Failure of Insulin Infusion to Stimulate Fractional Muscle Protein Synthesis in Type I Diabetic Patients

Anabolic Effect of Insulin and Decreased Proteolysis

PAUL J. PACY, KARTHA S. NAIR, CHARLES FORD, AND DAVID HALLIDAY

We evaluated the influence of insulin on fractional mixed skeletal muscle protein synthesis (FMPS) in eight type I (insulin-dependent) diabetic patients in the postabsorptive state. FMPS was calculated from the increment in [^{13}C]leucine in mixed skeletal muscle protein obtained by serial percutaneous needle biopsy during a continuous 8-h intravenous infusion of L-[^{13}C]leucine. We used the plasma [^{13}C]-α-ketoisocaproate (representing intracellular leucine labeling) as the precursor pool of protein synthesis for our calculations. FMPS during the insulin treatment (0.0472 ± 0.0046%/h; plasma glucose 4.6 + 1.0 mM) was not different from FMPS during insulin deprivation (0.0499 ± 0.0046%/h; plasma glucose 16.4 + 0.5 mM). Using plasma [^{13}C]-α-ketoisocaproate at isotopic plateau for calculation of leucine flux and as the precursor for leucine oxidation, we further confirmed the findings of our group and others that insulin treatment decreases leucine flux, leucine oxidation, and the nonoxidative portion of leucine flux. Our data on direct measurement of FMPS provide further evidence that the anabolic effect of insulin in the postabsorptive type I diabetic patient is mediated via reduction of proteolysis rather than by increasing protein synthesis. *Diabetes* 38:618–24, 1989

Insulin treatment rapidly reverses the negative nitrogen balance of insulin-dependent (type I) diabetic patients. The mechanism of insulin-induced protein anabolism must reflect increased protein synthesis, reduced proteolysis, or a combination of both (1). Recently, there have been attempts to elucidate this mechanism in humans by use of COOH-labeled leucine as the tracer (2–8). Initially, we demonstrated that, compared with a matched nondiabetic control group, insulin withdrawal in postabsorptive type I diabetic patients was associated with significantly increased whole-body proteolysis (2). Since this original observation, insulin reduction of whole-body leucine flux has been clearly demonstrated in control subjects and type I diabetic patients, indicating decreased proteolysis (3–8). In contrast, the influence of insulin on the nonoxidative portion of flux, assumed to represent protein synthesis, is much less clear-cut. There are reports of insulin having no effect on (5,7) or significantly decreasing (6,8) the nonoxidative portion of leucine flux.

Recently, Gelfand and Barrett (9), in an elegant forearm study, used labeled leucine and phenylalanine to evaluate the influence of a physiologically increased insulin level on muscle protein kinetics in postabsorptive control males. They demonstrated that insulin promoted net muscle anabolism primarily by inhibiting breakdown, with no influence on muscle protein synthesis. Our preliminary data suggested that direct incorporation of [^{13}C]leucine into quadriceps muscle may, in fact, be decreased during insulin infusion in type I diabetic patients (10). Most evidence from animal studies demonstrates that insulin promotes muscle protein synthesis (11,12) so the above findings in humans are somewhat surprising. The aim of this study was to evaluate further the influence of insulin on the fractional mixed skeletal muscle protein synthesis (FMPS) rate as well as whole-body leucine kinetics determined with the reciprocal pool model (13) in postabsorptive type I diabetic patients.

Glucose	1 mM = 18 mg/dl	α-Ketoisocaproic acid	1 μM = 0.013 mg/dl
β-Hydroxybutyrate	1 μM = 0.01 mg/dl		

From the Nutrition Research Group, Clinical Research Centre, Harrow, Middlesex, United Kingdom; and the Department of Endocrinology, College of Medicine, University of Vermont, Burlington, Vermont.

Address correspondence and reprint requests to Dr. Paul J. Pacy, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, UK.

Received for publication 9 November 1987 and accepted in revised form 9 December 1988.

MATERIALS AND METHODS

Subjects. Eight type I diabetic patients were recruited from the Northwick Park Hospital diabetic clinic. Their clinical features are shown in Table 1. None of these patients had clinically apparent diabetic micro- or macrovascular complications, and all renal- and hepatic-function tests were normal. Each patient consumed a weight-maintaining dia-

TABLE 1
Clinical details of type I diabetic subjects in muscle-biopsy study

Sex	Age (yr)	Weight (kg)	Body mass index (kg/m²)	Duration of diabetes (yr)	Glycosylated hemoglobin (5.6–7.6%)	Total insulin dose (U/day)
M	26	78.1	24.9	5	11.1	56
M	35	68.2	22.0	22	10.2	60
M	25	86.8	23.3	14	9.4	91
M	35	62.1	21.2	17	7.7	36
F*	19	59.8	26.6	17	9.0	45
F	47	71.9	26.7	13	9.0	60
M*	33	84.9	25.6	6	12.1	60
M	25	64.2	22.2	2	8.6	40
Mean ± SE	30.6 ± 3.1	73.3 ± 3.5	24.1 ± 0.8	12.0 ± 2.5	9.6 ± 0.5	56.0 ± 6.0

*Subjects with initial insulin infusion.

betic diet that supplied 45% of daily calorie intake as complex carbohydrate and 20% as protein. The study was outpatient based, and normal daily activity and level of exercise were continued throughout the study. The protocol was approved by Northwick Park Hospital Ethical Committee, and all patients gave informed consent.

