet indicated the need for a more extensive study of the effects of increasing protein intake in acid maltase deficiency. We have, therefore, measured whole body protein turnover in five patients with acid maltase deficiency and examined the effect of 6 months of high protein diet.

Address for reprint requests: Dr A M Umpleby, Department of Medicine, UMDS, St Thomas' Hospital, London SE1 7EH, UK.

Present address of Dr J V Conaglen: Waikato Hospital, Hamilton, New Zealand.

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Methods

Five patients (aged 42–50 yr) with adult onset acid maltase deficiency were recruited from the Respiratory Unit at St Thomas' Hospital. Clinical details of four of these patients have previously been reported.[3] Details are shown in table 1. All patients were studied (1) prior to therapeutic intervention, (2) after 6 months on an isocaloric high protein diet and (3) after 6 months on a placebo tablet. The selection of patients for the order of these two regimes was random. Patients 1, 4, 5, received the diet followed by the placebo study and patients 2, 3, the placebo study followed by the diet. On each occasion patients were admitted to a metabolic ward for seven days. The patients' normal dietary protein intake was assessed by a dietitian by keeping an accurate daily food diary for 1 week prior to the study. The subjects were encouraged to remain on their normal diet during the

Table 1 *Clinical details of acid maltase patients and control subjects*

Acid Maltase Patients	*Sex*	*Age (yr)*	*Ht (cm)*	*Wt (kg)*	*% Ideal Body Weight*	*Lean Body Mass (kg)*
1	M	42	193	84	100	32
2	M	50	171	69	103	28
3	F	43	172	53	83	28
4	M	42	185	64	82	27
5	M	43	176	61	87	
$\bar{x}$, SEM		44, 2	179, 4	67, 6	91, 10	27, 4
Control subjects (leucine turnover study)						
1	M	34	188	84	104	65
2	M	43	179	93	126	62
3	F	52	165	70	116	42
4	M	35	178	83	115	60
5	M	44	183	74	98	57
$\bar{x}$, SEM		42, 3	179, 4	81, 4	112, 11	57, 4
Control subjects (alanine turnover study)						
1	M	38	188	80	100	63
2	F	57	177	81	117	49
3	F	31	172	65	100	47
4	F	25	174	63	95	48
$\bar{x}$, SEM		38, 7	178, 4	72, 5	103, 10	52, 4

Table 2 *Dietary composition of the acid maltase patients during the placebo and the high protein diet*

Patient	*Carbohydrate (g)*	*Protein (g)*	*Fat (g)*	*Total calories*	*% Calories from protein*
a Normal diet					
1	387	94	111	3008	12·5
2	248	74	75	1952	15·0
3	266	65	99	2320	11·2
4	284	97	127	2373	16·3
5	159	74	97	2155	13·6
b High protein diet					
1	359	117 (24%)	141	3032	15·4
2	252	87 (18%)	88	2119	16·3
3	267	105 (62%)	110	2423	17·3
4	207	155 (60%)	128	2790	22·2
5	101	88 (19%)	61	1432	22·3

The percentage increase in protein intake is shown in parentheses.

initial assessment and whilst on the placebo. During the diet treatment phase patients were asked to increase the proportion of calories provided by protein to 25% of their total caloric intake. None of the patients were able to do this but all, except one, were able to increase the proportion of calories taken as protein by 6%, one subject managed an increase of over 9% (table 2). This protein intake was maintained during their assessment in the metabolic ward. Twenty four hour urine samples were collected on days 4, 5 and 6 of the admission period for measurement of creatinine. Blood samples were taken to measure metabolites, amino acids, insulin, growth hormone, creatinine kinase and creatinine. During each admission measurements were made of leucine turnover, alanine turnover, oral glucose tolerance and lean body mass. Clinical assessment of the patients was undertaken by a blind observer and included measurement of the maximum voluntary isometric knee extension force, the time to walk 30 metres and the time to walk up and down 10 steps, erect and supine vital capacity, maximal static inspiratory and expiratory mouth pressures,[13] the trans-diaphragmatic pressure,[14] estimates of cardiac output determined from echocardiogram tracings and the maximum oxygen utilisation rate. Measurements of leucine and alanine metabolism were also made in two separate groups of control subjects on their usual caloric intake. Details of sex, age, weight, height and body mass are shown in table 1.

