Skeletal Muscle Myosin Heavy Chain Synthesis in Type 1 Diabetes

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Although insulin's anticatabolic effect on protein metabolism in type 1 diabetes has been clearly shown to be related to the inhibition of protein breakdown, insulin's effect on muscle protein synthesis remains controversial. Cross-limb studies and measurements of synthesis rates of mixed muscle protein have yielded conflicting results. These measurements represent the mean synthesis of several muscle proteins and may miss changes in the synthesis rates of individual muscle proteins. We measured the fractional synthesis rates of myosin heavy chain (MHC), the principal muscle contractile protein, and mixed muscle protein (MMP) in six type 1 diabetic patients during insulin deprivation and insulin treatment. Comparisons were made with six healthy control subjects. Muscle biopsies were taken at 2 h and 8 h during a primed continuous infusion of L-[1-^{13}C]leucine. MHC was purified by a preparative continuous elution gel electrophoresis, and fractional synthesis rates were calculated. We found that in type 1 diabetic subjects, the fractional synthesis rates of MHC and MMP during insulin treatment are similar to those of control subjects. Acute insulin deprivation did not affect either the synthesis rate or the ratio of MHC to MMP in type 1 diabetic subjects. In the postabsorptive state, acute insulin deprivation has no effect on MHC or MMP synthesis in type 1 diabetic patients. *Diabetes* 46:1336–1340, 1997

Insulin's potent anticatabolic effect is well known. The insulin treatment of patients with type 1 diabetes is associated with the normalization of body composition (1), characterized by increased lean body mass, diminished urinary excretion of nitrogen (2,3), and lowered concentrations of circulating amino acids (4,5). As net protein accretion is determined by the balance of protein breakdown and synthesis, the inhibition of protein breakdown, without changes in protein synthesis, may result in an increase in muscle mass. Insulin is a well-known inhibitor of whole-body and muscle protein breakdown (6–9). Despite the clear demonstration of insulin's stimulatory effect on protein synthesis during in vitro experiments, there are no conclusive data to support a stimulatory effect of insulin on muscle protein synthesis in humans. When the effects of insulin on muscle protein synthesis have been studied in patients with type 1 diabetes, no changes have been detected (6,9–13). Investigators have found either no effect (14–22), an increase (23), or an inhibition (24) of muscle protein synthesis with insulin administration in healthy subjects.

All human studies in normal and type 1 diabetic subjects have been based on the effects of insulin on mixed-muscle protein (MMP) (10,12) or cross-limb measurements (6,9,11,13). Skeletal muscle comprises a variety of constituent proteins, such as sarcoplasmic, myofibrillar (e.g., actin and myosin), and mitochondrial proteins, which have disparate rates of synthesis. Whole-body, cross-limb, and even whole-muscle protein turnover studies may thus fail to detect important changes in the synthesis rates of individual skeletal muscle proteins.

We investigated whether insulin deprivation or insulin treatment stimulates synthesis of an individual muscle protein in type 1 diabetic patients. Myosin heavy chain (MHC) is the major structural protein of skeletal muscle, comprising ~25% of muscle protein (25). We have previously described a method of isolating MHC from small skeletal muscle samples by preparative continuous elution gel electrophoresis (25). Utilizing this technique, we set out to determine the effects of insulin treatment and acute insulin deprivation on the synthesis rates of MHCs and MMPs in patients with type 1 diabetes in the postabsorptive state.

RESEARCH DESIGN AND METHODS

Subjects. We studied six volunteers with type 1 diabetes (two men and four women), whose average age was 28.3 years (21–39 range). The mean weight was 67.8 ± 5.92 kg and heights ranged from 157.4 to 189.0 cm (165.1 ± 4.52 cm). Six healthy control volunteers were also studied (four men and two women), whose average age was 27.7 years (range, 20–39 years). The mean height and weight of the control subjects was 174.2 cm and 70.3 kg, respectively. All volunteers were within 10% of ideal body weight for height and were screened by detailed history, physical examination, and biochemical profile (urine and blood) for any other overt illness or complication from diabetes. Subjects with chronic renal failure or on medication for hypertension were excluded.

