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Effect of Insulin on Leucine Kinetics in Maple Syrup Urine Disease

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ABSTRACT. Leucine turnover was measured using [1-^{14}C] L-leucine in three patients with classical maple syrup urine disease. There was measurable leucine oxidation although it was lower than in normal adults. Leucine production rate was greater than normal in all three patients with an increased rate of incorporation of leucine into protein and increased protein catabolism. These fluxes were both relatively insensitive to exogenous insulin. (*Pediatr Res* 21: 10–13, 1987)

Abbreviations

MSUD, Maple syrup urine disease
BCAA, branched-chain amino acids
MCR, metabolic clearance rate

In MSUD the BCAA leucine, valine, and isoleucine, and their respective oxoacids are elevated as a result of deficient activity of the branched chain oxoacid dehydrogenase (E.C. 1.2.4.4) (1). Most patients with the severe (classical) form of the disease present in the neonatal period with overwhelming illness. Once the acute metabolic disorder has been controlled they are treated with a strict diet limiting the intake of the BCAA and yet providing sufficient of these and other essential amino acids for normal growth.

During intercurrent illness plasma concentrations of BCAAs and their oxoacids may rise rapidly owing to increased protein catabolism. The accumulation of metabolites may cause metabolic acidosis and potentially fatal encephalopathy. Insulin reduces leucine production rate (a measure of protein breakdown) in diabetic patients (2, 3) and an inverse relationship between plasma insulin and the BCAA concentration has been demonstrated (4). Insulin has also been shown to have important protein sparing effects in severely ill and traumatized patients, such as those with extensive burns, although the effective dose may need to be as much as 0.4 U.kg.$^{-1}$h^{-1} because of relative insulin resistance (5, 6). Thus insulin may be useful in the management of patients with MSUD (7).

We report three patients with classical MSUD in whom we have measured leucine production and turnover before and during an insulin infusion.

PATIENTS AND METHODS

The project was approved by the Ethical Committee of the Joint Research Board of the Hospitals for Sick Children and the Institute of Child Health. The use of radioactive isotopes was approved by the Administration of Radioactive Substances Advisory Committee of the Department of Health and Social Security. The investigations were explained in detail to the patients and parents. Written consent was obtained in all cases.

Patients. Classical MSUD was diagnosed in the neonatal period in all three patients. All had grossly elevated plasma BCAAs at presentation (plasma leucine >3000 μmol.l^{-1}) and were treated with a diet restricting leucine intake to less than 4.6 mmol.day^{-1} (603 mg.day^{-1}) (8).

Patients 1 and 2 were 12 yr of age at the time of the study with an IQ on the WISC-R scale of 69 and 63, respectively. Patient 3 was 23 yr of age, living independently, and employed full-time. All three patients were of normal height and weight. Patient 1: weight, 47 kg (75%), height, 152 cm (75%); patient 2: weight, 45 kg (75%), height, 156 cm (10%); and patient 3: weight, 47 kg (3%), height, 160 cm (25%).

The patients were studied 3 h after a light protein-free breakfast. Each received an infusion of [1-^{14}C]L-leucine (50–59 mCi.mmol^{-1}) (Amersham International). In patients 1 and 2 a total dose of 50 μCi was given over 150 min with insulin being infused at a rate of 0.05 U.kg.$^{-1}$h^{-1} between 90 and 150 min. In patient 3 a total dose of 80 μCi was administered over 210 min with an insulin infusion of 0.1 U.kg.$^{-1}$h^{-1} from 150 min. Plasma was collected for the measurement of leucine specific activity and other metabolites and expired air for the measurement of CO_2 specific activity immediately before the study and every 15 min in patients 1 and 2 and every 20 min in patient 3 until the start of the insulin infusion when the time interval was decreased to 10 min in each patient. Urine was collected for the measurement of leucine excretion in patient 3.

In a separate experiment in patient 1 the effect of an insulin infusion of 0.3 U.kg.$^{-1}$h^{-1} on plasma leucine was studied but no isotope was given and the patient's blood glucose was maintained in the normal range using a glucose clamp (9).

Normal adults. Ten normal adults (two female and eight male) with an age range of 18–52 yr (mean 36, SD 11 yr) and a weight range of 55–92 kg (mean 73, SD 11 kg) were studied the morning after an overnight fast using a 25 μCi bolus of [1-^{14}C]L-leucine. Plasma was collected immediately before the injection for baseline measurements and then every minute to 5 min, at 8 and 10 min, and then every 10 min to 120 min. Expired air was collected every 4 to 20 min and then every 20 min until the end of the study.

