Eur J Pediatr (1990) 149:346–350

European Journal of
Pediatrics

The contribution of protein catabolism to metabolic decompensation in 3-hydroxy-3-methylglutaric aciduria

G. N. Thompson[1], R. A. Chalmers[2], and D. Halliday[1]

[1]Nutrition Research Group and [2]Division of Perinatal Medicine and Child Health, Clinical Research Centre, Harrow, UK

Abstract. Leucine and protein metabolism were studied using stable isotope techniques in 6-year-old twins with 3-hydroxy-3-methylglutaric aciduria during acute metabolic decompensation. The decompensation was preceded by prolonged fasting in twin 1 and by an upper respiratory infection in twin 2. Twin 2 was also studied when well (control study). During infection, leucine oxidation (36 μmol/kg per hour), protein catabolism (6.0 g/kg per day) and urinary excretion of major leucine metabolites (104 μmol/kg per hour) were all increased compared with the control study (16 μmol/kg per hour, 4.7 g/kg per day and 28 μmol/kg per hour respectively). During fasting, leucine oxidation (18 μmol/kg per hour) was unchanged and protein catabolism (4.1 g/kg per day) was decreased despite substantially increased urinary metabolite excretion (87 μmol/kg per hour) compared with the control study. These results indicate that protein mobilisation and leucine oxidation played important roles in metabolic decompensation during infection but not during fasting. It is likely that the increased metabolite excretion during fasting arose primarily from fatty acid catabolism, indicating the importance of this substrate in metabolic decompensation in 3-hydroxy-3-methylglutaric aciduria.

Key words: 3-Hydroxy-3-methylglutaric aciduria – Protein – Fat – Stable isotope – Decompensation

Introduction

3-Hydroxy-3-methylglutaric aciduria results from a deficiency of 3-hydroxy-3-methylglutaryl CoA lyase (HMG CoA lyase), the final enzyme of the leucine catabolic pathway [2] (Fig. 1). The disorder is of particular interest because the deficient enzyme plays a key role in ketone body and, therefore, fatty acid metabolism [10]. Treatment has, however, tended to concentrate on restriction of dietary protein, with relatively little emphasis on the defect in ketone body and fatty acid metabolism.

3-Hydroxy-3-methylglutaric aciduria usually presents in infancy with vomiting, lethargy, encephalopathy, hypoglycaemia, metabolic acidosis, hyperammonaemia and hepatomegaly [16, 21]. The clinical course may resemble that of a recurring Reye syndrome, with elevation of serum levels of hepatic enzymes [7, 12]. Presentation with acute pancreatitis has also been reported [20]. Hypoglycaemia is an apparently universal feature of acute metabolic decompensation and may be life-threatening [14]. The diagnosis may be made by urinary organic acid analysis, which is characterised by increased 3-hydroxyisovaleric, 3-methylglutaconic, 3-methylglutaric and 3-hydroxy-3-methylglutaric acids during acute metabolic decompensation. Elevation of urinary 3-hydroxy-3-methylglutaric acid persists during periods of good metabolic control. The diagnosis can be confirmed by direct enzyme assay [16, 18].

There are now over 20 reported cases of HMG CoA lyase deficiency [1, 2, 5–7, 12, 14, 16, 20, 21]. Most children have made normal developmental progress with treatment. However, one child sustained severe neurological damage at initial presentation [7] and 2 have died [7, 14]. Treatment regimes have varied, and optimal management is not yet agreed. While some authors have recommended restriction of dietary protein, leucine and fat [7], others have concentrated on the restriction of protein and/or leucine alone [6]. Supplementation of carnitine has also been recommended [1, 6, 16].

We have used stable isotopically labelled leucine and phenylalanine to study the relationships between leucine and protein kinetics in twins with 3-hydroxy-3-methylglutaric aciduria. The rate of oxidation of leucine was used as a measure of the irreversible breakdown of leucine into the abnormal pathway, i.e. the metabolic load on that pathway. Phenylalanine, being metabolised normally in this condition, was employed as a tracer of whole body protein metabolism.