C-peptide levels were drawn after a standard protein meal to document endogenous insulin deficiency in all subjects. Volunteers with an insulin requirement of <35 U/day or >80 U/day were excluded from the study. Informed written consent was obtained after detailed explanation of the protocol, which had been approved by the institutional Human Investigation Committee of the University of Vermont, where all studies were carried out.

Materials. L-[1-^{13}C]leucine and [^{13}C]sodium bicarbonate were obtained from Cambridge Isotopes (Cambridge, MA). Chemical isotopic and optical purity of the L-[1-^{13}C]leucine were confirmed before use. Solutions of the isotopes were prepared under sterile precautions and were tested for pyrogens and sterility before use.

All electrophoresis reagents except SDS were purchased from Bio-Rad Laboratories (Richmond, CA). SDS was purchased from Sigma Chemical Company (St. Louis, MO), and trifluoroacetic acid was purchased from Pierce (Rockford, IL).

Study design. All subjects were placed on a weight-maintaining diet for the duration of the study (2,400 ± 200 kcal/day). Three days before each study, the volunteers were placed on regimens of regular insulin every 6 h at the exclusion of all longer-acting forms of insulin to avoid a carry-over effect from long-acting insulin during the study period. They were admitted to the General Clinical Research Center 1 day before each study. Two studies, each separated by at least 3 weeks, were carried out on each volunteer after an overnight fast. Women volunteers were always studied during the luteal phase of the menstrual cycle (i.e.,

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Received for publication 5 August 1996 and accepted in revised form 8 April 1997.

BOHB, β-hydroxybutyrate; FSR, fractional synthesis rate; KIC, ketoisocaproic acid; MHC, myosin heavy chain; MMP, mixed muscle protein.

studies in the same woman were separated by at least 1 month). The order of the two studies in the volunteers with type 1 diabetes (insulin treated and insulin deprived) was assigned in a random fashion.

Study 1: insulin treatment. At 7:00 P.M. on the evening of admission, an infusion of regular insulin was started and adjusted half-hourly to maintain blood glucose levels between 4.4 and 5.6 mmol/l. Between 8:00 A.M. and the end of the study, this infusion was adjusted every 15 min to maintain the blood glucose level in this range.

Study 2: insulin deprivation. No insulin was administered after 6:00 P.M. on the day of admission for the remainder of this study. At 7:00 P.M. on the evening of admission, an infusion of normal saline was commenced at a rate of 1 ml · kg^{-1} · h^{-1}, instead of insulin. This infusion was continued throughout the study. Water intake was encouraged, and blood glucose measurements were obtained hourly.

Common protocol for both studies. On the morning of each study day, an 18-gauge venous catheter was placed in a retrograde fashion in a dorsal hand vein. The hand with this catheter was then rested in a "hot box" that circulated air at a temperature of 60°C to arterialize the venous blood. Arterialized venous blood accurately reflects labeled leucine and ketoisocaproic acid (KIC) concentrations in arterial blood (26,27).

A second 18-gauge catheter was placed in the contralateral forearm for infusions. Once baseline blood and expired air samples were obtained, bolus doses of [^{13}C]sodium bicarbonate (1.5 μmol/kg) and L-[1-^{13}C]leucine (7.5 μmol/kg) were given. These boluses were given to prime the respective pools to achieve early isotopic plateaus (28). Immediately following the administration of the priming dose, a continuous infusion of L-[1-^{13}C]leucine was started at a rate of 7.5 μmol · kg^{-1} · h^{-1} and continued for 480 min. Leucine was chosen as a tracer because it is an essential amino acid in humans.

Arterialized venous blood samples for isotope analysis were drawn at 0, 120, 240, and 360 min, and then every 30 min until the end of the study at 480 min. Expired air for isotope analysis was collected at the same intervals as blood isotopes.

