Insulin-mediated Reduction of Whole Body Protein Breakdown

Dose-response Effects on Leucine Metabolism in Postabsorptive Men

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Abstract

In vivo effects of insulin on plasma leucine and alanine kinetics were determined in healthy postabsorptive young men (n = 5) employing 360-min primed, constant infusions of L-[1-^{13}C]leucine and L-[^{15}N]alanine during separate single rate euglycemic insulin infusions. Serum insulin concentrations of 16.4±0.8, 29.1±2.7, 75.3±5.0, and 2,407±56 μU/ml were achieved. Changes in plasma 3-methyl-histidine (3-MeHis) were obtained as an independent qualitative indicator of insulin-mediated reduction in proteolysis. Hepatic glucose output was evaluated at the lowest insulin level using D-[6,6-$^{2}H_2$]glucose.

The data demonstrate a dose-response effect of insulin to reduce leucine flux, from basal values of 77±1 to 70±2, 64±3, 57±3, and 52±4 μmol(kg · h)$^{-1}$ at the 16, 29, 75, and 2,407 μU/ml insulin levels, respectively ($P < 0.01$). A parallel, progressive reduction in 3-MeHis from 5.8±0.3 to 4.3±0.3 μM was revealed. Leucine oxidation estimated from the ^{13}C-enrichment of expired CO_2 and plasma leucine (12±1 μmol[kg · h]$^{-1}$) and from the ^{13}C-enrichment of CO_2 and plasma α-ketoisocaproate (19±2 μmol[kg · h]$^{-1}$) increased at the 16 μU/ml insulin level to 16±1 and 24±2 μmol(kg · h)$^{-1}$, respectively ($P < 0.05$ for each), but did not increase at higher insulin levels. Alanine flux (206±13 μmol(kg · h)$^{-1}$) did not increase during the clamp, but alanine *de novo* synthesis increased in all studies from basal rates of 150±13 to 168±23, 185±21, 213±29, and 187±15 μmol(kg · h)$^{-1}$ at 16, 29, 75, and 2,407 μU/ml insulin levels, respectively ($P < 0.05$). These data indicate the presence of insulin-dependent suppression of leucine entry into the plasma compartment in man secondary to a reduction in proteolysis and the stimulation of alanine synthesis during euglycemic hyperinsulinemia.

Introduction

While insulin and glucose have long been recognized as playing important roles in body protein and amino acid homeostasis, their specific in vivo effects have not been quantified. Plasma amino acid concentrations represent the net balance of entry and removal from plasma and thus provide only indirect estimates of the dynamic status of amino acid metabolism. Rates of amino acid entry into and removal from plasma are determined by amino acid utilization for protein synthesis, liberation from tissue proteins via proteolysis, oxidation, and in the case of the dispensable amino acids, via their *de novo* synthesis. Insulin produces reductions in plasma amino acids; the effect is most marked for the branched-chain amino acids (BCAA)[1] and minimal for alanine (1–3). To clarify the specific mechanisms responsible for the insulin-dependent reduction in plasma BCAA levels and to examine the subsequent effects on alanine metabolism, we have combined stable isotope tracer dilution kinetics in combination with the euglycemic insulin clamp technique in healthy young men. The observed kinetic data indicate an insulin-dependent suppression of the rate of leucine entry into the plasma compartment without major changes in leucine oxidation. In addition, *de novo* alanine synthesis is stimulated by euglycemic hyperinsulinemia.

Methods

Subjects. Five nonobese healthy young men (21–34 yr) whose relative body weight[2] ranged from 95 to 117% participated in these studies. Each subject had normal hepatic and renal function. None of the participants had a family history of diabetes mellitus, and none were taking medications on an acute or chronic basis. Fasting blood glucose levels (92±3 mg/dl, mean±SE), and 1-h (155±10 mg/dl) and 2-h (97±0.5 mg/dl) plasma glucose levels following an oral glucose ingestion (40 g/m^2) were within normal limits. The subjects received a weight-maintaining diet, which supplied 47% of energy from carbohydrate and 1.5 g protein/kg body wt for 3 d before the studies. The average caloric intake was 3,382±139 kcal/d. All subjects maintained their usual level of physical exercise. The studies were conducted after subjects had experienced a 12-h overnight fast in the Massachusetts Institute of Technology Clinical Research Center.