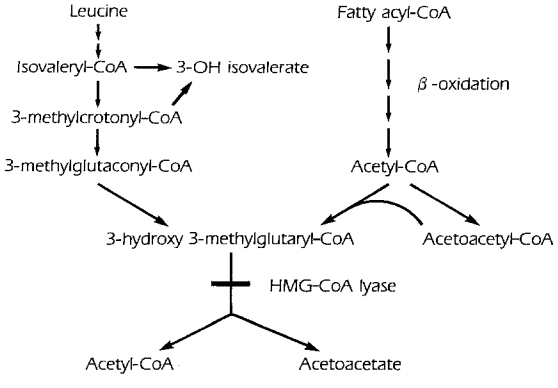

Fig. 1. Metabolic pathways in 3-hydroxy-3-methylglutaric aciduria

Offprint requests to: G. N. Thompson, The Murdoch Institute, Royal Children's Hospital, Flemington Road, Parkville, Victoria 3052, Australia

Abbreviations: HMG CoA lyase = 3-hydroxy-3-methylglutaryl CoA lyase; KIC = alpha-ketoisocaproic acid

Methods

Subjects

Six-year-old twins with HMG CoA lyase deficiency were studied during acute metabolic decompensation and, in the case of twin 2, when well. The twins have been previously described in detail [16]. Twin 2 (male) was noted to be hypotonic at 4 days of age. The hypotonia progressed over the next 4 months, after which the diagnosis of HMG aciduria was made in both twins by urinary organic acid analysis and direct enzyme assay in cultured skin fibroblasts [16]. Twin 1 (female) was asymptomatic prior to diagnosis, but both twins subsequently had repeated episodes of vomiting, hypotonia, convulsions, hypoglycaemia, metabolic acidosis and hyperammonaemia resembling Reye syndrome. These episodes occurred during treatment which restricted leucine intake to 100 mg/kg per day, total protein (including leucine-free amino acid supplements) to 2 g/kg per day and total fat to 5 g/kg per day.

At the time of study both twins were receiving a diet restricted in protein (1.2 g/kg per day, energy intake 60 kcal/kg per day) but with no specific restriction in leucine or fat. On this regime they both had occasional episodes of metabolic decompensation (about two per year) characterised by vomiting, drowsiness, metabolic acidosis and hypoglycaemia and responding to intravenous dextrose infusion.

Twin 1 was studied following an overnight fast. The child was well when the study commenced, but became drowsy, hypoglycaemic (blood glucose 1.2 mmol/l) and began to vomit towards the end of the study. The child had eaten little during the evening preceding the study because of excitement about the impending hospital admission. Effectively she had fasted for 18 h prior to the study.

Twin 2 was studied when well (control study) and during metabolic decompensation preceded by an upper respiratory infection. One day prior to the latter study the child became drowsy and lethargic with frequent vomiting. Blood glucose was 1.6 mmol/l on admission. Treatment with intravenous dextrose 0.5 g/kg per hour was begun 16 h prior to commencing the stable isotope study and was continued throughout the study, maintaining a blood glucose concentration of 8–10 mmol/l.

Values from the study performed in twin 2 when metabolically stable were used for control comparison in both twins. Comparison with protein and leucine kinetics in normal children may not have been appropriate because the treatment regime used in HMG-CoA lyase deficiency, or even the condition itself, may induce changes in protein or leucine metabolism. It was not possible to perform stable isotope studies in twin 1 when well, but as she was closely matched to her sib both genetically and nutritionally, comparison between her values and those of her sib are likely to be more valid than comparison with normal controls. Metabolic similarity between the twins was also suggested by similar urinary metabolite excretion patterns when well (see Results). The studies were approved by the Harrow District Ethical Committee.

Measurement of protein and leucine metabolism

Isotopes. L-[1-^{13}C]leucine (99% ^{13}C), L-[ring-$^{2}H_5$]phenylalanine (98% ^{2}H), L-[ring-$^{2}H_4$]tyrosine (98% ^{2}H) and sodium [^{13}C]bicarbonate (99% ^{13}C) were obtained from Cambridge Isotopes Laboratories (Woburn, Massachusetts, USA).

Procedure. Following an overnight fast, an intravenous cannula was inserted into a peripheral vein (hand or foot) for blood collection. Isotopes were infused into a vein in a separate limb. After collection of baseline blood and expired air samples, priming bolus doses of L-[1-^{13}C]leucine (0.7 mg/kg), L-[$^{2}H_5$]phenylalanine (0.5 mg/kg), [$^{2}H_4$]tyrosine (0.08 mg/kg) and sodium [^{13}C]bicarbonate (0.08 mg/kg) were given. A continuous infusion of L-[1-^{13}C]leucine (0.7 mg/kg per hour) and [$^{2}H_5$]phenylalanine (0.5 mg/kg per hour) was then given over 4 h. Blood and expired air samples were collected at 15–20 min intervals in the final 2 h of each infusion. A timed urine collection was commenced at the start of the stable isotope study and continued for at least 5 h. A separate 5 h urine collection was made afer an overnight fast in twin 1 when she was well. Measurement of expired air total CO_2 production rate was made by indirect calorimetry over at least 20 min in the final 2 h of each infusion using a ventilated hood system as described previously [4].