The patients and control subjects gave informed written consent. The study was approved by the Ethical Committee of St Thomas' Hospital, the Isotope Advisory Panel of the Medical Research Council and the Department of Health and Social Security.

Leucine and alanine turnover were measured the morning after an overnight fast. Thirty minutes after cannulation of an antecubital vein for blood sampling, a rapid injection of 0·93 MBq (25 μCi) [1-^{14}C] leucine and 2·8 MBq (75 μCi) [3-^{3}H] alanine (Radiochemical Centre, Amersham, England) was administered into a contralateral antecubital vein. Blood samples were taken immediately before the injection of the

isotopic tracers for baseline measurements, then every minute to 5 minutes, at 8 and 10 minutes, then at every 10 minutes to 120 minutes. Collections of expired air for measurement of $^{14}CO_2$ and % CO_2 were made at intervals over the 2 hour period via a 2-way valve into Douglas Bags every 4 minutes to 20 minutes and every 10 minutes thereafter. Inspiratory volume was measured by a Wright Respirometer (British Oxygen Company, Harlow, England) attached to the inspiratory end of the 2-way valve.

An oral glucose tolerance test was performed the morning after an overnight fast. Following ingestion of 100 g glucose, blood samples were taken over a 2 hour period for the measurement of plasma glucose and insulin.

Total body potassium (TBK) measured by whole body counting of potassium-40 was used to estimate lean body mass (LBM).[15] Normal values of TBK for the control subjects were calculated from equations based on weight, height and age.[16]

^{14}C leucine and ^{3}H alanine were measured on 1·5 ml plasma samples as described previously.[17] Plasma glucose was measured on a Clandon Scientific glucose analyser (Yellow Springs Instrument Company, USA). Blood ketones, pyruvate, lactate and glycerol were determined enzymatically.[18,19] Plasma non-esterified fatty acids were extracted and measured using standard methods.[20,21] Insulin and growth hormone were measured by radioimmunoassay[22] and urinary creatinine was measured by the Jaffe reaction on an autoanalyser.

Plasma leucine and alanine activity curves (dpm/ml) were fitted with the sum of three exponentials using a least squares error optimisation algorithm.[23] Leucine and alanine metabolic clearance rates (MCR) were calculated from the area under their respective activity curves.

$$\text{Leucine or alanine MCR (ml/min/kg)} = \frac{\text{Injected dose of tracer (dpm/kg)}}{\text{Area under activity curve (dpm/ml} \times \text{min)}}$$

Leucine and alanine turnover (or production rate, μmol/min/kg) were then calculated by multiplying MCR by concentration. Leucine oxidation rate was calculated using a model of leucine metabolism which includes a bicarbonate subsystem (fig 1) as previously described.[17] The parameters for the leucine subsystem model were calculated in each subject from the three exponential equations describing the leucine specific activity curve. The parameters for k_{42} and the bicarbonate subsystem were determined by a process of adaptive fitting minimising the sum of squares error between model derived and experimental CO_2 specific activity curve.[17] Leucine oxidation rate was then calculated from the following equation:

$$\text{Leucine oxidation rate (}\mu\text{mol/min/kg)} = \frac{k_{42}(\text{min}^{-1})}{k_{42} + k_{p2}(\text{min}^{-1})} \times \text{leucine turnover (}\mu\text{mol/min/kg)}$$

where k_{42} is the rate constant for leucine oxidation and k_{p2} the rate constant for leucine incorporation into protein as shown in fig 1. The rate of leucine incorporation into protein was calculated from leucine turnover minus leucine oxidation rate.