Subjects were awake and supine throughout the isotope tracer studies. The volumes of blood removed were similar to the total fluid infused and were similar for all subjects at the various levels of insulin infusion. The protocols used were approved by the Massachusetts Institute of Technology Committee on the Use of Humans as Experimental Subjects

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1. *Abbreviations used in this paper:* BCAA, branched-chain amino acids; KIC, leucine oxidation with [1-^{13}C]ketoisocaproic acid as precursor enrichment; L, leucine oxidation with [1-^{13}C]leucine as precursor enrichment; 3-MeHis, 3-methyl-histidine.

2. Relative body weight was calculated as the ratio of actual to desirable weight using the middle of the weight range for men of medium frame from the 1959 Metropolitan Life Insurance Table for desirable weights.

and the Executive Committee of the Clinical Research Center. Written informed consent was obtained from all subjects before the study.

Experimental design. A total of 19 isotope tracer/euglycemic insulin clamp studies were performed on the 5 subjects. Each study consisted of a 360-min primed (0.62 mg/kg), constant (0.06 μmol[kg · min]$^{-1}$) infusion of L-[1-^{13}C]leucine and a primed (2.6 mg/kg), constant (0.130 μmol[kg · min]$^{-1}$) infusion of L-[^{15}N]alanine (99% ^{13}C and ^{15}N, respectively; MSD Isotopes, Pointe-Duval, Canada) (4). In addition, 0.087 mg/kg of $NaH^{13}CO_3$ (90% ^{13}C; Kor Isotopes, Cambridge, MA) was given as a bolus to prime the bicarbonate pool (5) at the beginning of each study. 180 min after initiation of the tracer infusion, a 180-min euglycemic insulin clamp was started. All five subjects participated in separate studies at insulin infusion rates of 6, 10, and 30 mU(m^2 · min)$^{-1}$, and four participated in the studies at 400 mU(m^2 · min)$^{-1}$. Each subject's studies were separated by at least 3 wk, and the different insulin infusion rates were used in random order. For the 6 mU(m^2 · min)$^{-1}$ study, a primed (4 mg/kg), constant infusion (0.05 mg[kg · min]$^{-1}$) of D-[6,6-$^2H_2$]glucose (98% ^{2}H, MSD Isotopes) was administered with the leucine and alanine tracers to permit determination of endogenous glucose production (6).

Sampling procedures, tracer methods, and calculations. Leucine, alanine, and glucose fluxes were computed based on primed, constant tracer dilution methods (4, 6, 7). Arterialized venous blood was withdrawn every 15 min during the last hour of the basal and insulin clamp periods, centrifuged at 4°C, and the plasma stored at −20°C until assayed for isotope enrichment. Blood for determination of plasma amino acid concentrations including 3-methyl-histidine (3-MeHis) was obtained every 30 min throughout the 360-min study.

Rates of CO_2 production were determined during the study using eight 6–10-min expired air collections. Volumes were measured with a gasometer (Collins, Inc., Braintree, MA) and CO_2 concentration with an LB2 CO_2 gas analyzer (Beckman Instruments, Inc., Fullerton, CA). Values were corrected to standard temperature and pressure. Separate aliquots of expired air were collected in four liter anesthesia bags every 15 min during the last hour of the basal and insulin clamp periods and stored in Venoject Vacutainer tubes for later analysis of $^{13}CO_2$ enrichment. The glucose used for infusion has a natural abundance of ^{13}C which is slightly higher than that of expired natural abundances of $^{13}CO_2$. Under conditions of the euglycemic insulin clamp, the ^{13}C-enrichment of expired CO_2 is increased by 0.0019 atoms % excess when natural glucose is infused at rates equal to or greater than 4 mg(kg · min)$^{-1}$. Therefore, this value has been subtracted from the measured ^{13}C-enrichment of expired CO_2 obtained during the clamp period to estimate leucine oxidation when exogenous glucose is infused. Rates of $^{13}CO_2$ recovery following administration of $NaH^{13}CO_3$ were unaffected by the insulin clamp (Fukagawa, N. K., and K. L. Minaker, unpublished observations).