Materials. L-[1-^{13}C]leucine (99 atom %) and $NaH^{13}CO_3$ (99 atom %) were obtained from Cambridge Isotopes (Cambridge, MA). Isotopic solutions of both materials were prepared in sterile pyrogen-free saline (150 mM NaCl).

Experimental design. All participants were studied randomly with the order shown in Table 1, during insulin infusion (2–3 U/h) or during its withdrawal (≥18 h). Their normal insulin regimen was changed to one supplying soluble (Actrapid) insulin every 6 h on the day before study. The dose given was dependent on self-monitored blood glucose concentrations by means of BM-test-glycemie 1-44 strips (Boehringer, East Sussex, UK). The final insulin dose before the withdrawal study was given at 1800 on the previous evening, while at 2300 before the infusion study. All subjects attended the metabolic ward of the clinical research center at 0800 after a 12- to 14-h overnight fast, having taken no insulin on the morning of the study.

A diagrammatic representation of the protocol is shown in Fig. 1. Two intravenous catheters were inserted, one in a superficial forearm vein, for infusion of the tracer, the other into a vein in the dorsum of the contralateral hand for sampling of arterialized blood (14). Arterialized blood and expired-breath CO_2 were taken before tracer infusion to determine basal ^{13}C enrichment. A priming dose of $NaH^{13}CO_3$ (0.16 mg/kg) and L-[1-^{13}C]leucine (0.9 mg/kg) was injected, followed by the continuous infusion of L-[1-^{13}C]leucine (1 mg · kg^{-1} · h^{-1}) over 8 h. The L-[1-^{13}C]leucine was made up in 500 ml of 150 mM saline so on both occasions the diabetic patient was infused with approximately this volume of fluid. The insulin-infusion study consisted of a 5-U bolus of soluble insulin (Actrapid) followed by continuous infusion (2–3 U/h) by a syringe Driver type M16 pump (Graseby Dynamics, Watford, UK).

Two hours after the start of the leucine infusion, a muscle biopsy was performed under local anesthesia (2% lignocaine) with either the UCH (15) or Bergstrom (16) biopsy needle. Muscle (150–200 mg) was taken ~6 inches above the knee joint from the lateral aspect of the thigh. Visible fat was removed from the sample, which was then immediately frozen in liquid nitrogen. This procedure was repeated 6 h later (with both biopsies taken from the same leg).

Arterialized blood and expired air were collected every 30 min from 2 to 8 h during the infusion. Expired air was collected into a 2-L latex bag and rapidly transferred into preevacuated glass sample bottles fitted with greaseless taps. Blood glucose concentrations were monitored every 30 min during insulin infusion, and a glucose solution (prepared from C-3 and C-4 plant sugars such that its mean ^{13}C natural-abundance enrichment mimicked that of an individual subject's basal expired $^{13}CO_2$) was given orally (mean 84 ± 15 g) as required to prevent symptomatic hypoglycemia. In three diabetic patients, 100 g of glucose solution, prepared as described, was given orally over 8 h without L-[1-^{13}C]leucine to evaluate its effect on expired-breath $^{13}CO_2$ enrichment, given that change in substrate utilization is one of the potential errors in determining oxidation rates (17). Expired-breath $^{13}CO_2$ enrichment tended to increase during ingestion of the solution but overall was only 0.0006 ± 0.00005 atom % excess (n = 47) higher than basal enrichment. Blood glucose was 6.4 ± 0.6 mM (n = 42) during the three studies.

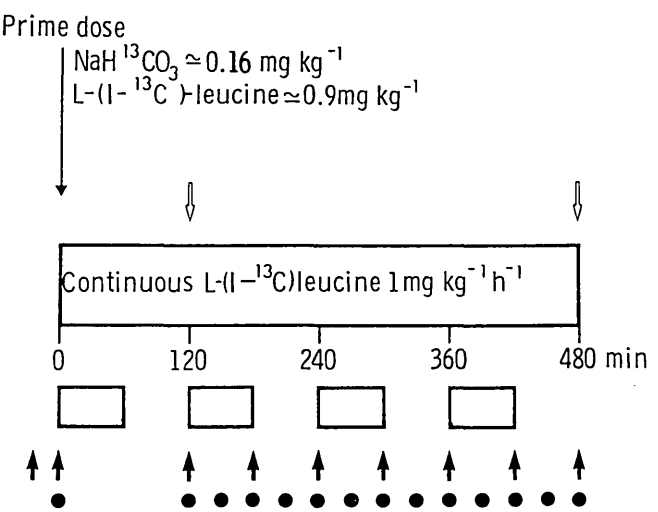

FIG. 1. Protocol during continuous infusion of L-[1-^{13}C]leucine in postabsorptive type I diabetic patients. *Open bars*, indirect calorimetry; *up arrows*, expired-breath sample for $^{13}CO_2$; ●, blood sample; *down arrows*, quadriceps muscle biopsy. In 1 study, insulin (2–3 U/h) was continuously infused after administration of prime doses.

The subjects lay quietly on beds throughout the study, and indirect calorimetry was performed on alternate hours with a ventilated-hood system (18).

Analytical techniques. Blood for glucose estimation was collected in fluoride/oxalate tubes (2.0 ml) and analyzed within 24 h by a standard hexokinase method. Blood for ketone body analysis (1 ml) was mixed with 1 M perchloric acid (1 ml). Blood for glucagon estimation was collected into tubes to which 0.1 ml of 20,000 KIU aprotinin (Bayer UK, Newbury, UK) had been added previously. These and all other blood samples were centrifuged immediately after collection at 4°C, and the plasma was stored at −70°C until analyzed.

