Defective Suppression by Insulin of Leucine-Carbon Appearance and Oxidation in Type 1, Insulin-dependent Diabetes Mellitus

Evidence for Insulin Resistance Involving Glucose and Amino Acid Metabolism

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Abstract

To determine whether a resistance to insulin in type 1, insulin-dependent diabetes mellitus (IDDM) is extended to both glucose and amino acid metabolism, six normal subjects and five patients with IDDM, maintained in euglycemia with intravenous insulin administration, were infused with L-[4,5-^{3}H]leucine (Leu) and [1-^{14}C]αketoisocaproate (KIC). Steady-state rates of leucine-carbon appearance derived from protein breakdown (Leu + KIC Ra) and KIC (~ leucine) oxidation were determined at basal and during sequential euglycemic, hyperinsulinemic (~40, ~90 and ~1,300 μU/ml) clamps. In the euglycemic postabsorptive diabetic patients, despite basal hyperinsulinemia (24±6 μU/ml vs. 9±1 μU/ml in normals, $P < 0.05$), Leu + KIC Ra (2.90±0.18 μmol/kg · min), and KIC oxidation (0.22±0.03 μmol/kg · min) were similar to normal values (Leu + KIC Ra = 2.74±0.25 μmol/kg · min) (oxidation = 0.20±0.02 μmol/kg · min). During stepwise hyperinsulinemia, Leu + KIC Ra in normals decreased to 2.08±0.19, to 2.00±0.17, and to 1.81±0.16 μmol/kg · min, but only to 2.77±0.16, to 2.63±0.16, and to 2.39±0.08 μmol/kg · min in the diabetic patients ($P < 0.05$ or less vs. normals at each clamp step). KIC oxidation decreased in normal subjects to a larger extent than in the diabetic subjects. Glucose disposal was reduced at all insulin levels in the patients. In summary, in IDDM: (*a*) Peripheral hyperinsulinemia is required to normalize both fasting leucine metabolism and blood glucose concentrations. (*b*) At euglycemic hyperinsulinemic clamps, lower glucose disposal rates and a defective suppression of leucine-carbon appearance and oxidation were observed. We concluce that in type 1 diabetes a resistance to the metabolic effects of insulin on both glucose and amino acid metabolism is present.

Introduction

In recent years, type 1, insulin-dependent diabetes has been recognized as a disease characterized not only by insulin deficiency, but also by a resistance to exogenously administered insulin. In increasing numbers of reports (1–3) insulin-mediated glucose utilization has been found to be reduced in type 1 diabetic subjects. However, as insulin influences the metabolism of a variety of substrates (4), a resistance to the metabolic effects of insulin may be extended to substrates other than glucose.

Insulin modulates the metabolism of the amino acids and, consequently, of proteins. Both in vitro (5, 6) and in vivo (7, 8) studies have shown that insulin decreases protein degradation, while it stimulates protein synthesis in isolated tissue preparations (6, 9) and in animals (10, 11). Oxidation of essential amino acids was reduced by insulin in vivo (12) and in isolated tissues from fasted (9) and from fed (13) animals. Insulin administration to diabetic animals restored growth rate and reduced miofibrillar protein degradation (14). On the whole, the experimental data confirm the general, clinical observation that insulin exerts a major anabolic effect on protein metabolism.

Whether insulin effectiveness as regards amino acid metabolism is normal in human, insulin-dependent diabetes, is not known. Therefore, the aim of this study was to characterize the response, in insulin-dependent diabetic patients, of the metabolism of the essential amino acid leucine, to stepwise increments in insulin concentrations. As leucine, in the postabsorptive state, derives only from body proteins, this amino acid was taken as representative of whole-body protein metabolism. Leucine-carbon is extensively interconverted and partially released into the circulation as the corresponding α-ketoanalogue, α-ketoisocaproate (KIC)[1] (15, 16). Therefore, both leucine and KIC rates of appearances (Ra) were measured by means of the simultaneous infusions of two independent tracers of leucine and KIC, in order to estimate "total" leucine carbon appearance. Leucine-carbon (i.e., Leucine + KIC) Ra, oxidation, and nonoxidized leucine-carbon flux, possibly representing an index of leucine entering protein(s) (Leu → P), were estimated at steady state and at the different insulin plateaus by means of conventional isotope-dilution techniques. Glucose concentrations were clamped at euglycemic levels by means of variable glucose infusions.

Methods

Isotopes. L-[4,5-^{3}H]leucine ([^{3}H]leucine) (55.1 Ci/mmol) and L-[1-^{14}C]leucine (57 mCi/mmol) were purchased from Amersham Corp. (Amersham, England). [1-^{14}C]αketoisocaproate ([^{14}C]KIC) was prepared by the method of Rüdiger et al. (17), with final purification by high pressure liquid chromatography (HPLC) (18), with the exception that ethanol was substituted for acetonitrile in the running buffer. [^{14}C]HCO_3^- (50 mCi/mmol) was obtained from Amersham Corp. Isotopes were proven to be sterile and pyrogen-free before use.