Methods. Plasma leucine was measured by split stream analysis on a Chromaspek (Hilger Analytical) amino acid analyzer. $^{14}CO_2$ was measured using the method of Kaihara and Wagner (10). The total volume of distribution of leucine was calculated from the metabolic clearance rate and mean residence time. Measurements of leucine metabolism were calculated using a six compartment model of leucine and bicarbonate kinetics (Fig. 1), the number of compartments being determined from the number of exponentials fitting the curve (3). The leucine subsystem is represented by compartments 1–3 and the bicarbonate subsystem

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by compartments 4–6. Compartment 1 was fixed as plasma volume and compartment 2 as the site for leucine oxidation (represented by the rate constant K_{42} in Fig. 1) and protein synthesis (K_{P2}). Compartment 2 therefore contains both extracellular and intracellular components. Compartment 3 may represent a site of proteolysis but not protein synthesis which has been described in several models of amino acid metabolism in isolated tissues (11). The site of net inflow of unlabelled leucine is unknown. The bicarbonate subsystem is based on the model of Steele (12) and adapted by Waterhouse *et al.* (13) in which the central compartment is the blood pool. This model which is both mathematically identifiable and physiologically sound was previously established in normal subjects (3, 14). The parameters of the leucine subsystem and the bicarbonate subsystem were estimated by a process of adaptive fitting for the minimum sum of squares error (15) to the plasma leucine specific activity curve and the CO_2 specific activity curve, respectively. In the studies in which a steady state level of leucine activity was not achieved before insulin administration (patients 1 and 2) the steady state level of leucine activity was predicted using the parameter estimates of the leucine subsystem. Leucine MCR, production rate, percent leucine oxidation and the rate of leucine incorporation into protein were calculated from the following equations:

$$\text{Leucine MCR} = \frac{\text{infusion rate (dpm.min}^{-1})}{\text{leucine at steady state (dpm.ml}^{-1})} \times \frac{1}{\text{body wt (kg)}} \quad (1)$$

$$\text{Leucine production rate } (\mu\text{mol.min.}^{-1}\text{kg}^{-1}) = \text{leucine MCR (ml.min.}^{-1}\text{kg}^{-1}) \times \text{leucine concentration } (\mu\text{mol.l}^{-1}) \quad (2)$$

$$\%\ \text{Leucine oxidation rate} = \frac{K_{42}}{K_{P2} + K_{42}} \times 100 \quad (3)$$

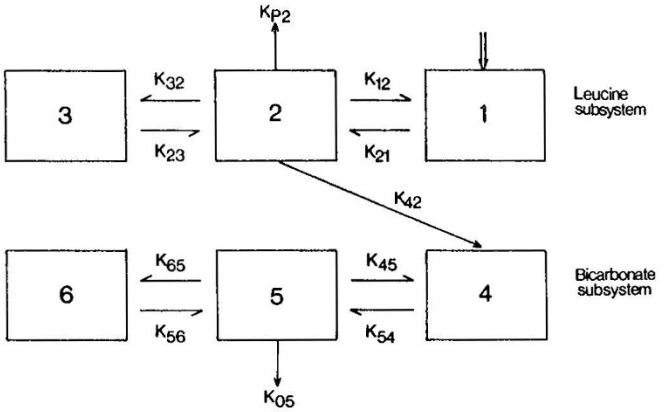

Fig. 1. Compartmental model of leucine and bicarbonate kinetics (10). The leucine subsystem is represented by compartments 1–3 and the bicarbonate subsystem by compartments 4–6. K_{P2} is the rate constant for protein synthesis and K_{42} the rate constant for leucine oxidation.

$$\text{Rate of leucine incorporation into protein } (\mu\text{mol.min.}^{-1}\text{kg}^{-1}) = \text{leucine production rate } (\mu\text{mol.min.}^{-1}\text{kg}^{-1}) - \text{leucine oxidation rate } (\mu\text{mol.min.}^{-1}\text{kg}^{-1}) \quad (4)$$

Leucine oxidation rate was calculated assuming any recycling of $^{14}CO_2$ into other metabolites was negligible. Recovery of $^{14}CO_2$ has been found to be 95% following administration of $NaH^{14}CO_3$ in man (Umpleby AM, Boroujerdi MA, Sonksen PH, unpublished observations).

Following insulin administration there was no change in leucine specific activity (dpm.ml^{-1}) implying no change in leucine MCR. Plasma leucine concentration (μmol.ml^{-1}) was then simulated by reducing the leucine production rate (with a step change and a time constant of 20 min) until an optimal fit to the concentration curve was obtained. Leucine disappearance rate was calculated from the product of the MCR and the simulated plasma leucine concentration curve (3). Intermediary metabolites and insulin were measured using standard methods.