Plasma glucagon levels were determined with the Novo radioimmunoassay kit (catalog no. 32, Novo Research Institute, Copenhagen) and free plasma insulin radioimmunoassay after treatment with polyethylene glycol (19). The interassay C.V. for plasma glucagon was 11%, whereas the interassay C.V. for insulin was 9%. Plasma amino acids were quantitated in an LKB Biochrom 4400 amino acid analyzer (with norleucine as an internal standard) and ketone body concentrations were estimated enzymatically (20).

Plasma [^{13}C]-α-ketoisocaproate (α-KIC) enrichment was measured by gas chromatography/mass spectrometry with chemical ionization and selected ion monitoring. Plasma α-KIC concentration was simultaneously determined with ketovaleric acid as the internal standard (21), and the enrichment of $^{13}CO_2$ in expired breath was measured by isotope-ratio mass spectrometry (22). This analysis was always carried out within 24 h of the infusion.

Separation of protein-bound amino acids before determination of L-[1-^{13}C]leucine incorporation into quadriceps muscle was performed as previously described (23). However, this technique has been significantly modified and improved by isolating amino acids with preparative gas-liquid rather than ion-exchange chromatography. The volatile amino acid isobutyl esters were separated on a packed column with argon as the carrier gas and collected after stream splitting by condensation in a liquid nitrogen–cooled U-shaped tube. This procedure resulted in isolation of a pure, dry sample of leucyl ester that was hydrolyzed to the free amino acid; the CO_2 was evolved by the Van Slyke ninhydrin reaction, and the $^{13}CO_2$ enrichment was measured in an isotope-ratio mass spectrometer.

Whole-body leucine kinetics were calculated with a steady-state two-pool model. Flux (Q) is calculated from the rate of dilution of the tracer by unlabeled tracee by

$$Q = i\left(\frac{E_i}{E_p} - 1\right)$$

where i is the infusion rate (μmol · kg^{-1} · h^{-1}), E_i is the enrichment of the tracer, and E_p is the enrichment of plasma plateau α-KIC. This is the reciprocal pool model and was chosen to calculate whole-body leucine kinetics because it appears to have several advantages over that of plasma [^{13}C]leucine enrichment. Plasma α-KIC enrichment has been demonstrated to reflect more precisely total leucine flux (13), is the more immediate precursor of leucine oxidation (24), minimizes sample-site error, and closely resembles intracellular muscle [^{13}C]leucine enrichment (25). Oxidation was calculated from the excretion rate of $^{13}CO_2$ and plasma plateau of [^{13}C]-α-KIC enrichment as

$$F^{13}CO_2 = \frac{FCO_2 \times E^{13}CO_2 \times 44.6 \times 60}{\text{body weight (kg)} \times 0.81 \times 100}$$

where FCO_2 is CO_2 production rate (ml/min), $E^{13}CO_2$ is plateau $^{13}CO_2$ enrichment, the factor of 100 converts this to a fraction, 44.6 × 60 converts CO_2 ml/min to μmol/h, and 0.81 accounts for CO_2 retention within the body. Whole-body leucine oxidation (μmol · kg^{-1} · h^{-1}) was then determined from

$$F^{13}CO_2 \times \left(\frac{1}{E_p} - \frac{1}{E_i}\right) \times 100$$

Postabsorptively in the steady state, Q = breakdown = oxidation + the nonoxidative portion of leucine flux (synthesis). Net balance represents the difference between breakdown and the nonoxidative portion of Q. Leucine metabolic clearance rate (26) was calculated from Q divided by leucine concentration (ml · kg^{-1} · min^{-1}). The FMPS rate was calculated from

$$\frac{\Delta E \text{ muscle}}{[^{13}\text{C}]\text{-}\alpha\text{-KIC } E_p \times \text{time between biopsies}} \times 100\ \%/\text{h}$$

where ΔE muscle represents the increase in [^{13}C]leucine enrichment in muscle protein between the biopsies. Inherent in this calculation is the assumption that, during plasma plateau enrichment of α-KIC, incorporation of the tracer into muscle protein is linear.

The two-tailed paired t test was used to compare insulin withdrawal and infusion in the same individual. Linear regression analysis was used to determine the correlation coefficients.

RESULTS

A steady-state model, which requires plateau enrichment of expired CO_2 and plasma α-KIC, was used to determine whole-body leucine kinetics. We defined plateau as a C.V. (SD/mean) of <10%, an established criterion (27), which was always fulfilled during the insulin withdrawal and infusion components of the study. The mean C.V.s (±SE) for expired $^{13}CO_2$ during insulin withdrawal and infusion were 6.0 ± 0.8% and 6.1 ± 0.8%, whereas for α-KIC enrichment the respective results were 4.4 ± 0.4% and 4.9 ± 0.4%. The time course of ^{13}C enrichment in plasma α-KIC, expired-breath CO_2 and plasma α-KIC concentration are shown in Table 2.

The effect of insulin infusion and withdrawal on whole-body leucine kinetics is shown in Table 3. Insulin withdrawal was associated with significantly greater whole-body leucine flux ($P < .001$), oxidation ($P < .001$), and net breakdown ($P < .001$). In contrast, plasma α-KIC concentration was higher ($P < .001$), as was the leucine metabolic clearance rate ($P < .001$). There was a 14% increase in the nonoxidative portion of flux ($P < .05$) during insulin withdrawal.