Experimental design. Six male normal volunteers (age, 26±2 yr; body mass index (BMI), 24.4±0.2) and five male insulin-dependent diabetic

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1. *Abbreviations used in this paper:* BMI, body mass index; HPLC, high pressure liquid chromatography; KIC, ketoisocaproate; Leu → P, leucine entering proteins; Ra, rates of appearance.

patients (age, 32±4 yr; BMI, 23.1±0.6) were studied in the postabsorptive state. All the diabetic subjects were documented to have no β-cell function, as indicated by no plasma C-peptide response (19) to glucagon stimulation. All subjects were healthy at the time of the study. None had clinical history of diabetic autonomic neuropathy. The diabetic subjects had an average insulin requirement of 57±8 U/d, subdivided into at least two daily administrations of regular and intermediate-acting insulin. Their metabolic control before the study was fairly good, as testified by hemoglobin A_1c values (20) (7.3±1.0%), not markedly higher than the normal values of our laboratory (4.8±1.0%). The individual data of the patients are reported in Table I. The purposes and the potential risks of the study were explained in detail to each subject. Informed, written consent to the study was then signed by each volunteer. The study was part of a more comprehensive project approved by the "Consiglio Nazionale delle Ricerche" of Italy. The total amount of radioactive isotopes administered was within the range approved by other institutions (16) for human use. The diabetic subjects consumed a diet containing 45% carbohydrates, 30% lipids, and 25% proteins for at least 3 d before the study. On the evening before the day of the study, the diabetic patients were given ~50% of their usual short-acting insulin dose, and no intermediate insulin was administered. During the night, an intravenous insulin infusion (~1–2 U/h) was started, to normalize blood glucose concentrations and to maintain euglycemia. Between 7 and 8 a.m., one forearm vein was cannulated with an 18-gauge plastic catheter (Terumo, Japan), connected with a three-way stopcock, and used for isotopes, insulin, and glucose infusion. The subjects were also connected to the Biostator GCIIS (Miles Laboratories, Elkhart, IN) via a double lumen catheter placed in an opposite forearm vein, for continuous blood glucose monitoring. An 18-gauge plastic catheter was also placed in a retrograde fashion in a wrist vein on the same side of the vein used for the blood glucose monitoring, and kept patent with a slow drip of 0.9% saline infusion. The hand was kept in a warming box, to obtain arterialized blood samples (21). Priming doses of [^{3}H]leucine (~10.9 μCi in the diabetics, ~14.0 μCi in normals), of [^{14}C]KIC (~3.1 μCi in the diabetic patients, ~3.9 μCi in normals) and of [^{14}C]HCO_3^- (~3 μCi) (22), were administered, followed by constant infusions of [^{3}H]leucine (~0.10 μCi/min in the diabetic subjects, ~0.13 μCi in normals). After the beginning of tracers administration, 2 h were allowed to reach steady state conditions for leucine and KIC specific activities and expired $^{14}CO_2$. Thereafter, four breath and plasma samples were taken over 30 min to determine basal substrate and hormone concentrations, specific activities, and expired $^{14}CO_2$. Thereafter, multiple euglycemic insulin-clamp studies were performed sequentially on each subject. Plasma glucose concentrations were maintained at similar values in normals (84±2 mg/dl, coefficient of variation: ~10%) and in the diabetic subjects (90±3 mg/dl, coefficient of variation: ~11%). Blood glucose concentrations were continuously monitored with the Biostator, and frequently measured also with a Beckman Glucose Analyzer II (Beckman Instruments, Inc., Fullerton, CA). When necessary, glucose concentrations measured by the Biostator were adjusted to the values of the Glucose Analyzer using the Pump Ratio dial.

Insulin was infused with a Harvard Pump (Harvard Apparatus Co., S. Natick, MA) at rates of 0.02, 0.05, and 0.5 U/m^2 · min. A priming dose was administered (23). Euglycemia was maintained by the Biostator using 20% dextrose infusions, and applying an algorithm for glucose clamping (24). After the start of each insulin infusion, 110 min were allowed to reach a new steady state for insulin concentrations, glucose requirements to maintain euglycemia, leucine, and KIC plasma specific activities, and expired $^{14}CO_2$. Thereafter, four breath and blood samples were taken over 30 min for the determinations at the new steady state. The insulin infusion periods lasted, therefore, 140 min for each insulin plateau.