Analyses. Blood samples were centrifuged at 4°C immediately after collection and the plasma stored at −70°C until analysed. Plasma alpha-ketoisocaproic acid (KIC) concentration and ^{13}C-enrichment were measured using a quinoxalinol-trimethylsilyl derivative and a Finnigan 4500 gas chromatograph/mass spectrometer as previously described [3]. Plasma phenylalanine and tyrosine were derivatised to their tertiary-butyl dimethylsilyl derivatives and their concentrations and deuterium enrichments determined by electron ionisation gas chromatography/mass spectrometry using selected ion monitoring and beta-methyl-phenylalanine or alpha-methyl-tyrosine as internal standards respectively in a similar manner to that described previously [15]. Plasma leucine concentration was determined by the same method using norleucine as internal standard. Plasma enrichment of [$^{2}H_5$]phenylalanine was corrected to the expected 'mixed venous' value for protein turnover calculations as described previously [17]. ^{13}C-enrichment of expired air CO_2 was determined by isotope ratio mass spectrometry.

Urine was frozen at −20°C immediately after collection until analysed. Excretion of 3-hydroxyisovaleric, 3-methylglutaconic and 3-hydroxy-3-methylglutaric acids was quantitated by gas chromatography as described previously [16].

Whole body protein and leucine turnover. Protein turnover was determined from the continuous infusion of [$^{2}H_5$]phenylalanine as described previously [17]. In the fasting, steady state the phenylalanine flux (Q μmol/kg per hour) was calculated by isotope dilution:

$$Q = i \cdot \left[\frac{Ei}{Ep} - 1\right]$$

where i is the rate of infusion of tracer (μmol/kg per hour) and Ei and Ep the respective enrichments of [$^{2}H_5$]phenylalanine in the infusate and at plateau in plasma. The rate of conversion of phenylalanine to tyrosine (Qpt μmol/kg per hour) was calculated from the plasma enrichments of [$^{2}H_5$]phenylalanine and [$^{2}H_4$]tyrosine using similar principles to those applied in the above equation [17].

In the steady, fasted state:

$$Q = S + Qpt = C$$

where S is the loss of phenylalanine from the free amino acid pool to protein synthesis (μmol/kg per hour) and C the rate of

entry of phenylalanine into the free amino acid pool from protein catabolism (μmol/kg per hour). Rates of protein synthesis and catabolism (g/kg per hour) were calculated by assuming the phenylalanine content of protein is 280 μmol/g protein [11]. The difference between S and C was used to calculate the net loss of protein from the whole body in the fasting state.

Whole body leucine kinetics were determined using similar principles to those above as described previously [8]. Leucine turnover was calculated from the plateau plasma enrichment of [^{13}C]KIC, which is presumed to closely reflect the enrichment of the leucine intracellularly [9]. Leucine oxidation was calculated from the plateau enrichments of [^{13}C]KIC in plasma and $^{13}CO_2$ in expired air and from the total expired air CO_2 production rate measured by indirect calorimetry.

In each of the three studies, steady state of KIC, phenylalanine and tyrosine enrichment and concentration and leucine concentration was achieved as defined by plateau coefficient of variation of less than 10%.

Results

Changes in protein turnover calculated using the [$^2H_5$]phenylalanine continuous infusion technique are shown in Table 1. Metabolic decompensation precipitated by fasting was accompanied by a decrease in protein catabolism compared with the control study. However, a smaller decrease in synthesis resulted in increased net loss. The net loss of whole body protein may be expected to relate closely to the net production of leucine from whole body protein breakdown, assuming that the amino acid composition of whole body protein in man is constant [19]. Net protein breakdown may therefore have produced a slight increase in leucine production during the prolonged fast.

Metabolic decompensation precipitated by acute infection was accompanied by substantially increased protein catabolism compared with the control study. A similar increase in synthesis resulted in only a small increase in the calculated net loss. Plasma phenylalanine concentration was similar in each study (control 78 μmol/l, fasting 64 μmol/l, infection 87 μmol/l).