The levels of statistical significance for differences between values for control subjects and the acid maltase patients were calculated using Student's *t* test. A paired *t* test was used to calculate the significance of differences between the placebo and treatment studies in the acid maltase patients. For each

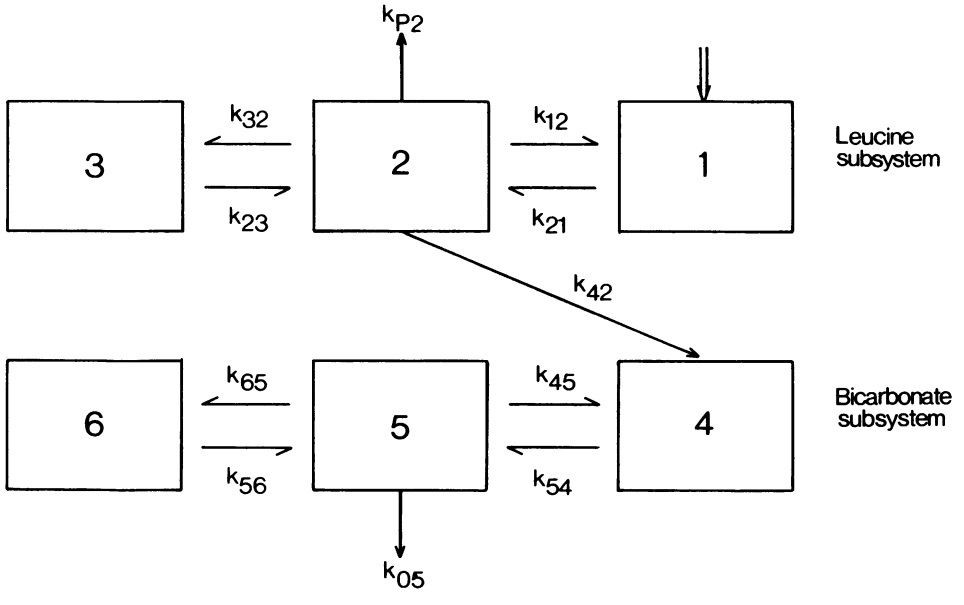

Fig 1 *Compartmental model of leucine and bicarbonate metabolism. The single arrows represent the direction of flux between compartments or out of the system. The double arrow indicates the site of injection.*

Table 3 *Measurements of blood metabolites, insulin and C peptide ($\bar{x}$, SEM) in the acid maltase patients and control subjects)*

	Acid maltase patients (n = 5)			
	Baseline	*Placebo*	*Diet*	*Control subjects† (n = 5)*
Acetoacetate (mmol/l)	0·06, 0·04	0·07, 0·02	0·09, 0·03	0·05, 0·07
B Hydroxybutyrate (mmol/l)	0·19, 0·13	0·14, 0·04	0·18, 0·06	0·05, 0·01
Pyruvate (mmol/l)	0·04, 0·01	0·07, 0·01	0·07, 0·01	0·04, 0·01
Lactate (mmol/l)	0·84, 0·18*	0·83, 0·08*	0·82, 0·08*	0·58, 0·05
Glycerol (mmol/l)	0·13, 0·04	0·12, 0·03	0·16, 0·02	0·19, 0·03
FFA (mmol/l)	0·63, 0·10	0·92, 0·10	0·71, 0·03	0·51, 0·05
Glucose (mmol/l)	5·40, 0·54	5·42, 0·15	5·42, 0·31	5·12, 0·22
Insulin (uU/ml)	17·27, 7·70	14·10, 6·59	12·90, 5·41	4·58, 1·01
C peptide (nmol/l)	0·54, 0·18	0·61, 0·13	0·54, 0·08	0·52, 0·04

*Significantly different from control subjects $p < 0·05$.
†Leucine turnover study.

clinical assessment the differences between the values following the high protein diet and the values following the placebo were plotted as histograms and the distribution shown to be normal.

Results

As shown in table 1, all of the acid maltase patients studied were within 20% of their ideal body weight. However, the lean body mass of all the patients was significantly lower ($p < 0·05$) than in a group of age and height matched normal subjects (table 1). Body weight and lean body mass did not alter following the high protein diet or placebo study.