The individual values and 95% CI of mixed quadriceps muscle protein synthesis rate are shown in Table 4. There was no difference in FMPS rate during insulin withdrawal or

TABLE 2
Time course of ^{13}C enrichment in plasma and expired-breath CO_2, plasma concentration of α-ketoisocaproate, glucose ketone bodies, glucagon, and insulin in withdrawal and infusion studies

	Time (min)										
	150	180	210	240	270	300	330	360	390	420	450
Plasma [^{13}C]-α-KIC enrichment (APE)											
A	5.9 ± 0.2	5.8 ± 0.1	6.0 ± 0.3	6.1 ± 0.2	6.4 ± 0.1	6.2 ± 0.2	6.5 ± 0.2	6.4 ± 0.2	6.4 ± 0.2	6.3 ± 0.2	6.5 ± 0.2
B	7.3 ± 0.4	7.3 ± 0.3	7.2 ± 0.3	7.4 ± 0.2	7.4 ± 0.5	7.4 ± 0.3	7.4 ± 0.3	7.7 ± 0.3	7.5 ± 0.3	7.5 ± 0.3	7.7 ± 0.5
Plasma α-KIC concentration (μM)											
A	61.1 ± 9.5	61.6 ± 5.5	63.1 ± 6.9	61.0 ± 6.3	65.7 ± 8.7	66.8 ± 7.5	62.4 ± 7.5	56.2 ± 2.9	61.7 ± 5.8	62.3 ± 8.7	55.4 ± 9.3
B	24.1 ± 3.5	24.0 ± 2.9	21.4 ± 3.4	23.5 ± 4.0	21.7 ± 3.9	22.6 ± 3.7	22.8 ± 3.5	22.0 ± 2.5	18.5 ± 2.3	20.4 ± 2.3	18.0 ± 1.3
Expired $^{13}CO_2$ enrichment (APE)											
A	0.017 ± 0.002	0.017 ± 0.001	0.017 ± 0.001	0.017 ± 0.001	0.018 ± 0.002	0.018 ± 0.001	0.018 ± 0.001	0.019 ± 0.001	0.019 ± 0.001	0.019 ± 0.002	0.019 ± 0.002
B	0.014 ± 0.001	0.013 ± 0.001	0.014 ± 0.001	0.014 ± 0.001	0.014 ± 0.001	0.015 ± 0.001	0.014 ± 0.001	0.014 ± 0.001	0.015 ± 0.001	0.015 ± 0.001	0.013 ± 0.002
Blood glucose (mM)											
A	18.8 ± 0.7	17.5 ± 0.6	17.2 ± 0.6	16.4 ± 0.5	16.4 ± 0.4	16.1 ± 0.4	16.0 ± 0.4	16.0 ± 0.5	16.0 ± 5.0	15.8 ± 0.4	15.5 ± 0.5
B	6.0 ± 1.2	4.8 ± 1.2	4.1 ± 1.2	4.5 ± 1.2	5.6 ± 1.3	4.6 ± 1.3	4.3 ± 1.1	3.8 ± 0.9	4.1 ± 0.7	3.8 ± 0.6	3.1 ± 0.5
Plasma β-hydroxybutyrate (mM)											
A		2.5 ± 0.5		2.7 ± 0.6		2.3 ± 0.4		3.1 ± 0.6		3.1 ± 0.4	
B			0.1 ± 0.02		0.1 ± 0.02			0.07 ± 0.02		0.04 ± 0.01	
Plasma acetoacetate (mM)											
A		0.3 ± 0.05		0.4 ± 0.06		0.3 ± 0.06		0.4 ± 0.07		0.4 ± 0.05	
B			0.06 ± 0.01		0.05 ± 0.01			0.04 ± 0.01		0.04 ± 0.01	
Plasma glucagon (pg/ml)											
A		217 ± 24						160 ± 22		180 ± 25	
B		134 ± 23						151 ± 26		172 ± 15	
Free plasma insulin (μU/ml)											
A		8 ± 1								7 ± 1	
B		15.8 ± 1.4								14.7 ± 1.5	

Values are means ± SE. A, insulin withdrawal; B, insulin infusion; α-KIC, α-ketoisocaproate; APE, atom % excess.

TABLE 3
Whole-body leucine kinetics during insulin withdrawal and infusion in postabsorptive type I diabetic patients

	Insulin withdrawal	Insulin infusion
Flux (μmol · kg^{-1} · h^{-1})	104.6 ± 3.3*	84.5 ± 2.4
Oxidation (μmol · kg^{-1} · h^{-1})	27.0 ± 2.2†	16.4 ± 1.3
Nonoxidative portion of flux (μmol · kg^{-1} · h^{-1})	77.6 ± 2.9‡	68.1 ± 2.8
Protein synthesis (g/day)	232.4 ± 17.3§	204.3 ± 15.7
α-KIC concentration (μM)	61.0 ± 6.8*	22.3 ± 3.0
Leucine metabolic clearance (ml · kg^{-1} · min^{-1})	7.3 ± 0.5*	16.4 ± 2.2

Values are means ± SE. Statistics were determined by paired *t* test. α-KIC, α-ketoisocaproate.
*$P < .001$, †$P < .01$, ‡$P < .05$, §$P < .02$.

continuous insulin infusion. The proportion of muscle to whole-body protein synthesis was calculated by assuming that 40% of body weight is muscle (28), 18% of muscle is protein, 8% of protein is leucine (29), quadriceps muscle is representative of muscle in general, and the nonoxidative portion of flux represents synthesis. This proportion was 27.6 ± 3.0% during insulin withdrawal and 29.4 ± 3.0% during insulin infusion (mean ± SE).