Analytical methods. The concentrations and the specific activities of plasma leucine and KIC were determined by HPLC (18). Plasma free insulin concentrations were determined by radioimmunoassay, after polyethyleneglycol precipitation (25). Plasma amino acids concentrations were determined by HPLC as dansyl-derivatives (26) with some modifications (27). Plasma glucose was determined with a Beckman Glucose Analyzer II. Expired air was collected into Douglas bags over 1-min periods, through a three-way valve. Bags were subsequently aspirated slowly through a 1-M ethanolamine solution. Aliquots of 1 ml of the resulting solution were then counted in triplicates for the determination of $^{14}CO_2$ radioactivity. All radioactive samples, i.e., those eluting from the HPLC (18) and the ethanolamine solution containing the ^{14}C-bicarbonate, were mixed into glass vials with scintillation cocktail, allowed to equilibrate, and counted in a Packard TRI Carb Liquid Scintillation Spectrometer (Packard Instruments Co., Inc., Downers Grove, IL). Radioactivity was corrected for quench and ^{14}C-spillover into the tritium channel with external standard methods. Correction curves were plotted as percent of the less-quenched standards. Therefore, the disintegrations per minute (dpms) so calculated do not represent the actual dpms of the samples. For example, the relative efficiency of the tritium channel, within our usual quenching range, was between 55 and 65%, while the absolute efficiency (i.e., the percent of the actual dpms), was ~30%. As both samples and infusates were counted in the same way, and the numerators and the denominators of the equations reported in the appendix are divided by the same factor (i.e., the percent difference from the actual and the calculated dpms), no bias in the calculations were introduced. The curve depicted in Fig. 4 was graphically interpolated by plotting the best fit by hand on semilog paper, using the four values obtained during the sequential euglycemic clamps.

Calculations. Glucose disposal was defined as the glucose infusion rate necessary to maintain euglycemia at each insulin concentration. It was calculated either as mean values in the last 30 min of each insulin-infusion period (3), or in the integrated area from 30 to 140 min (2). Leucine carbon kinetics were analyzed at steady state by applying conventional isotope dilution techniques. Leucine Ra (or "flux") was calculated using the following standard formula: r[^{3}H]leu/SA[^{3}H]leu, where

Table I. Characteristics of Normal and Type 1 Diabetic Subjects Studied

Diabetic subjects	Age	BMI	Duration of diabetes	Daily insulin requirement	Hemoglobin A_1c	C-peptide after glucagon i.v.
	yr		*yr*	*U*	*percent*	*ng/ml*
1	26	17.9	17	53	9.5	<0.02
2	26	23.2	15	70	8.6	<0.02
3	40	23.2	10	50	6.6	<0.02
4	41	22.8	20	34	6.5	<0.02
5	27	25.2	21	80	5.2	<0.02
Mean±SEM	32±4	23.1±0.6	17±2	57±8	7.3±1.0	
Normals (n = 6)						
Mean±SEM	26±2	24.4±0.2	—	—	4.8±1.0	—

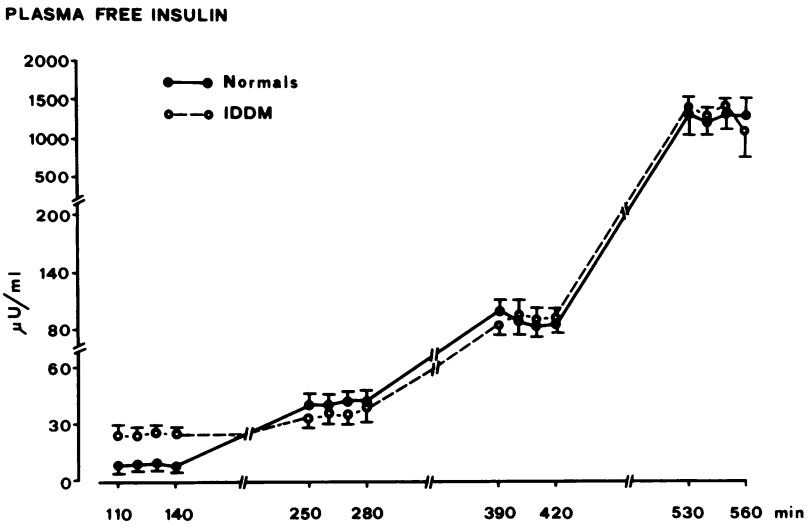

Figure 1. Plasma free-insulin concentrations at basal and during stepwise euglycemic, hyperinsulinemic clamps, in patients with insulin-dependent, type 1 diabetes (*IDDM*) and in normals.

r[^{3}H]leu is the rate of isotope infusion (DPM/kg per min) and SA[^{3}H]leu is the specific activity of [^{3}H]leucine in plasma at steady state. KIC Ra (or "flux") was calculated similarly. We calculated also the sum of leucine and KIC Ra, as an index of total leucine carbon release from endogenous proteolysis. Leucine-carbon oxidation was calculated using the standard product-precursor relationship: $^{14}CO_2$/SA[^{14}C]KIC × 0.8, where $^{14}CO_2$ is the rate (DPM/kg · min) of $^{14}CO_2$ expired, and SA[^{14}C]KIC is the plasma specific activity of [^{14}C]KIC. The constant 0.8 represents the fractional recovery of $^{14}CO_2$ in expired air during a [^{14}C]HCO_3^- infusion (28), and corrects for CO_2 fixation and other losses. Nonoxidized leucine flux, representing rates of leucine incorporation into protein(s), was calculated by subtracting the rate of leucine oxidized from leucine flux. Leucine "clearance" was calculated by dividing leucine flux over the prevailing leucine concentrations at each insulin plateau. As leucine "clearance", calculated in this way, may not be linear over a wide range of leucine concentrations, as those achieved in the present study, this estimate may not be quantitatively correct. Statistical analyses were performed using two-tailed *t* tests for paired data (differences from basal values of each group), or for unpaired data (differences between groups). A *P* values equal to or less than 0.05 was taken as statistically significant. All data were expressed as mean±SEM.