RESULTS

Before the insulin infusion. All three patients had elevated plasma leucine concentrations before the start of the study but the total volume of distribution of leucine was normal (Table 1). The percentage of leucine oxidized and the MCR were lower and the leucine production rate greater than in normal adults (Table 1). Thus the rate of leucine incorporation into protein was greater than in the normal subjects. The rate of leucine excretion was not measured in patients 1 and 2 because they were unable to pass urine but in patient 3 the rate of leucine excretion was negligible at 0.0095 μmol.min.$^{-1}$kg^{-1} [normal adults: mean 0.00046, SD 0.0002 μmol.min.$^{-1}$kg^{-1} (16)]. Plasma glucose, lactate, free fatty acids and ketone bodies were all normal (Table 2).

Effect of the insulin infusion. An insulin infusion of 0.05 U.kg.$^{-1}$h^{-1} in patients 1 and 2 produced no significant change in plasma leucine or leucine oxidation, metabolic clearance rate, production rate, or leucine incorporation into protein (Fig. 2). When 0.3 U.kg.$^{-1}$h^{-1} of insulin was infused in patient 1 plasma leucine fell from a mean of 324 to 276 μmol.l^{-1} in 30 min. In patient 3 when 0.1 U.kg.$^{-1}$h^{-1} of insulin was infused plasma leucine fell from a mean of 755 to 587 μmol.l^{-1} and the leucine production rate decreased from 2.51 to 1.96 μmol.min.$^{-1}$ kg^{-1} after 1 h (Figs. 2 and 3). There was no effect on leucine MCR (3.34 ml.min.$^{-1}$kg^{-1}).

Plasma glucose fell by less than 1.5 mmol.l^{-1} in patients 1 and 2 when insulin was infused at a rate of 0.05 U.kg.$^{-1}$h^{-1} (Fig. 2). Patient 3 developed symptomatic hypoglycemia 50 min after the start of the insulin infusion (blood glucose 2.0 mmol.l^{-1}) and required a glucose infusion (Fig. 2). In all patients there was a slight fall in 3-hydroxybutyrate and acetoacetate, a marked fall in free fatty acids, and a slight rise in lactate during the insulin infusion; the changes were similar at both infusion rates (Table 2).

Table 1. *Leucine kinetics before the insulin infusion*

Patient	Mean plasma leucine (μmol·l^{-1})	Total volume of distribution of leucine (ml·kg^{-1})	Leucine oxidation (%)	Leucine production rate (μmol·min^{-1}·kg^{-1})	MCR (ml·min^{-1}·kg^{-1})	Leucine incorporation into protein (μmol·min^{-1}·kg^{-1})
1	485	445	2.53	2.45	5.05	2.39
2	898	375	2.38	4.98	5.55	4.86
3	755	450	4.53	2.51	3.32	4.00
Normal adults (mean ± SD)	127 ± 18	466 ± 154	10.63 ± 2.38	1.78 ± 0.43	14.04 ± 4.29	1.59 ± 0.38
(Range)	(91–163)	(312–620)	(5.87–15.39)	(0.92–2.64)	(5.46–22.62)	(0.83–2.35)

Table 2. *Intermediary metabolites before and after the insulin infusion*

Patient	Lactate (mmol·l^{-1})		3 Hydroxybutyrate (mmol·l^{-1})		Acetoacetate (mmol·l^{-1})		Free fatty acids (mmol·l^{-1})	
	Before	After	Before	After	Before	After	Before	After
1	0.60	0.82	0.055	0.06	0.06	0.04	0.57	0.18
2	0.73	0.78	0.08	0.03	0.04	0.03	0.58	0.20
3	0.43	0.62	0.10	0.02	0.05	0.03	0.52	0.23
Normal adults (mean ± SD)	0.52 ± 0.37		0.05 ± 0.02		0.05 ± 0.02		0.51 ± 0.14	