Amino acid levels after 330 and 450 min during insulin withdrawal and infusion are shown in Table 5. Insulin withdrawal was associated with significantly elevated concentrations of all three branched-chain amino acids ($P < .001$ in each case), whereas glycine was reduced ($P < .001$).

Insulin withdrawal significantly elevated arterialized blood glucose (16.4 ± 0.5 vs. 4.6 ± 1.0 mM, $P < .001$), β-hydroxybutyrate (2.73 ± 0.5 vs. 0.049 ± 0.02 mM, $P < .001$), acetoacetate (0.36 ± 0.06 vs. 0.05 ± 0.008 mM, $P < .001$), and plasma glucagon (198.0 ± 29.5 vs. 145.0 ± 22.4 pg/ml, $P < .02$) with free plasma insulin levels reduced (15 ± 1 vs. 8 ± 1 μU/ml, $P < .001$).

Insulin withdrawal resulted in increased oxygen uptake (308 ± 14 vs. 278 ± 15 ml/min, $P < .02$), but CO_2 production was unchanged (218 ± 8 vs. 211 ± 10 ml/min). There was a significant correlation between oxygen uptake (ml/min) and nonoxidative portion of flux (mg/min during insulin infusion and its withdrawal [$n = 16$, $r = .85$, $P < .001$, $y = 1.09x + 127.4$]).

DISCUSSION

We demonstrated that insulin infusion in postabsorptive type I diabetic patients failed to promote the FMPS rate. We further confirmed our original hypothesis that the anabolic action of insulin in humans during the postabsorptive state depends on insulin's inhibition of proteolysis rather than a stimulation of protein synthesis (2,8). This hypothesis is supported by the failure of physiologically elevated insulin levels to promote protein synthesis across the forearm; rather this elevation significantly inhibited proteolysis (9).

Measurement of the rate of protein synthesis, as determined by use of a single labeled amino acid, requires knowledge of the precursor pool (amino acyl-tRNA) enrichment or specific activity (SA) at the site of protein synthesis. We are unaware of any direct measurement of this in humans, although several in vitro and in vivo animal studies have reported that leucyl-tRNA SA is similar to that of extracellular leucine (30–32). However, this conclusion is not unanimous (33). In humans, considerable variation in plasma isotope enrichments occurs, depending on whether samples are taken proximal (arterialized) or distal (deep venous) to forearm muscle, with the differences being larger for leucine than for α-KIC enrichment. This sampling-site variation appears more significant than physiological differences in the relationship between plasma α-KIC and leucine enrichment in humans (34). Sampling-site variation has not been considered in any of the aforementioned animal studies. Thus, although evidence for the most appropriate plasma component for estimation of precursor pool enrichment for muscle protein synthesis is inconclusive, we favor use of plasma α-KIC, if only because it appears more representative of the whole-body leucine pool (25). There are few data regarding the effect of insulin on the relationship between tRNA and intra- and extracellular leucine SA. However, in the presence and absence of insulin, the leucyl-tRNA SA was unchanged as was the leucyl-tRNA:extracellular leucine SA ratio of 80% in skeletal muscle cells (33).

We demonstrated that insulin infusion failed to increase FMPS. This contrasted with several animal studies, which have noted augmentation of protein synthetic rates with insulin (1). This difference in response may be species related. However, it has recently been suggested that maximal stimulation of protein synthesis might occur at basal insulin levels (35). If valid, the levels noted during insulin withdrawal might have been sufficient for such an effect, resulting in insulin infusion failing to further promote protein synthesis in quadriceps muscle.

The magnitude of FMPS in the postabsorptive type I patients during insulin withdrawal and infusion was comparable with recent values observed in control subjects by use of L-[1-^{13}C]leucine (36,37), although it contrasted with that of 0.09%/h calculated by the forearm approach with ring phenylalanine as the tracer (9). In that study, the proportion of muscle to whole-body protein synthesis was ~50%. This was higher than the values in this study during insulin withdrawal (25%) and infusion (29%), which accorded with those noted previously in animals (38) and humans (36,37).

The nonoxidative portion of flux has been used as an index of tracer incorporation into protein to provide an estimate of whole-body protein synthesis with the steady-state model.

TABLE 4
Synthesis of individual mixed quadriceps muscle protein during insulin withdrawal and infusion in postabsorptive type I diabetic patients

Subject no.	Insulin withdrawal	Insulin infusion
1	0.046	0.068
2	0.043	0.043
3	0.043	0.041
4	0.045	0.041
5	0.070	0.049
6	0.042	0.040
7	0.039	0.031
8	0.072	0.065
Mean ± SE	0.050 ± 0.0046	0.047 ± 0.0046
95% CI	(0.039, 0.061)	(0.036, 0.058)

Values are percent per hour.