Results

Plasma glucose and free-insulin concentrations and glucose disposal during multiple euglycemic insulin clamp studies. Plasma free-insulin concentrations were near steady state at least in the final 30 min of each insulin infusion period (Fig. 1). Basal free insulin concentrations (Table II) in the euglycemic diabetic patients were more than twofold higher (24±6 μU/ml) than in normals (9±1 μU/ml) ($P < 0.05$). After sequential insulin infusions, plasma free insulin rose to similar levels in the diabetics and in normals at the first (36±4 in the diabetics vs. 42±6 μU/ml in normals), the second (80±13 vs. 89±11 μU/ml), and at the third (1,249±107 vs. 1,295±85 μU/ml) insulin-infusion period. In Fig. 2, the amounts of glucose infused to maintain euglycemia, during the three sequential insulin infusions, are shown. Both in normals and in the diabetic subjects, an almost constant glucose infusion rate was attained at least in the final 30 min of each insulin infusion period, indicating that the insulin effect on glucose uptake had reached a steady state.

In Table II, the rates of glucose disposal (mg/kg · min), calculated as mean values either over the last 110 min (2) or in the final 30 min (3) of each 140-min insulin-infusion period, are also reported. Insulin-mediated glucose disposal was impaired in the diabetic patients at each insulin concentrations, particularly at the lower insulin levels (Table II). At maximal insulin concentrations, glucose disposal was ~30% lower in the diabetic than in normal subjects, albeit a statistical significance was not achieved.

Plasma leucine and KIC concentrations and specific activities, and expired $^{14}CO_2$. Plasma leucine and KIC concentrations (Table III) and specific activities (Fig. 3) were near steady state in the last 30 min of each insulin-infusion period. In normals, fasting leucine concentrations were 130±5 and 109±11 μM in

Table II. Plasma Free Insulin Concentrations and Glucose Disposal during Multiple Euglycemic Clamp Studies in Normal Subjects (N) and in the Diabetic Subjects (IDDM)

		Basal	First level	Second level	Third level
Free insulin (*μU/ml*)	N	9±1	42±6	89±11	1,295±185
	IDDM	24±6‡	36±4	80±13	1,249±107
Glucose disposal (*mg/kg · min*)	N	—	6.1±0.8	10.5±0.8	16.2±1.0
(last 110′ area)	IDDM	—	2.9±0.6§	6.7±1.2‡	12.2±1.6*
Glucose disposal (*mg/kg · min*)	N	—	8.8±0.9	11.9±1.1	16.6±1.1
(last 30′)	IDDM	—	2.9±0.9‖	7.3±1.3‡	13.5±2.6

* $P < 0.06$. ‡ $P < 0.05$. § $P < 0.02$. ‖ $P < 0.01$; all probability values are vs. normals.

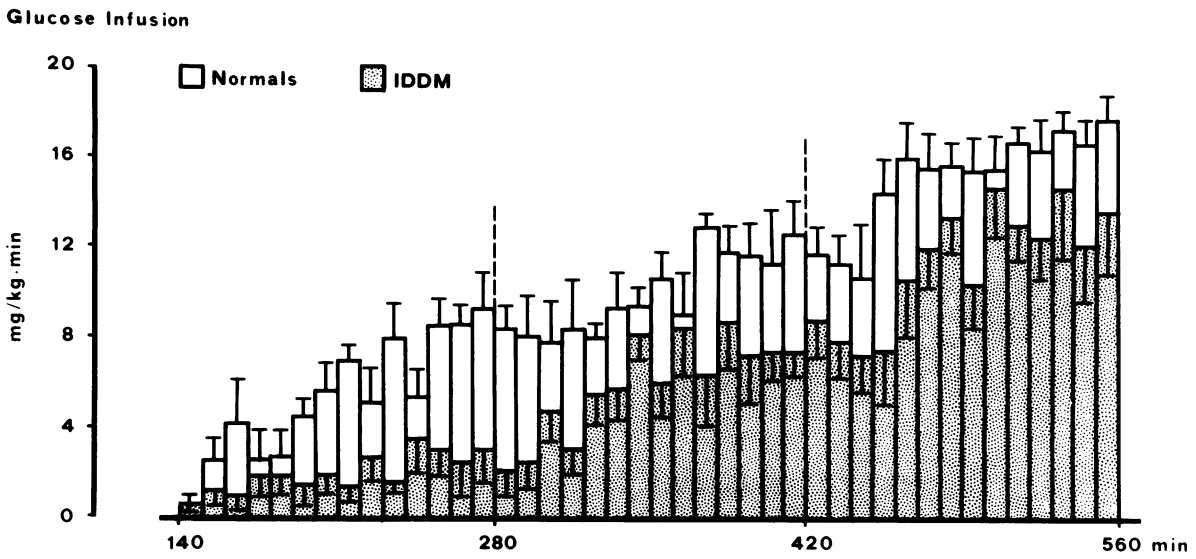

Figure 2. Rates of glucose infusions to maintain euglycemia during stepwise euglycemic, hyperinsulinemic clamps, in patients with insulin-dependent type 1 diabetes (IDDM) and in normals.