Needle muscle biopsies were carried out under local anesthesia at 2 and 8 h from the vastus lateralis, as previously described (29). Entry sites for the 2 and 8 h biopsies were separated by at least 2 in. All muscle samples were frozen in liquid nitrogen immediately after isolation and were stored at –80°C until analyzed.

Analysis of samples

Separation of MHC and MMP. The purification of MHC was performed as described previously (25). Briefly, the tissue samples (~150 mg) were homogenized in a solubilization buffer (100 mmol/l sodium pyrophosphate, 1% SDS, and 4 mmol/l EGTA, pH 7.4), using a Polytron homogenizer (Model PT 1200C, Brinkmann Instruments, Westbury, NY) and centrifuged at 2,200*g*. The supernatant containing mixed SDS-soluble tissue protein was electrophoresed using a preparative electrophoresis cell (491 Prep-Cell, Bio-Rad Laboratories, Richmond, CA) to separate MHC from other proteins. The purity of the MHC fractions in each sample was determined by analytical SDS-PAGE, followed by silver staining. Only those MHC fractions that showed a single band on silver staining were used for further analysis.

Mixed muscle protein was precipitated from a ~50-mg biopsy sample, as previously described (30).

Recovery and hydrolysis of protein. Eluent fractions containing MHC were pooled and the protein precipitated using trichloroacetic acid, as described previously (25). The MHC and mixed tissue protein precipitates were hydrolyzed using 6 N HCl, as previously described (30). The hydrolyzates were then passed through a cation exchange resin-packed column (Bio-Rad 50W-8X), and amino acids were eluted using 4 mol/l ammonium hydroxide (25). This was dried on a speed vac (Savant) and reconstituted in 200 μl of 0.01 N HCl.

Measurement of [^{13}C]leucine enrichment in tissue proteins. The amino acids in the protein hydrolyzates (both MHC and MMP) were derivatized as their *N*-heptafluorobutyryl, methyl ester, and the [^{13}C] enrichment was measured using an on-line gas chromatography/combustion/isotope-ratio mass spectrometer (Delta S IRMS, Finnigan MAT, Bremen, Germany), as previously described (31).

Plasma [^{13}C]KIC isotope enrichment. Plasma ketoacid was derivatized with a quinoxalinol bis-trimethylsilyl tri-fluoroacetic anhydride and pyridine (32). The [^{13}C]KIC enrichment was then determined by selected ion monitoring and electron impact ionization in a quadropole gas chromatograph mass spectrometer, monitoring ions m/z 259 and 260 (28).

Calculations. The fractional synthesis rate (FSR) for MHCs and MMPs were calculated using [^{13}C]KIC as the precursor pool (33). A previous study supported the use of KIC enrichment as a surrogate measure of leucyl-tRNA in skeletal muscle (30). The equation used for these calculations was as follows:

$$\text{FSR (\%/h)} = \frac{\text{Increment in isotopic enrichment in protein (2–8 h)}}{\text{Precursor pool enrichment} \times \delta_t \text{ (hours)}} \times 100$$

where δ_t is 6 h, the time between biopsies.

Hormones and substrates. Plasma glucose concentrations were measured with an autoanalyzer (Beckman Instruments, Fullerton, CA). Glucagon, free insulin, growth hormone, and C-peptide concentrations were measured by radioimmunoassay using a commercial kit (Diagnostic Products, Los Angeles, CA). Cortisol was also measured using a radioimmunoassay kit (Cortisol Kit, Diagnostic Products, Los Angeles, CA). Free insulin was measured after precipitation with polyethylene glycol (34). Cortisol was also measured using radioimmunoassay kit (Cortisol Kit, Diagnostic Products, Los Angeles, CA). Glycerol was measured using the free glycerol assay kit, and β-hydroxybutyrate (BOHB) was measured using the BOHB assay kit (Sigma Chemical, St. Louis, MO). Free fatty acids were measured using an in vitro enzymatic calorimetric kit (Wako Chemicals, Neuss, Germany). Plasma concentrations of norepinephrine and epinephrine were measured by radioenzymatic assay (35).