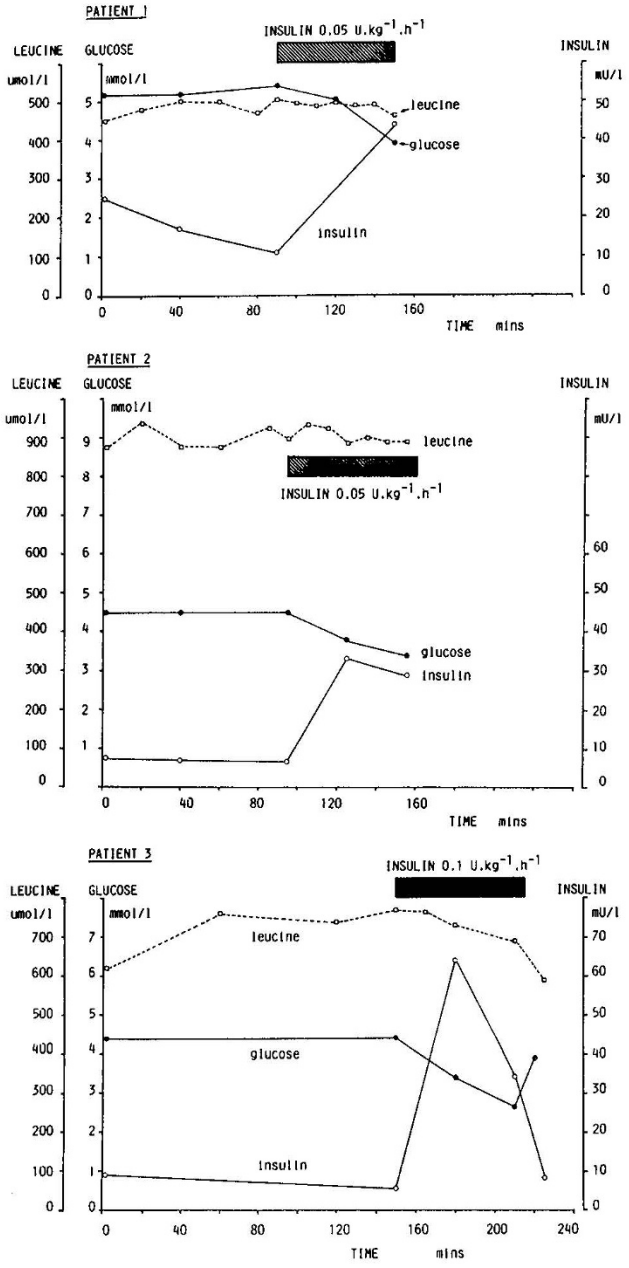

Fig. 2. The effect of an insulin infusion on plasma glucose, leucine, and insulin concentrations in the patients.

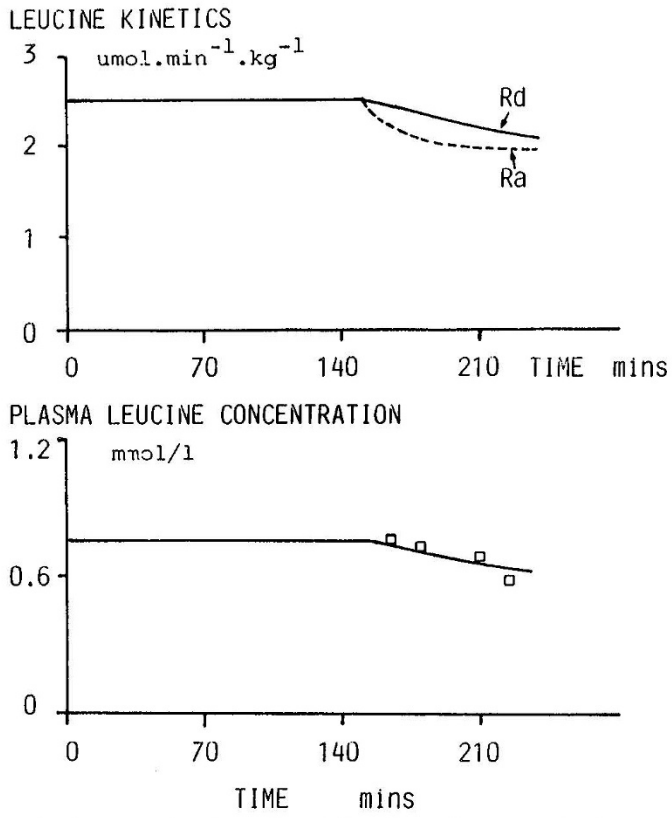

Fig. 3. Plasma leucine and model-predicted leucine production rate (*Ra*) and disappearance rate (*Rd*) in patient 3 before and during an insulin infusion of 0.1 U.kg.$^{-1}$h^{-1}.

DISCUSSION

In MSUD elevated concentration of BCAAs have been assumed to be due to reduced activity of the branched-chain oxoacid dehydrogenase complex (1) since the oxidation of ^{14}C labelled BCAA in intact fibroblasts from patients with classical MSUD is less than 2% (17). However, in this study the percentage oxidation in the three patients was not negligible, being between 22 and 43% of the normal mean. This unexpectedly high rate of leucine oxidation may have occurred for two reasons. At high plasma leucine concentrations there may be nonspecific oxidation by other enzymes or alternatively there may be residual enzyme activity.