TABLE 5
Influence of insulin infusion and withdrawal on amino acid concentrations in type I diabetic patients

Amino acid concentration	330 min		450 min	
	Insulin infusion	Insulin withdrawal	Insulin infusion	Insulin withdrawal
Alanine	232.2 ± 33.4	189.0 ± 24.0	239.7 ± 46.5	172.2 ± 15.8
Arginine	76.2 ± 12.7	69.4 ± 8.7	75.8 ± 14.7	59.2 ± 7.3
Citrulline	28.0 ± 15.8	37.0 ± 4.2	32.5 ± 3.3	33.4 ± 5.8
Glutamate	100.5 ± 18.8	106.8 ± 32.6	115.2 ± 17.2	76.4 ± 11.8
Glutamine	430.8 ± 27.7	435.6 ± 60.8	437.4 ± 28.5	461.1 ± 49.9
Glycine	224.7 ± 21.4*	151.4 ± 16.9	224.0 ± 23.0†	153.8 ± 18.5
Histidine	87.1 ± 17.4	98.5 ± 8.5	76.9 ± 16.3	83.9 ± 11.2
Isoleucine	29.9 ± 5.3‡	89.9 ± 6.6	26.8 ± 3.5‡	104.5 ± 4.1
Leucine	100.7 ± 13.1‡	234.2 ± 21.8	85.3 ± 8.3‡	254.2 ± 13.8
Lysine	142.5 ± 14.8	150.9 ± 24.4	144.9 ± 8.1	139.5 ± 17.0
Methionine	15.4 ± 3.9	14.7 ± 2.6	10.2 ± 2.0	17.8 ± 3.0
Phenylalanine	41.3 ± 6.5	45.0 ± 4.9	41.9 ± 3.9	48.2 ± 4.4
Serine	117.4 ± 10.4	100.1 ± 8.7	105.8 ± 5.1	96.5 ± 10.9
Threonine	81.9 ± 11.0	75.9 ± 10.5	74.5 ± 11.8	75.7 ± 10.1
Tyrosine	27.3 ± 3.3	38.4 ± 4.3	23.3 ± 2.3	37.6 ± 4.2
Valine	154.7 ± 21.5§	307.4 ± 25.7	135.4 ± 17.3§	347.6 ± 26.2

Values are micromoles per liter (means ± SE). Statistics obtained by paired *t* test.
*$P < .05$, †$P < 0.02$, ‡$P < .001$, §$P < .01$.

This assumption has been supported by data in dogs (39) and humans (37). This study demonstrated an increased nonoxidative portion of flux during insulin withdrawal, which confirmed previous findings (2,8), although this observation is not unanimous (5,7). Thus, the response in both whole-body and in individual tissue provided complementary evidence that insulin failed to stimulate protein synthesis. We and others have postulated that this finding may reflect substrate availability. Recent studies lend support to this thesis, because infusion of amino acids promotes the nonoxidative portion of flux (35,40,41). Important in the context of insulin-withdrawn type I diabetic patients, in whom only branched-chain amino acids were elevated, has been the demonstration that leucine per se has such a capability (42). Likewise, preliminary data have suggested that muscle protein synthesis is stimulated by amino acid infusion (43,44).

These data implied that the increased nonoxidative portion of leucine flux during insulin withdrawal was not due to events in skeletal muscle. Given that <30% of whole-body protein synthesis reflected events in muscle, this is not too surprising. Protein synthesis in the gut is less sensitive to insulin than other tissues in animals (45), a factor that may be relevant in the type I diabetic patients studied.

We have confirmed several reports that have demonstrated reduced proteolysis during insulin infusion in both control subjects and type I diabetic patients (2–8). In contrast, the influence of insulin on whole-body leucine oxidation is less well established. Short-term infusion (≤4 h) has been observed to exert a variable response ranging from no effect (8) to 103% reduction (7). More prolonged insulin infusion has generally reduced whole-body leucine oxidation (3,5,8), although this was not observed in the only previous study in which plasma α-KIC was used to represent the SA of the precursor pool (6). In this 9-h study, stepwise-increased insulin failed to reduce this parameter, which contrasted with the significant inhibition in controls. These investigators noted concomitant reduced glucose disposal in the diabetic patients, implying insulin resistance, which was suggested as the cause of the inability to decrease whole-body leucine oxidation. We believe this is unlikely, because previous evidence shows that amino acid metabolism, like that of fatty acids, is more sensitive than carbohydrate metabolism to insulin (46). The absolute values of whole-body leucine oxidation during insulin infusion were similar to those reported in controls (41), which suggests no significant insulin resistance of amino acid metabolism in this study. In addition, it was likely that providing oral glucose may have increased $^{13}CO_2$ enrichment ~4%, which would have elevated rates of oxidation to the same extent. This small increase must have resulted from failure to match precisely the enrichment of ingested glucose with that of the diabetic patient's basal expired breath.

Oxidation of the branched-chain amino acids is regulated by the multienzyme α-ketoacid dehydrogenase complex. Control of this complex is by phosphorylation (inactive) and dephosphorylation (active) by mechanisms not fully understood. Glucagon, which was elevated during insulin withdrawal, appears to result in increased enzyme complex activity (47). We have reported that somatostatin-induced hypoinsulinemia and hypoglucagonemia failed to influence leucine oxidation, whereas this was significantly promoted by selectively increasing glucagon (48). Although purely speculative, the apparent different responses of short and more prolonged insulin administration may reflect the true course of inactivation of the enzyme complex.

The increased resting metabolic rate during insulin withdrawal further confirms previous data (49,50). We tentatively suggest that the positive correlation between this and the nonoxidative portion of flux supports the suggestion that these two variables might be causally related.

In summary, we demonstrated that FMPS was not promoted by insulin in postabsorptive type I diabetic patients. We also confirmed that insulin inhibited proteolysis in the postabsorptive state but failed to promote protein synthesis. This study is further evidence that the principal anabolic effect of insulin is to reduce protein breakdown.