Changes in leucine kinetics and plasma concentration during metabolic decompensation due to prolonged fasting and to infection are documented in Table 2. Fasting resulted in little change in leucine kinetics or concentration, the small changes in turnover and oxidation being similar in magnitude and direction to the changes in protein catabolism and net loss measured by the phenylalanine model. Leucine turnover, oxidation and plasma concentration were, however, substantially increased in response to infection. The increased leucine oxidation may be expected to increase the production of isovaleryl-CoA and, subsequently, 3-hydroxy-3-methylglutaric acid.

Both prolonged fasting and infection resulted in increased excretion of 3-hydroxy-3-methylglutaric acid and other leucine metabolites compared with the control study (Fig. 2). Following prolonged fasting the major metabolites were 3-methylglutaconic and 3-hydroxy-3-methylglutaric acids. By contrast substantially greater amounts of 3-hydroxyisovaleric acid were excreted during metabolic decompensation due to infection, in addition to 3-methylglutaconic and 3-hydroxy-3-methylglutaric acids. Urinary organic acid excretion in twin 1 when she was well (3-hydroxy-3-methylglutaric acid 8.5 μmol/kg per hour, 3-methylglutaconic acid 10.1 μmol/kg per hour, 3-hydroxyisovaleric acid 3.5 μmol/kg per hour) was similar to that in twin 2 when well (control).

Table 1. Protein turnover. Fasting values are taken from studies in twin 1 during acute metabolic decompensation following an 18 h fast, while infection values were obtained in twin 2 during acute metabolic decompensation due to an upper respiratory infection. Control values are taken from twin 2 when metabolically stable. All values are expressed in g/kg per 24 h. Calculations were made from plateau plasma enrichments of [$^2H_5$]phenylalanine and [$^2H_4$]tyrosine during continuous infusion of [$^2H_5$]phenylalanine

	Control	Fasting	Infection
Synthesis	3.55	2.58	4.52
Catabolism	4.67	4.11	5.95
Net loss	1.13	1.53	1.43

Table 2. Leucine kinetics and plasma concentration. Control, fasting and infection values as in Table 1. Calculations of leucine kinetics were made from the plateau enrichments of [^{13}C]alpha-ketoisocaproic acid in plasma and of $^{13}CO_2$ in expired air during continuous infusion of [^{13}C]leucine

	Control	Fasting	Infection
Turnover (μmol/kg/h)	119.0	104.7	150.8
Oxidation (μmol/kg/h)	16.5	18.3	41.7
Concentration (μmol/l)	108	119	390

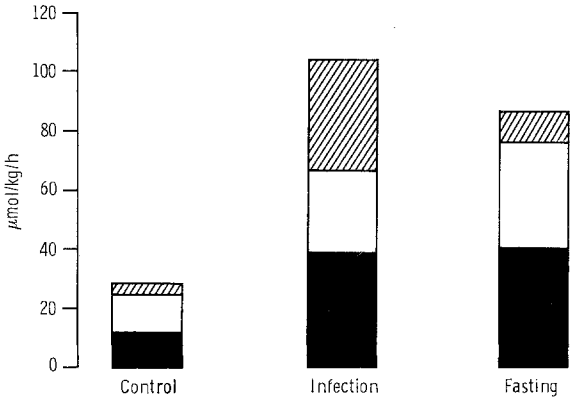

Fig. 2. Excretion of urinary metabolites. Urine was collected over at least 5 h at the same time as the stable isotope study in each case. Small increases in excretion of 3-methyl glutarate and adipate were also noted. Control, fasting and infection values are as in Table 1. The urinary metabolite excretion pattern was similar in both twins when well (control period). ■, HMG; □, methylglutaconate; ▨, 3-OH isovalerate

Discussion

The need for rapid institution of intravenous glucose infusion in the treatment of acute metabolic decompensation in 3-hydroxy-3-methylglutaric aciduria makes it possible to study changes in protein metabolism in acute episodes only after the commencement of treatment when patients present with hypoglycaemia. Intravenous glucose has significant effects on protein metabolism in children, decreasing protein catabolism and net loss (Thompson GN, Walter JH, Leonard JV, Halliday D, unpublished observations). The administration of glu-

cose may explain the similarity in net protein loss in the current control and acute infection studies, despite the increase in total catabolism during infection. By contrast, leucine turnover, oxidation and plasma concentration were markedly increased during infection. These changes most likely reflect changes in protein metabolism prior to the administration of glucose. While glucose infusion appeared to rapidly normalise net protein loss, the clearance of accumulated metabolites of the leucine catabolic pathway was not complete at the time of study. The plasma leucine concentration was increased, and presumably in response to this increased leucine load, leucine oxidation remained high. This proposed sequence of events is supported by the relatively high proportion of 3-hydroxyisovaleric acid in urinary metabolites excreted during infection. This metabolite arises early in the leucine catabolic pathway and would therefore be more likely to be increased when leucine, rather than fat, is the major precursor to 3-hydroxy-3-methylglutaric acid production.