the euglycemic diabetic patients (NS from normals). Fasting KIC concentrations in normals were 42±4 μM, and 32±2 μM in the euglycemic diabetic subjects (NS from normals). At the three sequential steps of the euglycemic insulin clamps, leucine concentrations fell in normals to 91±10 μM, to 61±6 μM, and to 48±4 μM ($P < 0.01$ or less from basal) (Table III). In the diabetic subjects, it did not change at the first clamp step (112±12 μM), then it decreased to 81±9 μM and to 58±7 μM ($P < 0.01$ from basal). Thus, leucine concentrations tended to be lower in the diabetic patients, except for the basal period, even if the differences did not reach statistical significance. KIC concentrations fell in normal subjects to 26±2 μM, to 20±2 μM, and to 17±1 μM ($P < 0.01$ or less from basal) at the three clamp steps, while in the diabetic subjects it did not change at the first insulin level (32±1 μM, $P < 0.05$ vs. normals), then it decreased ($P < 0.01$ from basal) to 26±3 μM and to 18±1 μM (Table III).

[^{3}H]leucine and [^{14}C]KIC specific activities rose more in normals than in the diabetic patients (Fig. 3), at the three clamp steps. The ratios of [^{3}H]KIC over [^{3}H]leucine specific activities remained relatively constant both in normal subjects (0.78, 0.73, 0.83, and 0.77) and in the diabetics (0.67, 0.68, 0.68, and 0.75) throughout the studies. On the contrary, the ratios of [^{14}C]leucine over [^{14}C]KIC specific activities decreased in normal subjects from 0.39 to 0.26, 0.22, and 0.20 at the three clamp steps, while in the diabetics they did not change or slightly increased (0.37, 0.40, 0.53, and 0.41).

Table III. Leucine and KIC Concentrations in Normal Subjects and in Diabetic Subjects (IDDM) at Basal and During the Euglycemic Hyperinsulinemic Clamps

		Basal	First level	Second level	Third level
Leucine	Normals	130±5	91±10‡	61±6‡	48±4‡
(μM)	IDDM	109±11	112±12	81±9‡	58±7‡
KIC	Normals	42±4	26±2‡	20±2‡	17±1‡
(μM)	IDDM	32±2	32±1*	26±3‡	18±1‡

* $P < 0.05$ vs. normals; ‡ $P < 0.01$ vs. basal.

Expired counts in $^{14}CO_2$ were near steady state at basal and in the last 30 min of each insulin infusion period (Fig. 3). Labeled $^{14}CO_2$ decreased significantly from baseline in normals at the second ($P < 0.05$) and at the third ($P < 0.02$) clamp step, while it did not change in the diabetic patients.

Leucine kinetics. In the euglycemic diabetic patients, despite basal hyperinsulinemia with respect to normals, the sum of basal leucine and KIC Ra (Leu + KIC Ra), representing total leucine-carbon release from endogenous proteins, was not different from that observed in normal subjects (2.90±0.18 vs. 2.74±0.25 μmol/kg·min, respectively) (Fig. 4). At each euglycemic, hyperinsulinemic clamp step, Leu + KIC Ra was suppressed to a larger extent in the normal than in the diabetic subjects, and resulted significantly higher ($P < 0.05$–$P < 0.01$) at each step in the diabetic group (2.77±0.16 vs. 2.08±0.19 μmol/kg·min, 2.63±0.16 vs. 2.00±0.17 μmol/kg·min, and 2.39±0.08 vs. 1.81±0.16 μmol/kg·min, respectively). Maximal suppression from basal values was 33% in normals, but only 18% in the diabetic patients.

In Table IV, individual flux rates of leucine and KIC, KIC oxidation, and the estimate of leucine incorporation into protein(s) (Leu → P), are shown. Again, in the basal state no differences were detected between the two groups, despite higher plasma free-insulin concentrations in the diabetic group. At each clamp step, leucine flux, KIC flux, and KIC oxidation were higher in the diabetic than in the normal subjects. Statistically significant differences between the groups were found at the third step for leucine flux ($P < 0.03$), at all three steps for KIC flux ($P < 0.05$–$P < 0.01$), and at the second and the third step for KIC oxidation ($P < 0.01$ and $P < 0.02$, respectively), while it approached statistical significance at the first step ($0.05 > P < 0.1$) (Table IV). Rates of leucine-carbon flux, not accounted for by oxidation (Leu → P) were not significantly different between the two groups at any clamp level, even if they tended to be slightly higher in the diabetic group. In both groups, hyperinsulinemia resulted in a slight decrease in Leu → P from basal values (Table IV). Leucine "clearance" was stimulated by hyperinsulinemia to a