ACKNOWLEDGMENTS

We thank Dr. R.F. Mahler for allowing us to study his patients; S. Heels, J. Webster, C. Hicks, and J. Harpin for technical support; the Biochemistry Department at Northwick Park Hospital for glucose and ketone body analysis; and all of the patients who took part in this study.

REFERENCES

1. Jefferson LS: Role of insulin in the regulation of protein synthesis. *Diabetes* 29:487–96, 1980
2. Nair KS, Garrow JS, Ford C, Mahler RF, Halliday D: Effect of poor diabetic control and obesity on whole body protein turnover in man. *Diabetologia* 25:400–403, 1983
3. Fukagawa NK, Minaker KL, Rowe JW, Goodman MN, Matthews DE, Bier DM, Young VR: Insulin mediated reduction of whole body protein breakdown: dose response effects of leucine metabolism in post absorptive man. *J. Clin Invest* 76:2306–11, 1985
4. Tessari P, Trevisan R, Inchiostro S, Biolo G, Nosadini R, De Kreutzenberg SV, Duner E, Tiengo A, Crepaldi G: Dose-response curves of effects of insulin on leucine kinetics in humans. *Am J Physiol* 251:E334–42, 1986
5. Robert JJ, Beaufrere B, Koziet J, Desjeux JF, Bier DM, Young VR, Lestradet H: Whole body de novo amino acid synthesis in type I (insulin-dependent) diabetes studied with stable isotope-labeled leucine, alanine, and glycine. *Diabetes* 34:67–73, 1985
6. Tessari P, Nosadini R, Trevisan R, De Kreutzenberg SV, Inchiostro S, Duner E, Biolo G, Marescotti MC, Tiengo A, Crepaldi G: Defective suppression by insulin of leucine-carbon appearance and oxidation in type I insulin-dependent diabetes mellitus. *J Clin Invest* 77:1797–804, 1986
7. Umpleby AM, Boroujerdi MA, Brown PM, Carson ER, Sonksen PH: The effect of metabolic control on leucine metabolism in type I (insulin-dependent) diabetic patients. *Diabetologia* 29:131–41, 1986
8. Nair KS, Ford GC, Halliday D: Effect of intravenous insulin treatment on in vivo whole body leucine kinetics and oxygen consumption in insulin-deprived type I diabetic patients. *Metabolism* 36:491–95, 1987
9. Gelfand RA, Barrett EJ: Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. *J Clin Invest* 80:1–6, 1987
10. Nair KS, Read WWC, Ford GC, Halliday D: Insulin withdrawal does not reduce skeletal muscle protein synthesis in type I diabetic patients (Abstract). *Diabetes* 33 (Suppl. 1):166A, 1984
11. Hay AM, Waterlow JC: The effect of alloxan diabetes on muscle and liver protein synthesis in the rat measured by constant infusion of L-(^{14}C)lysine. *J Physiol* 191:111–12, 1967
12. Pain VM, Albertse EC, Garlick PJ: Protein metabolism in skeletal muscle, diaphragm and heart of diabetic rats. *Am J Physiol* 245:E604–10, 1983
13. Schwenk W, Beaufrere B, Haymond MW: Use of reciprocal pool specific activities to model leucine metabolism in humans. *Am J Physiol* 249:E646–50, 1985
14. Abumrad NN, Rabin D, Diamond MP, Lacy WW: Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism* 30:936–40, 1981
15. Edwards R, Young A, Wiles M: Needle biopsy of skeletal muscle in the diagnosis of myopathy and the clinical study of muscle function and repair. *New Engl J Med* 302:261–71, 1980
16. Bergstrom J: Muscle electrolytes in man: determined by neutron activation analysis on needle biopsy specimens: a study in normal subjects, kidney patients and patients with chronic diarrhea. *Scand J Clin Lab Invest* 14 (Suppl. 68):1–110, 1962
17. Robert JJ, Koziet J, Chauvet D, Darmaun D, Desjeux JF, Young VR: Use of ^{13}C-labeled glucose for estimating glucose oxidation: some study design considerations. *J Appl Physiol* 63:1725–32, 1987
18. Garrow JS, Webster JD: A computer-controlled indirect calorimeter for the measurement of energy expenditure in one or two subjects simultaneously. *Hum Nutr Clin Nutr* 40:315–21, 1986
19. Albano JDM, Ekins RP, Martiz G, Turner RC: A sensitive precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. *Acta Endocrinol* 70:487–509, 1972
20. Hultman E: Carbohydrates and related compounds: A glucose, galactose, fructose, insulin and ketone bodies in blood and urine. In *Clinical Biochemistry. Principles and Methods*. Vol. II. Curtius HC, Roth M, Eds. New York, de Gruyter, 1974, p. 908–30
21. Ford GC, Cheng KN, Halliday D: 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* 12:432–36, 1985
22. Halliday D, Read WWC: Mass spectrometric assay of stable isotopic enrichment for the estimation of protein turnover in man. *Proc Nutr Soc* 40:321–34, 1981
23. Read WW, Read MA, Rennie MJ, Griggs RC, Halliday D: Preparation of CO_2 from blood and protein-bound amino acid carboxyl groups for quantification and ^{13}C-isotope measurements. *Biomed Mass Spectrom* 11:40–44, 1984