Data analysis. Data analysis was carried out with the SAS software package (SAS, Cary, NC). Comparisons of fractional synthesis rates, hormone and substrate levels were made with paired *t* tests (for comparisons of the two studies in each of the subjects with diabetes) and unpaired *t* tests (for comparisons of the subjects with diabetes and the healthy control subjects). By power table calculations, the sample size, six subjects in each group with paired samples in the diabetic subjects, allowed the detection of an insulin treatment effect of 15% or greater on myosin heavy chain synthesis to be detected.

All results are expressed as means ± SE.

RESULTS

Hormones and substrates. Plasma glucagon levels were at plateau during the last 2 hours of the study. The mean plateau glucagon levels were higher ($P < 0.05$) during insulin deprivation (190 ± 20 ng/l) than during insulin treatment (110 ± 10 ng/l). Plateau free insulin levels during insulin treatment were (87 ± 15.5 pmol/ml) greater than during insulin deprivation, during which free insulin levels were <35 pmol/ml (i.e., undetectable) in five subjects and 37.5 pmol/ml in one subject.

The mean plateau glucose concentration was lower ($P < 0.001$) during insulin treatment (4.85 ± 0.04 mmol/l) than during insulin deprivation (19.0 ± 0.6 mmol/l).

The plateau values of other hormones and substrates are given in Table 1. Mean plateau growth hormone levels were higher ($P < 0.05$) during insulin deprivation (5.05 ± 1.0 ng/l) than during insulin treatment (3.53 ± 1.1 ng/l). There were no significant differences in the mean plateau levels of cortisol, epinephrine, or norepinephrine between insulin deprivation and insulin treatment.

Plasma levels of BOHB, glycerol, and free fatty acids all were lower ($P < 0.001$) during insulin treatment than during insulin deprivation (Table 1).

Amino acids. Alanine, threonine, serine, lysine, and tyrosine all were similar during insulin deprivation and insulin treatment (Table 2). In contrast, the branched chain amino acids, leucine, isoleucine, and valine, were all statistically significantly lower ($P < 0.05$) during insulin treatment than during insulin deprivation. Glycine concentrations were higher ($P < 0.01$) during insulin treatment than during insulin deprivation alone.

Isotopes. [^{13}C]KIC isotopic enrichment (atom percentage excess) was stable from 360 to 480 min in both protocols (Fig. 1).

Leucine flux was lower during insulin treatment (93.59 ± 5.04 μmol · kg^{-1} · h^{-1}) than during insulin deprivation (111.99 ± 6.36 μmol · kg^{-1} · h^{-1}) ($P < 0.001$). Leucine flux was 96 ± 4.5 μmol · kg^{-1} · h^{-1} in the control subjects.

The FSR of MHCs was 0.035 ± 0.004%/h during insulin treatment and 0.033 ± 0.003%/h during insulin deprivation in subjects with type 1 diabetes. In the control subjects, the FSR of MHC was 0.035 ± 0.004%/h. None of these values were significantly different from each other (Fig. 2). Individual enrichment values for MHC, MMP, and KIC are given in Table 3.

TABLE 1
Plasma values of growth hormone, cortisol, epinephrine, norepinephrine, glucose, insulin, glucagon, glycerol, BOHB, and free fatty acids during the baseline (mean values obtained between 0 and 120 min) and the plateau periods (mean values obtained between 360 and 480 min) of each study