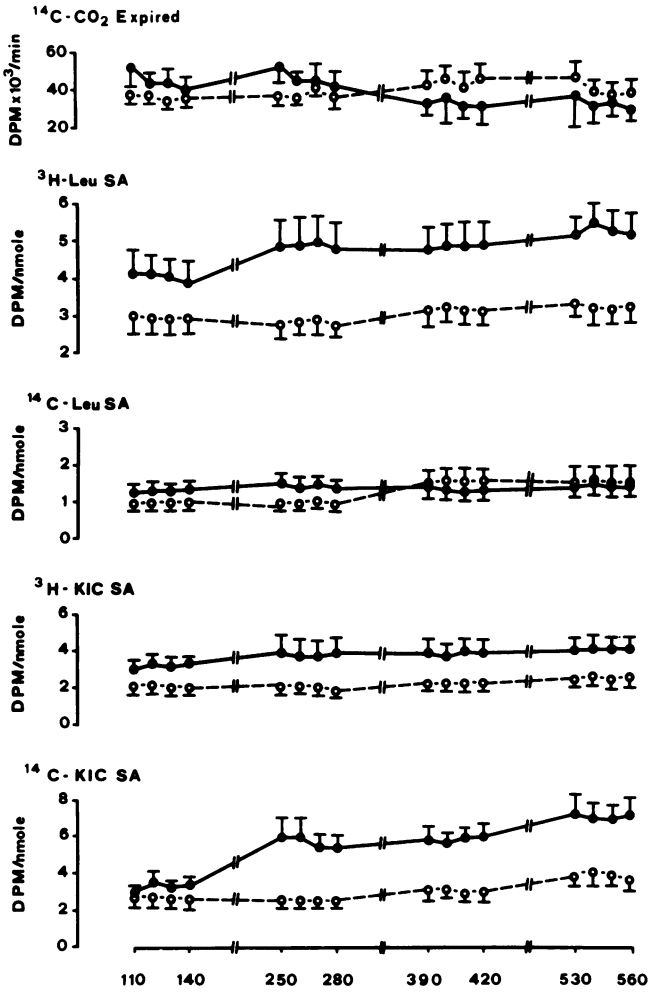

Figure 3. Plasma leucine (Leu) and KIC ^{3}H and ^{14}C specific activities, and expired $^{14}CO_2$, at basal and during stepwise euglycemic, hyperinsulinemic clamps, in patients with insulin-dependent, type 1 diabetes (IDDM; o) and in normal subjects (•). Abscissa units of measure, min.

similar extent in both groups. The glucose infusion rate to maintain euglycemia (M) correlated inversely with Leucine + KIC Ra ($r = -0.40$, $P < 0.05$), when all the subjects were analyzed together. A correlation between M and KIC oxidation did not reach statistical significance ($r = -0.35$, $P < 0.1$).

Discussion

This study shows that in type 1 diabetes a resistance to the metabolic effects of insulin is present, involving not only glucose but also leucine and KIC metabolism. Insulin-mediated glucose utilization was reduced in the diabetic patients at physiological and pharmacological insulin concentrations, thus confirming previous reports from our (2) and other laboratories (1, 3, 29). Moreover, the suppressive effect of insulin on endogenous leucine-carbon Ra (leucine + KIC Ra) and on KIC (~ leucine-carbon) oxidation was impaired in the diabetic patients. As leucine is an essential amino acid, and therefore it derives only from endogenous proteins in the postabsorptive state, the alterations observed in the insulin-diabetic patients are likely to reflect alterations in whole-body proteolysis.

As a significant portion of leucine-carbon is apparently released into the circulation as KIC (15, 16), the determinations of the kinetics of both leucine and KIC could be a better index of leucine-carbon appearance and, possibly, of proteolysis. In the present study, leucine and KIC Ra were measured with the simultaneous infusions of two different labels of leucine and KIC. All the kinetic data were derived from plasma ^{3}H and ^{14}C specific activities of leucine and KIC. Therefore, as the corresponding intracellular specific activities might be lower than those of plasma, the kinetic data calculated from plasma measurements may set a minimum for the estimates of leucine-carbon metabolism, in particular for the release of leucine-carbon from endogenous proteolysis.

In the diabetic patients, basal leucine metabolism was determined during an intravenous infusion of insulin at rates sufficient to maintain euglycemia. Under such conditions, despite peripheral hyperinsulinemia, leucine and KIC Ra, oxidation and nonoxidized leucine carbon flux (Leu → P), a possible index of leucine incorporation into protein(s), were similar in normal and in diabetic subjects. Thus, higher peripheral plasma free-insulin levels were apparently necessary to normalize basal leucine metabolism in the diabetic patients. In regard to this, it might be argued that, if a threshold dose for the effects of insulin on leucine kinetics does exist, at a level below the first clamp step of the normal subjects (~40 μU/ml), comparable rates of leucine kinetics in normal and diabetic subjects could have been observed also at degrees of hyperinsulinemia of ~25 μU/ml of plasma insulin in the normal subjects, i.e., at the same insulin concentration necessary to maintain the diabetic patients at euglycemia. However, in a recent study performed in normal subjects (30), degrees of hyperinsulinemia of ~20 and of ~30 μU/ml induced significant changes from the basal, postabsorptive state values, of plasma leucine concentration and flux. Therefore, no threshold dose of insulin on leucine kinetics can be postulated. As a consequence, the similarity in basal leucine + KIC Ra in the normal and in the euglycemic diabetic patients, despite a relative hyperinsulinemia in the latter, can itself suggest the presence of insulin-resistance in diabetes.