In the fasting state lactate concentrations were significantly greater ($p < 0·05$) in the acid maltase patients than in the control subjects but the means of other metabolite measurements were normal (table 3). Fasting insulin concentrations, although normal in patients 1, 4 and 5, were elevated in patient 2 (34 μU/ml) and patient 3 (14 μU/ml) and were not affected by either the diet or the placebo treatment.

The response of plasma glucose, insulin and C peptide to an oral glucose tolerance test (OGTT) is shown in fig 2. Two patients [2 and 3] were within WHO criteria for impaired glucose tolerance,[24] two patients [4 and 5] displayed borderline impaired glucose tolerance and patient 1 was normal. Patient 2 had a raised fasting glucose of 6·4 mmol/l and a very high insulin response to the OGTT suggesting that this patient was insulin resistant. The response of C peptide to the oral glucose paralleled that of insulin in each case, the ratio of insulin to C peptide being unusually high in patient 2. The high protein diet and placebo treatment had no effect on the response to an OGTT in each patient (data not shown).

Fasting leucine concentration was significantly lower in the acid maltase patients ($p < 0·05$) than in the control subjects in the baseline study and after six months of placebo or high protein diet (table 4). In the baseline study and after 6 months of placebo study,

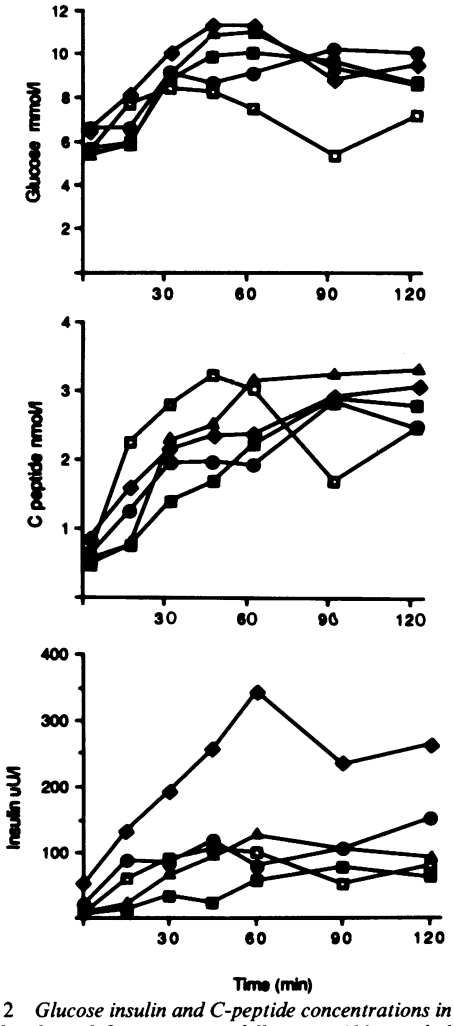

Fig 2 *Glucose insulin and C-peptide concentrations in the 5 acid maltase deficient patients following a 100 g oral glucose tolerance test. The shaded area indicates the normal range.*

leucine metabolic clearance rate (MCR), production rate (Ra) and the rate of leucine incorporation into protein were not significantly different from the subjects when expressed in terms of whole body control weight. However, when expressed in terms of lean body mass, these measurements of leucine metabolism were significantly greater ($p < 0.05$) than normal (table 4). Although there were no statistically significant changes in these measurements of leucine metabolism following the high protein diet, plasma leucine, Ra, MCR and the rate of leucine incorporation into protein were all reduced in 4 of the 5 patients [1, 3, 4 and 5] as shown in fig 3.

Alanine MCR and production rate expressed as lean body mass were significantly greater ($p < 0.05$) than the controls in the baseline study and following the placebo (table 5). As shown in fig 3, alanine MCR was lower in four of the patients after the high protein diet than after the placebo. Alanine production rate and concentration (table 5) was reduced in all five patients by high protein therapy ($p < 0.05$).