	Insulin (–)		Insulin (+)	
	Basal	Plateau	Basal	Plateau
Growth hormone (μg/l)	6.5 ± 1	5.0 ± 1	4.0 ± 1	3.5 ± 1
Cortisol (nmol/l)	430 ± 60	450 ± 100	380 ± 30	310 ± 80
Epinephrine (pmol/l)	190 ± 40	290 ± 40	360 ± 60	350 ± 40
Norepinephrine (nmol/l)	190 ± 40	310 ± 50	240 ± 30	210 ± 60
Glucose (mmol/l)	16.2 ± 0.5*	19.0 ± 0.6*	5.20 ± 0.15	4.85 ± 0.04
Insulin (pmol/ml)	35 ± 1*	35 ± 1*	102 ± 13	87 ± 16
Glucagon (ng/l)	145 ± 14*	190 ± 20*	111 ± 11	110 ± 10
Glycerol (mmol/l	1.1 ± 0.3*	2.4 ± 0.5*	0.6 ± 0.4	0.9 ± 0.5
BOHB (mmol/l)	1,990 ± 180*	3,170 ± 830*	640 ± 85	680 ± 80
Fatty free acids (g/l)	1.3 ± 0.3*	1.7 ± 0.5*	115 ± 9	0.73 ± 0.2

Data are means ± SE. Plasma free insulin values <35 pmol/l were not detectable with the assay used in this study. *Significantly greater during insulin deficiency than during insulin treatment ($P < 0.05$).

The FSR of MMPs was 0.046 ± 0.007%/h during insulin treatment and 0.043 ± 0.007%/h during insulin deprivation in subjects with type 1 diabetes. In the control subjects, the FSR of MMP was 0.047 ± 0.004%/h. None of these values were significantly different from each other. The FSR of MHC is ~25% slower than that of MMP ($P < 0.02$).

DISCUSSION

The current study represents the first report of measurement of the effect of insulin treatment and insulin deprivation on the synthesis of skeletal muscle MHC in type 1 diabetic patients. MHC was chosen because of its role as the major protein of the contractile apparatus of muscle, responsible for the conversion of chemical energy (adenosine triphosphate) to mechanical energy. Direct measurement of the synthesis rate of MHC has been difficult in humans due to the large sample sizes required. In this study, we applied a recently published method of isolating MHC from small skeletal muscle samples by preparative continuous elution gel electrophoresis (25). This method enabled the recovery of sufficient amounts of MHC from 150-mg muscle biopsy samples for the determination of the enrichment of [^{13}C]leucine. Using this technique, we found the FSR of MHC to be unaffected by acute changes in insulin levels. Specifically, we found that the fractional synthesis rate of MHC in subjects with type 1 diabetes during acute insulin deprivation is similar to the FSR during insulin treatment. The FSR of MHC in patients with type 1 diabetes

TABLE 2
Mean amino acid concentrations obtained during the plateau periods (between 360 and 480 min) of each study

	Insulin deprivation	Insulin treatment
Asp	153 ± 32	172 ± 35
Glu	131 ± 9	150 ± 14
Ser	110 ± 12	129 ± 8
His	79 ± 4	76 ± 4
Lys	131 ± 5	150 ± 6
Tyr	55 ± 3	48 ± 2
Met	7 ± 1	11 ± 1
Gly	275 ± 19	349 ± 24*
Thr	146 ± 16	148 ± 11
Ala	245 ± 19	252 ± 11
Phe	53 ± 2	48 ± 2
Ile	122 ± 12	44 ± 4*
Leu	255 ± 27	116 ± 7*
Val	325 ± 31	175 ± 11*

Data are means ± SE. All concentrations are mg/dl. *Significantly different during insulin deprivation ($P < 0.05$).