Stepwise hyperinsulinemia decreased leucine + KIC Ra to a larger extent in the normal than in the diabetic subject. Also, the relative decrements from baseline were less pronounced in the diabetic patients. Thus, endogenous proteolysis as reflected in the postabsorptive state by leucine + KIC Ra, was less suppressed in the diabetic than in the normal subjects, further indicating the presence of insulin resistance. After sequential insulin infusions, KIC oxidation in normals decreased also to lower values than in the diabetics. As oxidation represents the irreversible loss of an essential amino acid, possibly indicating net protein loss, our data suggest that also a resistance to the suppressive effect of insulin on irreversible leucine-carbon (~ protein) catabolism is present in type 1 diabetes. In the present study, leucine-carbon oxidation was calculated by dividing the expired $^{14}CO_2$ over plasma [^{14}C]KIC specific activity. In regard to this, some considerations should be made. First, expired $^{14}CO_2$ may require a longer equilibration time to achieve an incontestable steady state after perturbation. In our study, however, expired counts in $^{14}CO_2$ appeared to be nearly constant in the last 30 min of each experimental period (Fig. 4). Moreover, as they tended, if anything, to decrease after stepwise hyperinsulinemia in normal subjects, while they did not change in the

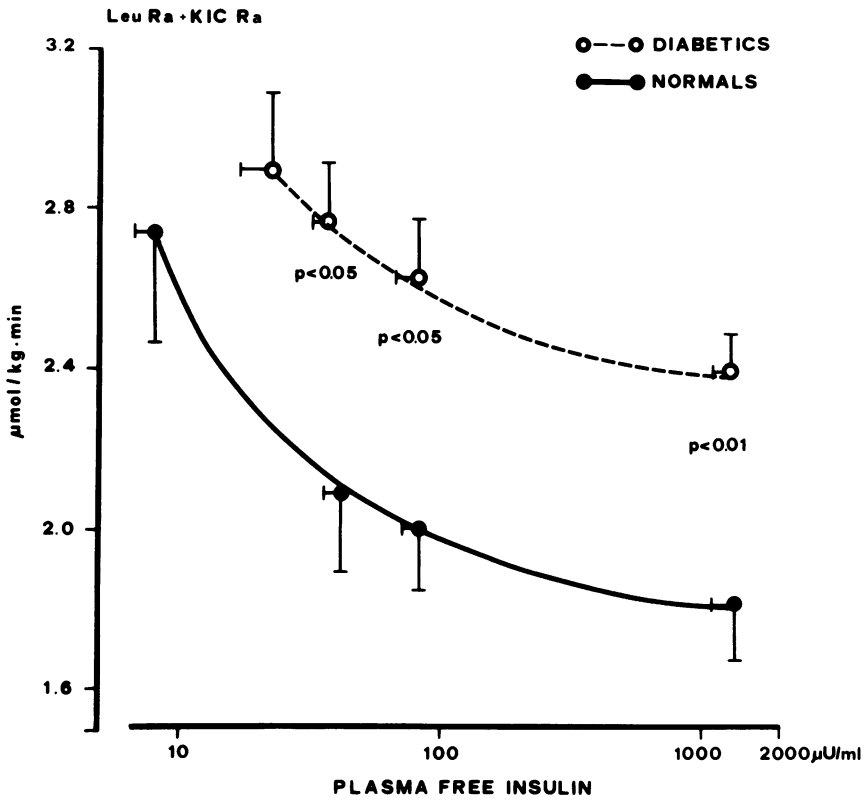

Figure 4. Leucine carbon flux, calculated as the sum of leucine (Leu) and KIC rates of appearance (Ra), at basal and during stepwise euglycemic, hyperinsulinemic clamps, in patients with insulin-dependent diabetes mellitus (IDDM) and in normals.

diabetics, lack of a complete equilibration of labeled bicarbonate would have led to an overestimation of absolute rates of leucine-carbon oxidation in normals and, therefore, to an underestimation of the differences between the two groups. Second, an underestimation of absolute rates of leucine-carbon oxidation in both groups might have occurred, because of the use of plasma [^{14}C]KIC specific activity during the infusion of [^{14}C]KIC as the direct precursor of oxidation of leucine-carbon. As dilution of the infused label may occur intracellularly, it might be preferable to infuse [^{14}C]leucine and use plasma [^{14}C]KIC specific activity as a reflection of the intracellular specific activity of leucine (31).