Urinary creatinine excretion in the acid maltase

Table 4 *Measurements of leucine metabolism ($\bar{x}$, SEM) in the acid maltase patients and control subjects, expressed in terms of body weight (BW) and lean body mass (LBM)*

		Acid Maltase Patients (n = 5)			
		Baseline	*Placebo*	*Diet*	*Control subjects (n = 5)*
Leucine MCR	BW	14·7, 3·04	14·75, 2·84	13·55, 2·70	11·69, 0·89
(ml/min/kg)	LBM	38·6, 5·10*	35·75, 13·28*	33·41, 6·28*	16·47, 1·16
Leucine Ra	BW	1·38, 0·35	1·37, 0·22	1·16, 0·12*	1·66, 0·15
(μmol/min/kg)	LBM	3·36, 0·85*	3·29, 0·32*	2·88, 0·29	2·33, 0·13
Leucine oxidation	BW	0·15, 0·04	0·21, 0·08	0·14, 0·03	0·20, 0·02
(μmol/min/kg)	LBM	0·34, 0·08	0·49, 0·16	0·34, 0·01	0·27, 0·03
Rate of leucine	BW	1·24, 0·11	1·20, 0·14	1·02, 0·10*	1·46, 0·12
incorporation into protein (μmol/min/kg)	LBM	3·04, 0·32*	2·80, 0·23*	2·51, 0·23	2·05, 0·11
Leucine concentration (μmol/l)		95, 12*	95, 7*	89, 7*	136, 8

*Significantly different from control subjects $p < 0.05$.

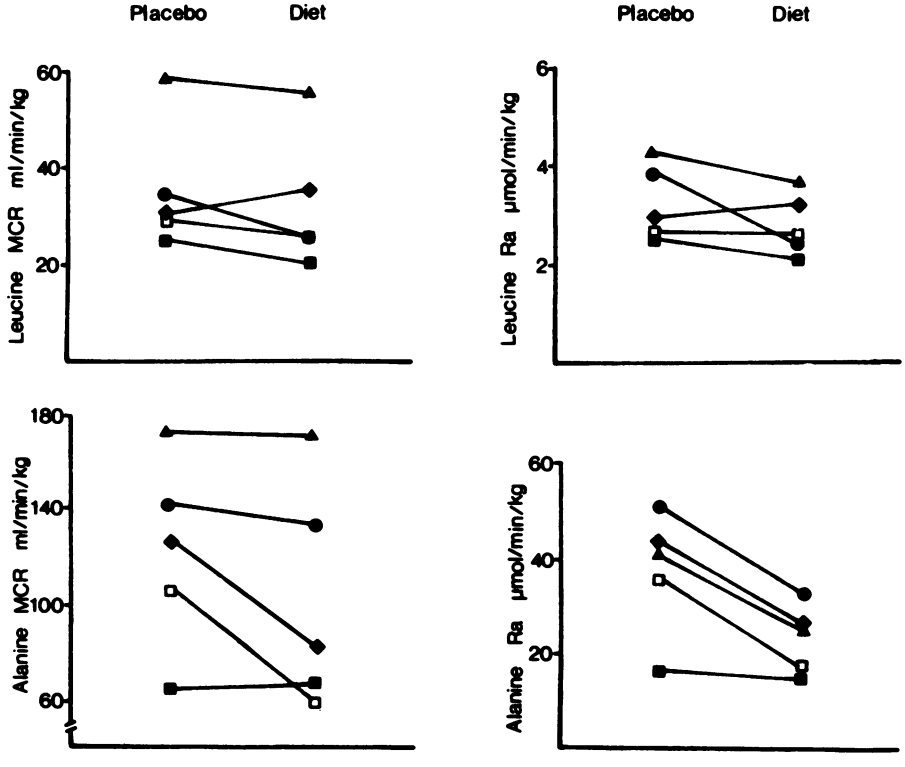

Fig 3 *Leucine and alanine metabolic clearance rate (MCR) and leucine and alanine production rate (Ra) following 6 months' treatment with a placebo and 6 months' on a high protein diet.*