24. Wolfe RR, Goodenough RD, Wolfe MH, Royle GT, Nadel ER: Isotopic analysis of leucine and urea metabolism in exercising humans. *J Appl Physiol* 52:458–66, 1982
25. Layman DK, Wolfe RR: Sample site selection for tracer studies applying a unidirectional circulatory approach. *Am J Physiol* 253:E173–78, 1987
26. Nissen S, Haymond MW: Effects of fasting on flux and interconversion of leucine and α-ketoisocaproate in vivo. *Am J Physiol* 241:E72–75, 1981
27. Galster AD, Bier DM, Cryer PE, Manafo WW: Plasma palmitate turnover in subjects with thermal injury. *J Trauma* 24:938–45, 1984
28. Rennie MJ, Edwards RHT, Halliday D, Matthews DE, Wolman SL, Millward DJ: Muscle protein synthesis measured by stable isotope techniques in man: the effects of feeding and fasting. *Clin Sci* 63:519–23, 1982
29. Block RJ, Weiss KW: *Amino Acid Handbook: Methods and Results of Protein Analysis*. Springfield, IL, Thomas, 1956
30. Davey PJ, Manchester KL: Isolation of labelled amino acyl transfer RNA from muscle studies of the entry of labelled amino acids into acyl transfer RNA linkage in situ and its control by insulin. *Biochim Biophys Acta* 182:85–97, 1969
31. Martin, AF, Rabinowitz M, Blough R, Prior G, Zak R: Measurements of half-life of rat cardiac myosin heavy chain with leucyl t-RNA used as precursor pool. *J Biol Chem* 252:3422–29, 1977
32. Everett AW, Prior G, Zak R: Equilibrium of leucine between the plasma compartment and leucyl t-RNA in the heart and turnover of cardiac myosin heavy chain. *Biochem J* 194:365–68, 1981
33. Airhart J, Arnold JA, Stirewalt WS, Low RB: Insulin stimulation of protein synthesis in cultured skeletal and cardiac muscle cells. *Am J Physiol* 243:C81–86, 1982
34. Thompson GN, Pacy PJ, Ford GC, Merritt H, Halliday D: Relationships between plasma isotopic enrichments of leucine and α-ketoisocaproic acid during continuous infusion of labelled leucine. *Eur J Clin Invest* 18:639–43, 1988
35. Castellino P, Luzi L, Simonson DC, Haymond M, DeFronzo RA: Effect of leucine and plasma amino acid concentrations on leucine metabolism in man. *J Clin Invest* 80:1784–93, 1987
36. Halliday D, Pacy PJ, Cheng KN, Dworzak F, Gibson JNA, Rennie MJ: Rate of protein synthesis in skeletal muscle in normal man and patients with muscular dystrophy: a reassessment. *Clin Sci* 74:237–40, 1988
37. Nair KS, Halliday D, Griggs RC: Leucine incorporation into mixed skeletal muscle protein in humans. *Am J Physiol* 254:E208–13, 1988
38. Lobley GE, Milne V, Reeds PJ, Pennie K: Whole body and tissue protein synthesis in cattle. *Br J Nutr* 43:491–502, 1980
39. Schwenk WF, Rubanyi E, Haymond MW: Effect of protein synthetic inhibitor on in vivo estimates of protein synthesis in dogs. *Am J Physiol* 252:E595–98, 1987
40. Tessari P, Inchiostro S, Biolo G, Trevisan R, Fantin G, Marescotti MC, Iori E, Tiengo A, Crepaldi G: Differential effects of hyperinsulinemia and hyperaminoacidemia on leucine-carbon metabolism in vivo. *J Clin Invest* 79:1062–69, 1987
41. Pacy PJ, Garrow JS, Ford GC, Merritt H, Halliday D: Influence of amino acid administration on whole body leucine kinetics and resting metabolic rate in postabsorptive normal subjects. *Clin Sci* 75:225–31, 1988
42. Schwenk WF, Haymond MW: Effects of leucine, isoleucine, or threonine infusion on leucine metabolism in humans. *Am J Physiol* 253:E428–34, 1987
43. Bennet WM, Connacher AA, Scrimgeour CM, Smith K, Rennie MJ: Whole body and muscle protein synthesis in man: the effect of amino acid infusion. (Abstract). *Diabetic Med* 4:570A, 1987
44. Lundholm K, Bennegard K, Zachrisson H, Lundgren F, Eden E, Moller-Loswick A-C: Transport kinetics of amino acids across the resting leg. *J Clin Invest* 80:763–71, 1987
45. McNurlan MA, Garlick PJ: Protein synthesis in liver and small intestine in protein deprivation and diabetes. *Am J Physiol* 241:E238–45, 1981
46. Umpelby AM, Sonksen PH: The chalonic action of insulin in man. In *Substrate and Energy Metabolism*. Garrow JS, Halliday D, Eds. London, Libbey, 1985, p. 169–78
47. Harris RA, Paxton R: Regulation of branched chain keto acid dehydrogenase complex by phosphorylation-dephosphorylation. *Fed Proc* 44:305–15, 1985
48. Nair KS, Halliday D, Matthews DE, Welle SL: Hyperglucagonemia during insulin deficiency accelerates protein catabolism. *Am J Physiol* 253: E208–13, 1987
49. Nair KS, Halliday D, Garrow JS: Increased energy expenditure in poorly controlled type I (insulin-dependent) diabetic patients. *Diabetologia* 27:13–16, 1984
50. Leslie P, Jung RT, Isles, TE, Bayt J, Newton RW, Illingworth P: Effect of optimal glycaemic control with continuous subcutaneous insulin infusion on energy expenditure in type I diabetes mellitus. *Br Med J* 293:1121–26, 1986