The reduction in leucine MCR was partly due to decreased leucine oxidation but may also be due to saturation of the cellular transport mechanisms at the high plasma leucine concentrations found in these patients (18). However, decreased leucine MCR was not the major cause of the raised plasma leucine. In this study the high leucine concentrations were mainly due to increased leucine production rate.

Patient 3 was in a steady state before the insulin infusion was started and the kinetic parameters can be analyzed without the need to consider the nature and volumes of distribution of leucine. From the leucine specific activity at plateau (1.71 ± 0.03 dpm.mmol^{-1}, mean ± SD) and $^{14}CO_2$ production (11007 ± 346 dpm.min^{-1}kg^{-1}) leucine oxidation (4.53%), leucine production rate (3.02 μmol.min.$^{-1}$kg^{-1}), and MCR (4.0 ml.min.$^{-1}$kg^{-1}) were calculated. However patients 1 and 2 did not reach a steady state before the insulin infusion and therefore the data from all three patients was analyzed using a six compartment model. This has

been validated in normal subjects and patients with diabetes mellitus, with and without an insulin infusion (3). Although patients with MSUD have elevated BCAA concentration their total volume of distribution of leucine was within the normal range and the results for patient 3 are in close agreement whether model derived or not. Therefore it appears valid to use the model in patients with MSUD.

The rate of leucine incorporation into protein was increased in these patients. This was calculated without subtraction of urinary losses of leucine. It was assumed that urine losses of leucine were negligible in all three patients since leucine excretion measured in patient 3 was low. The excretion of 2 oxoisocaproic acid (the oxoacid of leucine) was also assumed to be negligible. Since renal clearance of 2 oxoisocaproic acid has been reported to be between 0 and 0.355 $ml.min^{-1}$ (depending on the plasma leucine concentration) (19), patient 2 (with the highest plasma leucine concentration of 898 $\mu mol.l^{-1}$) would have a maximum calculated excretion rate of only 0.007 $\mu mol.min.^{-1}kg^{-1}$. Furthermore only 1% of the total dpm infused into patient 3 was recovered in the urine confirming that excretion of leucine and 2 oxoisocaproic acid were low and would have a negligible effect on leucine kinetics. Thus we conclude that both the rate of incorporation of leucine into protein, a measure of protein synthesis, and protein breakdown (as measured by leucine production rate) were increased.

Leucine has been demonstrated to stimulate protein synthesis in isolated rat skeletal muscle and perfused rat heart (20, 21). In MSUD the increased protein synthetic rate may be the result of the elevated plasma leucine concentrations with an increase in protein degradation maintaining protein balance. It should also be considered that patients 1 and 2 were 12 yr of age and still growing so they might be expected to have a higher rate of protein turnover than normal adults. Patient 3, however, was 23 yr of age and had a higher rate of protein turnover than patient 1, so it is more likely that the elevated protein turnover in all three patients was the result of their disease.

The insulin infusion had relatively little effect on protein turnover in these patients. This contrasts with studies in normal subjects in which an insulin infusion producing insulin concentrations similar to those achieved in patients 1 and 2 (*i.e.* an insulin infusion of 0.05 $U.kg.^{-1}h^{-1}$) has been shown to reduce leucine concentrations from 130 to 89 $\mu mol.l^{-1}$ (22). In insulin withdrawn diabetics an insulin infusion of 0.04 $U.kg^{-1}.h^{-1}$ for 1 h has also been shown to reduce the leucine concentration from 240 to 180 $\mu mol.l^{-1}$. This suggests that in these MSUD patients the rate of protein turnover was insensitive to insulin. The response of plasma glucose to insulin, however, was normal in patient 3 and at the lower end of the normal range in patients 1 and 2 (24). Basal insulin levels were not increased and there was a normal response of free fatty acids and ketone body concentrations to the insulin infusions in all three patients. Strictly the patients cannot therefore be considered to be insulin resistant. These results suggest that for insulin to be effective in lowering leucine concentrations during the treatment of acute illness in MSUD the dose of insulin will have to be greater than that used in diabetics. A simultaneous glucose infusion will be necessary to prevent hypoglycemia.

We have shown that in patients with MSUD not only is there considerable oxidation of leucine but that there is an increase in leucine production rate and an increase in leucine incorporation into protein. These rates appear to be relatively insensitive to insulin.

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