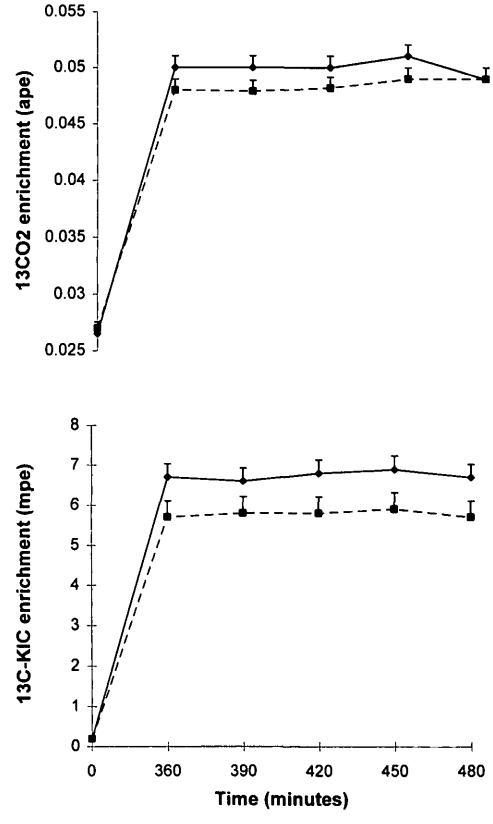

FIG. 1. Time-course enrichments (atoms percentage excess) for $^{13}CO_2$ and [^{13}C]KIC. Isotopic plateau was achieved by 120 min in each set of study conditions. —◆—, insulin treatment; ---■---, insulin deprivation.

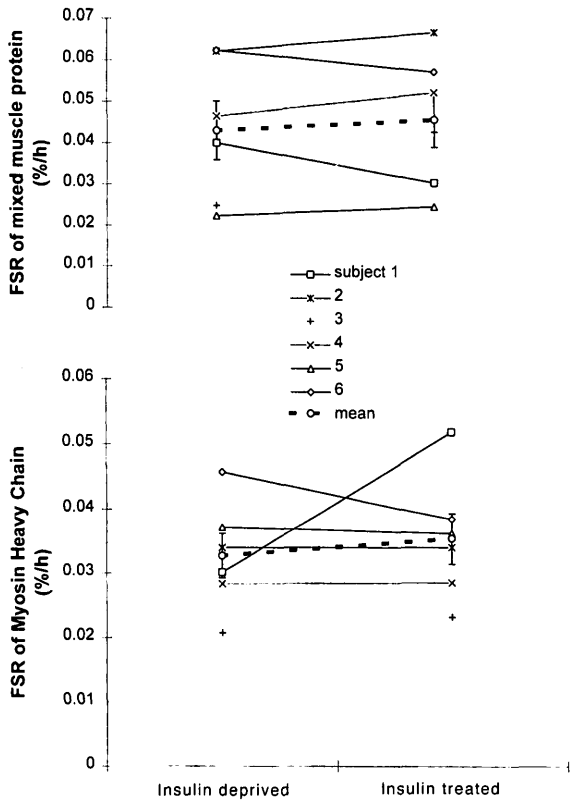

FIG. 2. FSR of MHC and MMP for each of the six study subjects (solid lines) and the mean values for these measurements (dotted lines). The FSR of MHC during insulin treatment was not significantly different from the FSR during insulin deprivation. This was also true for MMP. The FSRs of both MHC and MMP were similar in subjects with type 1 diabetes and in control subjects. In all subjects, the FSR of MHC was ~25% slower than that of MMP ($P < 0.02$).

(during both insulin treatment and acute insulin deprivation) was similar to that measured in healthy subjects.

There are several limitations to the interpretation of these data. First, small changes in the FSR of MHC could have been missed secondary to the small sample size. Furthermore, an independent effect of insulin on the fractional synthesis of MHC or MMP cannot be completely excluded since the levels of circulating branched chain and total amino acids were higher during insulin deprivation, possibly negating an inhibitory effect of isolated insulin withdrawal. In addition, glucagon levels were significantly higher during insulin deprivation, while there is evidence that glucagon may have a net catabolic effect on protein metabolism through, in part, the attenuation of the aminogenic protein stimulus of protein synthesis (36). Thus, hyperglucagonemia may have negated a stimulatory effect of insulin on the FSR of MHC. However, the experimental conditions did recreate the physiological conditions of acute insulin deprivation/deficiency. A chronic effect of insulin deprivation on the FSR of MHC was not studied. Most diabetic patients experience insulin deprivation on a short-term basis, since chronic insulin deprivation is not compatible with life in these C-peptide–negative patients. The lack of effect of short-term insulin deprivation suggests that MHC synthesis is not affected in most diabetic patients on insulin treatment. In addition, the effect of insulin deprivation on MHC breakdown remains to be investigated. An increased MHC breakdown in the face of a normal synthesis