Table 5 *Measurements of alanine metabolism ($\bar{x}$, SEM) in the acid maltase patients and control subjects, expressed in terms of body weight (BW) and lean body mass (LBM)*

		Acid Maltase Patients (n = 5)			
		Baseline	*Placebo*	*Diet*	*Control subjects (n = 4)*
Alanine MCR	BW	40·23, 5·4	50·8, 9·3	42·2, 10·4	42·1, 3·8
(ml/min/kg)	LBM	102·05, 11·6†	122·0, 16·4†	101·2, 20·7	59·3, 6·8
Alanine Ra	BW	11·78, 2·62	15·8, 3·0	9·3, 1·7*	9·9, 1·2
(μmol/min/kg)	LBM	27·21, 3·79†	38·0, 6·0†	22·8, 3·4*	13·7, 1·2
Alanine concentration (μmol/l)		283, 27	306, 46	237, 24*	247, 48

*Significantly different from placebo $p < 0.05$
†Significantly different from control $p < 0.05$.

Table 6 *Mean difference between treatment and placebo period in the clinical assessments of the patients*

Category	*No patients assessed*	*Mean*	*SE mean*	*95% confidence limits*	
Quadriceps strength (N)	5	0·6	3·3	−8·7	9·9
Timed 30 m walk (S)	4	1·3	1·6	−3·8	6·3
Timed 10 steps up/down (S)	3	−0·3	1·0	−4·6	3·9
VC fall (l)	5	0·14	0·29	−0·68	0·96
Pi_{max} ($cm.H_2O$)	4	1·8	3·1	−8·0	12·0
Pdi ($cm.H_2O$)	3	1·7	4·6	−18·0	22·0
CO (l/min)	5	−0·6	0·7	−2·5	1·3
VO_2max (l/min)	3	−0·003	0·18	−0·79	0·78
CK (iv/l)	5	−5·0	29·0	−86·0	76·0

patients was significantly lower in the baseline study (5·1, 0·6, $\bar{x}$, SEM) than in the control subjects (12·3, 0·7) ($p < 0.05$) and showed no significant change after the placebo and high protein diet.

There was no significant difference between treatment with a high protein diet and treatment with a normal diet plus a placebo tablet, in any of the clinical assessments. The numbers of patients in whom complete data were available, the mean differences, the standard errors of the mean differences and the values representing 95% confidence limits are presented in table 6. The maximum benefit of a high protein diet consistent with these data may be gleaned from inspection of the 95% confidence limit values. As examples, on the basis of these data, a high protein diet could not be expected to produce more than an increase of 10 Newtons in maximal isometric knee extension force, more than an increase of 12 cm in H_2O in maximal static inspiratory mouth pressure or more than a fall of 86 U/l in creatinine kinase.

Discussion

The adult onset form of acid maltase deficiency is a rare disease and we were fortunate to be able to recruit five patients for the study. As a result of the small number of patients, differences in leucine and alanine metabolism between patients and control subjects and in response to the high protein diet were not always clear cut.

Although four of the five patients studied were of apparently normal weight for their height, their lean body mass (fat free mass) was reduced to almost half that of normal. Therefore when comparing measurements of amino acid turnover in these patients with normal subjects it may be important to express measurements in terms of lean body mass rather than body weight. When expressed in terms of lean body mass, leucine production rate, a measure of whole body protein degradation, and the rate of leucine incorporation into protein, a measure of whole body protein synthesis, were significantly greater than in the control subjects. Since tissues other than muscle are not clinically affected in acid maltase deficiency this suggests that the muscle wasting in this disorder is due to an increase in muscle protein breakdown.