Leucine oxidation was quantitatively less than urinary metabolite excretion in all studies. It is likely that neither urinary organic acid analysis nor stable isotope oxidation rates accurately quantify the whole body production of metabolites. The two methods may, therefore, be compared qualitatively, but not necessarily quantitatively. In the control study leucine oxidation (16 μmol/kg per hour) accounted for 57% of the rate of urinary excretion of measured metabolites (28 μmol/kg per hour). During fasting, however, only 21% of measured urinary metabolites (87 μmol/kg per hour) could be accounted for by leucine oxidation (18 μmol/kg per hour). This suggests that during fasting in twin 1 there was another important source of urinary metabolites, the most likely being from fatty acid oxidation. The urinary organic acid excretion pattern again supported this proposal in that increases in the excretion of more distal metabolites in the leucine catabolic pathway, 3-methylglutaconic and 3-hydroxy-3-methylglutaric acids were much greater than the increase in excretion of the more proximal metabolite, 3-hydroxyisovaleric acid.

During infection, leucine oxidation (42 μmol/kg per hour) also formed a smaller percentage (40%) of the excretion rate of measured urinary metabolites (104 μmol/kg per hour) than during the control study (57%). It is likely that the glucose infusion decreased leucine oxidation more rapidly than urinary metabolite excretion; the latter tends to fall slowly in a wide range of metabolic conditions despite clinical improvement after episodes of metabolic decompensation. However, it is also probable that the decreased leucine oxidation as a percentage of urinary metabolite excretion arose from an increase in the contribution of fatty acid oxidation to 3-hydroxy-3-methylglutaric acid production during infection. While this would be consistent with the usual physiological metabolic response to hypoglycaemia and metabolic stress, the changes in leucine oxidation as a percentage of metabolite excretion during infection and fasting suggest that the importance of fatty acid oxidation to 3-hydroxy-3-methylglutaric acid production during infection is relatively much less than during prolonged fasting.

Gibson et al. [6] proposed that the mechanism of increased 3-hydroxy-3-methylglutaric acid production during fasting in HMG CoA lyase deficiency includes the increased catabolism of whole body protein. A similar mechanism was also proposed for the hyperammonaemia which is frequently seen during acute metabolic decompensation. The current study demonstrated a decrease in the absolute rate of protein catabolism during fasting with a small increase in the net loss of protein. The magnitude of these changes would suggest that alterations in protein metabolism contribute little to the increased production of abnormal metabolites during fasting, or to hyperammonaemia. An alternative explanation for the hyperammonaemia is that 3-hydroxy-3-methylglutaric acid or other accumulated metabolites may be directly toxic to the urea cycle in a manner similar to that described in methylmalonic aciduria [13].

These studies indicate that changes in protein metabolism cannot alone explain the accumulation of abnormal metabolites during metabolic decompensation in HMG CoA lyase deficiency, in particular when prolonged fasting is the major precipitating event. Changes in fat metabolism probably play a significant role in metabolic decompensation, and restriction of dietary fat intake may therefore be indicated in the treatment of HMG CoA lyase deficiency, particularly when response to protein/leucine restriction is not satisfactory. As recommended previously [6], the current study would also support the avoidance of prolonged fasting in the management of this condition.

Acknowledgements. We are grateful to Miss H. Merritt, Mr. G. C. Ford and Miss M. A. Jones for their technical assistance and to Dr. H. B. Valman for allowing us to study these patients.