TABLE 3
Individual MHC, MMP, and KIC enrichments expressed in atoms percentage excess

Subject	Study	MHC	MMP	KIC
1	Insulin	0.0214	0.0125	6.9180
	Treatment	0.0108	0.0143	6.2192
2	Insulin	0.0128	0.0273	6.7810
	Treatment	0.0139	0.0235	6.4120
3	Insulin	0.0065	0.0164	6.3700
	Treatment	0.0089	0.0077	5.2320
4	Insulin	0.0094	0.0200	6.3761
	Treatment	0.0072	0.0136	4.8400
5	Insulin	0.0138	0.0081	5.4800
	Treatment	0.0108	0.0057	4.3658
6	Insulin	0.0159	0.0236	6.8796
	Treatment	0.0197	0.0268	5.6560

KIC enrichments represent the mean of five paired samples taken at 360, 390, 420, 450, and 480 min.

rate could result in the depletion of the MHC content of skeletal muscle. The effects of insulin treatment and acute insulin deprivation on the synthesis rates of other constituent proteins of skeletal muscle are not known and should not be extrapolated from the current findings.

Our results are in contrast with some of the published data in animal models, which demonstrate a stimulatory effect of insulin on mixed skeletal muscle protein synthesis (37,38). These animal studies were carried out on growing streptozocin-induced diabetic rats. When adult diabetic rats were studied, no effect of insulin on skeletal muscle synthesis was noted (39). We suspect that the difference in results between our present study and the animal data is on the basis of the relative maturity of the subjects. In vitro experiments have demonstrated that insulin stimulates the synthesis rate of MHC (40–42).

The inhibitory effect of insulin on whole-body protein breakdown is well documented (6–9). The site of inhibition of protein breakdown by insulin appears to be chiefly skeletal muscle, although this is not without controversy. In vivo studies of human subjects with type 1 diabetes that have utilized tracer methodology have reported either no effect (11,13) or an inhibition (6,9) of skeletal muscle protein breakdown by insulin. In both of the studies in which no effect on skeletal muscle protein breakdown by insulin was seen (11,13), insulin infusion was associated with decreases in the average rate of protein breakdown across the forearm. Leucine flux fell from 147 ± 61 nmol · 100 ml^{-1} · min^{-1} during insulin deficiency to 82 ± 19 nmol · 100 ml^{-1} · min^{-1} during insulin treatment in the study by Pacy et al. (13). In the study by Tessari et al. (11), net leucine release across the forearm fell from 2.53 ± 0.72 μmol · kg^{-1} · min^{-1} during insulin deficiency to 1.89 ± 0.37 μmol · kg^{-1} · min^{-1} during insulin treatment. In both of these studies, the large amount of noise in the data, inherent to cross-limb studies, prevented the inhibition of skeletal muscle protein breakdown reaching statistical significance. On balance, it appears that insulin decreases skeletal muscle protein breakdown in type 1 diabetic patients.

The measurement of the MHC synthesis rate in type 1 diabetic patients has provided evidence that short-term insulin deprivation has no effect on the synthesis of MHC, the major

protein of muscle contractile apparatus. This study further demonstrated that insulin's anticatabolic effect is unlikely to be related to the stimulation of protein synthesis.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health Grants RO1-DK41973 and RO1-DK44248 and by Public Health Service Grants GCRC-RR00585, RO1-DK41973, and GCRC-RR109. We are grateful to our volunteers for diligent and generous participation and to Patricia Baumann, Janice Long, RN, and Dawn Morse for skilled technical support.

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