Leucine utilization was not decreased in the diabetic patients at each clamp step, as the estimates of nonoxidized leucine-carbon flux, a possible index of leucine entering protein(s) (Leu → P), and of leucine-carbon oxidation, as well as leucine "clearance", were normal or increased. This "normality" occurred in the presence of plasma leucine and KIC concentrations slightly higher than in the normal subjects. Thus, from our data it cannot be excluded that the normality of leucine utilization was sustained by the slightly elevated plasma concentrations of leucine and KIC, as a consequence of a mass-effect. On the other hand, glucose metabolism in type 1 diabetes is mainly impaired as regards insulin-mediated glucose utilization (1, 2, 29). These differences in insulin-resistance on aspects of glucose and amino acid metabolism might be due, at least in part, to the different experimental design with which these parameters

Table IV. Leucine Flux, KIC Flux, KIC Oxidation, Leucine Incorporation into Proteins (Leu → P), and Leucine Clearance, Estimated with the Single Isotope Model, in Normal Subjects and in the Diabetic Subjects (IDDM)

		Basal	First level	Second level	Third level
Leucine flux (Ra) (*μmol/kg·min*)	Normals	1.77±0.14	1.49±0.13*	1.50±0.12*	1.34±0.09*
	IDDM	1.86±0.11	1.76±0.11	1.73±0.12	1.68±0.07‡
KIC flux (Ra) (*μmol/kg·min*)	Normals	0.97±0.11	0.60±0.06*	0.50±0.06*	0.45±0.04*
	IDDM	1.04±0.09	1.01±0.09§	0.91±0.06*‖	0.71±0.05*§
KIC oxidation** (*μmol/kg·min*)	Normals	0.20±0.02	0.14±0.02¶	0.08±0.01*	0.06±0.01*
	IDDM	0.22±0.03	0.21±0.03	0.23±0.03§	0.17±0.03‡
Leu → P** (*μmol/kg·min*)	Normals	1.59±0.13	1.29±0.10¶	1.42±0.07	1.30±0.07*
	IDDM	1.55±0.12	1.43±0.20	1.45±0.14¶	1.47±0.09
Leucine clearance (*ml/kg·min*)	Normals	13.8±1.3	16.6±1.4	25.0±2.0	29.4±3.0
	IDDM	17.9±2.1	16.6±2.4	22.4±2.8	30.9±4.1

* $P < 0.01$ vs. basal values; ‡ $P < 0.03$ vs. normals; § $P < 0.01$ vs. normals; ‖ $P < 0.001$ vs. normals; ¶ $P < 0.05$ vs. basal values. ** Determined in four normals and in all ($n = 5$) the diabetic subjects.

are estimated. In fact, while glucose disposal is measured at euglycemia, leucine, KIC, and amino acid concentrations fall markedly during the euglycemic, hyperinsulinemic clamps. Possible effects of autoregulatory mechanisms by plasma glucose (32) and leucine (33) concentrations on their own rates of appearance might therefore modulate, in different ways, the effects of hyperinsulinemia per se.

Regarding the mechanism(s) accounting for the resistance to the insulin effects on leucine and KIC metabolism, it has to be pointed out that different rates of glucose infusions were required to maintain euglycemia in the diabetic vs. the control subjects. As competitions between glucose and amino acid peripheral utilization may occur (34–36), the increased leucine-carbon flux in type 1 diabetic subjects might also depend, at least in part, on their decreased glucose utilization. A weak but statistically significant inverse correlation was found between glucose disposal and leucine + KIC Ra, when all the subjects were analyzed together. Thus, the observed effects on leucine + KIC Ra could be at least in part related to the magnitude of glucose utilization. Further studies are required to investigate the effect of glucose utilization per se of leucine kinetics in vivo.

Finally, a criticism could be raised regarding the choice of sequential insulin infusions, lasting 140 min each, in our experimental design. It has been suggested that glucose utilization may increase progressively during more prolonged euglycemic, hyperinsulinemic clamps (37), and this time-related increase in the effects of insulin might also be present for leucine metabolism. Also, the effects of fasting on proteolysis (38), and a significant isotope recycling under such prolonged infusions (38), could influence the interpretations of the results, and might themselves be affected by diabetes per se. It has to be pointed out, however, that in the last 30 min of each 140 min infusion period, steady state conditions in plasma leucine and KIC concentrations and specific activities, in plasma free-insulin concentrations and in the rates of glucose disposal, were achieved in both groups, indicating that the insulin-induced effects on metabolism had reached an equilibrium. Moreover, the duration of the experiments were similar in both groups. In addition, during a 7-h insulin infusion in two normal subjects at a fixed insulin dose (0.05 $U/m^2 \cdot min$) (data not shown), we did not observe any further change in leucine kinetics after the first 2 h of the insulin infusion. On the other hand, if isotope recycling may constitute a potential problem in such experiments, it would underestimate the differences observed between normal and diabetic subjects. As isotope recycling is thought to arise from release of labeled substrate from labile proteins (38), it may be restrained by the suppressive effect of insulin on proteolysis. If this suppressive effect is diminished in diabetes, a relative increase in isotope recycling may lead to an underestimation of flux rates, and, therefore, to an overestimation of the effects of insulin in the diabetic vs. the normal subjects. Thus, the differences here observed between the two groups might have to be considered as conservative.

In conclusion, the present study demonstrates that type 1 diabetes is characterized not only by an impairment in insulin-mediated glucose disposal, but also by a resistance to the suppressive effects of insulin on leucine and KIC Ra (a reflection of proteolysis) and on leucine-carbon oxidation. Such defects in insulin action could be located at steps different from those of carbohydrate metabolic pathways.

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