Damage to muscle tissue as a result of disruption of lysosomes releasing glycogen and autolytic enzymes may result in loss of intracellular amino acids. Protein breakdown may, therefore, increase to preserve the intracellular amino acid pool with a compensatory increase in synthesis.[25] This is unlike the muscle wasting in Duchenne muscular dystrophy[26] and cancer cachexia[27] which has been shown to be the result of a decrease in protein synthesis. Increased protein degradation, however, has been reported in muscle

wasting due to skeletal trauma and burn injury.[28 29]

In a previous study, in a single patient with this disease, we also reported an increase in protein degradation expressed in terms of lean body mass which was decreased by treatment with a high protein diet. This was accompanied by a clinical improvement of the patient.[10] In the present study, we were not able to demonstrate a significant reduction in whole body protein degradation or significant improvements in any of the clinically relevant variables measured following 6 months' treatment with a high protein diet. Protein degradation, however, was lower, after the diet, in four out of the five patients studied. It is therefore possible that a higher protein intake and a longer study period is necessary for a significant clinical effect and a significant decrease in protein turnover to be demonstrated.

The failure of a high protein diet to reduce proteolysis in patient 2 may be due to the patient being "insulin resistant". This may have reduced amino acid uptake following a meal since the uptake of branched chain amino acids has been shown to be reduced in obese patients in whom insulin resistance is a well known feature;[30] alternatively, since Patient 2 was only able to increase his protein intake from 15% to 16·3% of his total caloric intake this may have been insufficient to produce a detectable effect.

If a high protein diet has an inhibitory effect on protein turnover in acid maltase deficiency, this is contrary to the effect of increased protein intake in normal subjects. There is an increase in protein synthesis and a decrease in protein breakdown following feeding in normal subjects and this response is greater when protein intake is increased. In the postabsorptive state, however, protein breakdown is increased with a high protein intake.[31] In the acid maltase patients any inhibitory effect of the high protein diet on protein breakdown during feeding appears to be maintained in the postabsorptive state.

Measurement of alanine turnover using [3-^{3}H] alanine should approximate the absolute rate of in vivo transamination since the ^{3}H is removed during or after alanine transamination.[32] Alanine turnover was lower after the diet than after the placebo which suggests the diet reduced the rate of transamination and the rate of alanine production. Since leucine production rate was reduced in four of the five patients a small proportion of the fall in alanine production in these patients can be accounted for by the decrease in proteolysis. However, most of the decrease must be due to reduced de novo alanine synthesis. A decrease in alanine flux, measured with L-[3,3,3,-$^2H_3$] alanine, in response to a high protein diet has previously been reported in normal subjects, whereas restricting protein intake was shown to increase flux.[33] It was proposed that these changes in alanine flux were due to the resultant changes in carbohydrate intake with these diets, that is decreased carbohydrate with the high protein diet and increased carbohydrate with the restricted protein intake. Although this could provide an explanation for the decrease in alanine production rate in three of the acid maltase patients, carbohydrate intake was unchanged in two patients. It is therefore more likely that there was a direct effect of protein intake on alanine turnover in these patients.

Abnormal glucose tolerance has been reported in other muscle wasting diseases such as myotonic dystrophy[34] and amyotrophic lateral sclerosis.[35] It has previously been reported as normal in acid maltase deficiency.[10 12] It is unclear why abnormal glucose tolerance was not found in all patients and why the degree of intolerance varied from patient to patient. Although there was no direct relationship between the amount of muscle wasting and the degree of glucose intolerance the patient with the normal OGTT had the highest lean body mass. It is possible that the response to an OGTT may be related to the proportion of muscle and fat in these patients since the rate of peripheral uptake by muscle and fat are the factors which determine the late phase of the plasma glucose curve.[36]

Contrary to previous studies which have investigated the effect of a high protein diet in single patients, a high protein diet was found to have no clinical effect in the present study. Measurement of protein turnover, however, demonstrated a decrease in protein catabolism in all but one patient who was found to be insulin resistant. Unfortunately we were only able to recruit five patients for this study and it may well be that the small numbers involved and the limited duration of the study in relation to the lifelong nature of this condition accounted for our failure to show a more clear and beneficial effect. It is possible that over a longer time period and with a larger increase in protein intake a more marked effect may be demonstrable.

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