References

1. Dasouki M, Buchanan D, Mercer N, Gibson KM, Theone J (1987) 3-Hydroxy-3-methylglutaric aciduria: response to carnitine therapy and fat and leucine restricion. J Inherited Metab Dis 10: 142–146
2. Faull KF, Bolton P, Halpern P, Hammond J, Danks DM, Hähnel R, Wilkinson SP, Wysocki SJ, Masters PL (1976) Patient with defect in leucine metabolism. N Eng J Med 294: 1013
3. Ford GC, Cheng KN, Halliday D (1985) Analysis of [1-^{13}C]leucine and [^{13}C]KIC in plasma by capillary gas chromatography/mass spectrometry in protein turnover studies. Biomed Mass Spectrom 12: 432–436
4. Garrow JS, Webster JD (1986) A computer-controlled indirect calorimeter for the measurement of energy expenditure in one or two subjects simulaneously. Hum Nutr Clin Nutr 40C: 315–321
5. Gibson KM, Breuer J, Nyhan WL (1988) 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency: review of 18 reported patients. Eur J Pediatr 148: 180–186
6. Gibson KM, Breuer J, Kaiser K, Nyhan WL, McCoy EE, Ferriera P, Greene CL, Blitzer MG, Shapira E, Reverte F, Conde C, Bagnell P, Cole DEC (1988) 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency: report of five new cases. J Inherited Metab Dis 11: 76–87
7. Leonard JV, Seakins JW, Griffin NK (1979) Beta-hydroxy-beta-methylglutaricaciduria presenting as Reyes syndrome. Lancet I: 680
8. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM (1980) Measurement of leucine metabolism in man from a primed continuous infusion of L-[1-^{13}C]leucine. Am J Physiol 238: E473–479
9. Matthews DE, Schwarz HP, Yang RD, Motil KJ, Young VR, Bier DM (1982) Relationship of plasma leucine and α-ketoisocaproic acid during a L-[1-^{13}C]leucine infusion in man: a method for measuring human intracellular leucine tracer enrichment. Metabolism 31: 1105–1112
10. McGarry JD, Foster DW (1980) Regulation of hepatic fatty acid oxidation and ketone body production. Annu Rev Biochem 49: 395–420
11. Munro HN, Fleck A (1969) Analysis of tissues and body fluids for nitrogenous constituents. In: Munro HN (ed) Mammalian protein metabolism. Academic Press, New York 3: 423–525

12. Robinson BH, Oei J, Sherwood WG, Slyper AH, Heininger J, Mamer OA (1980) Hydroxymethylglutaryl CoA lyase deficiency: features resembling Reye syndrome. Neurology 30:714–718
13. Rosenberg LE (1983) Disorders of propionate and methylmalonate metabolism. In: Stanbury JB, Wyngaarden JB, Frederickson DS, Goldstein JL, Brown MS (eds) The metabolic basis of inherited disease. McGraw-Hill, New York, pp 474–497
14. Schutgens RB, Heymans H, Ketel A, Veder HA, Duran M, Ketting D, Wadman SK (1979) Lethal hypoglycaemia in a child with a deficiency of 3-hydroxy-3-methylglutarylcoenzyme A lyase. J Pediatr 94:89–91
15. Schwenk WF, Berg PJ, Beaufrere B, Miles JM, Haymond MW (1984) Use of t-butyldimethylsilylation in the gas chromatographic/mass spectrometric analysis of physiologic compounds found in plasma using electron-impact ionisation. Anal Biochem 141:101–109
16. Stacey TE, Sousa C de, Tracey BM, Whitelaw A, Mistry J, Timbrell P, Chalmers RA (1985) Dizygotic twins with 3-hydroxy-3-methylglutaric aciduria: unusual presentation, family studies and dietary management. Eur J Pediatr 144:177–181
17. Thompson GN, Pacy PJ, Merritt H, Ford GC, Read MA, Cheng KN, Halliday D (1989) Rapid measurement of whole body and forearm protein turnover using a phenylalanine model. Am J Physiol 256:E631–9
18. Wanders RJA, Schutgens RBH, Zoeters PHM (1988) 3-Hydroxy-3-methylglutaryl-CoA lyase in human skin fibroblasts: study of its properties and deficient activity in 3-hydroxy-3-methylglutaric aciduria patients using a simple spectrophotometric method. Clin Chim Acta 171:95–102
19. Waterlow JC, Garlick PJ, Millward DJ (1978) Protein turnover in mammalian tissues and in the whole body. North-Holland, Amsterdam, pp 179–249
20. Wilson WG, Cass MB, Sovik O, Gibson KM, Sweetman L (1984) A child with acute pancreatitis due to 3-hydroxy-3-methylglutaryl-CoA lyase deficiency. Eur J Pediatr 142:289–291
21. Wysocki SJ, Hahnel R (1986) 3-Hydroxy-3-methylglutaryl-coenzyme A lyase deficiency: a review. J Inherited Metab Dis 9:225–233

Received January 30, 1989 / Accepted May 30, 1989