Plasma leucine kinetics were determined using steady state isotope dilution equations as previously described (4). Leucine oxidation was evaluated by two complimentary techniques. The apparent rate of leucine oxidation (L) was first estimated by dividing the ratio of expired $^{13}CO_2$ (atoms percent excess [kg · min]$^{-1}$), which was corrected for 20% CO_2 fixation (5, 8), by the ^{13}C-enrichment of plasma leucine. This technique has been extensively employed in studies of amino acid kinetics (4, 7, 9). Leucine oxidation (KIC) was also calculated for the 6 mU and 400 mU(m^2 · min)$^{-1}$ studies using the ^{13}C-enrichment of plasma α-KIC, as previously described (9). α-KIC acid is the immediate precursor for the oxidative decarboxylation of leucine and is presumably a preferable precursor enrichment estimate to use for estimating the rate of the irreversible decarboxylation of leucine. The enrichment of plasma leucine bears a constant relationship to the enrichment of plasma α-KIC (7, 9). Because the subjects were studied in the postabsorptive state without a dietary amino acid intake, the principal net source of leucine tracer dilution is unlabeled leucine entering plasma as a result of protein breakdown.

Estimates of alanine kinetics and *de novo* synthesis were calculated using a model described previously (4). Since an average value for the alanine content of mixed body protein can be approximated (8.5% for leucine and 6.4% for alanine) (10), the rate of alanine inflow into the free amino acid pool resulting from proteolysis can be quantitated by extrapolation from [1-^{13}C]leucine tracer kinetics (4). It should be noted that the absolute value for alanine flux depends upon whether a ^{13}C-, ^{15}N-, or ^{2}H-label is used to estimate alanine kinetics (11). However, additional studies from this laboratory have shown that relative changes in alanine metabolism due to nutritional treatments can be adequately determined irrespective of the choice of the particular isotope label (12).

In the basal state, rates of glucose production were calculated by the isotope dilution equation for the steady state (6). During the euglycemic insulin clamp, glucose kinetics were determined using Steele's equations (13) in their derivative form for the nonsteady state, as recently validated for both the steady and nonsteady states by Radziuk et al. (14).

Insulin clamp studies. Three basal samples for glucose and insulin were obtained at 150, 165, and 180 min after initiation of tracer infusions and before the start of the clamp study. Crystalline porcine insulin (Velosulin, Nordisk Inc., Bethesda, MD) was then infused for 180 min in all studies. 4 min after the start of the insulin infusion, a variable infusion of glucose in water (20%) maintained plasma glucose at the basal level in the studies at the three lower rates of insulin infusion. To maintain euglycemia during the 400 mU(m^2 · min)$^{-1}$ insulin infusion, glucose in water (50%) was infused via a peripherally inserted central venous line. Blood for estimation of plasma glucose was obtained at 5-min intervals and for insulin at 30-min intervals after the start of the clamp. Glucose was infused for an additional 30 min after termination of the insulin infusion. Euglycemia was maintained in all studies; mean coefficients of variation (%) for plasma glucose were 3.8, 5.7, 5.5, and 6.1 for the 6, 10, 30, and 400 mU(m^2 · min)$^{-1}$ studies, respectively. During the insulin clamp studies, glucose infusion rates were expressed as the mean of values observed during 20-min periods after corrections for overfilling and underfilling the glucose space (15). Steady state glucose disposal rates were calculated as the mean of the three 20-min segments from 300 to 360 min of the clamp study after correction for endogenous glucose output (15). Basal glucose production was 2.3±0.13 mg(kg · min)$^{-1}$ and was suppressed by ~50% to 1.19±0.24 mg(kg · min)$^{-1}$ during the lowest dose insulin clamp. Previous studies in our laboratory indicate that hepatic glucose output is 70% suppressed during 10 mU(m^2 · min)$^{-1}$ studies (Fukagawa, N. K., and K. L. Minaker, unpublished observations). Hence, mean values of 1.19 and 0.65 mg(kg · min)$^{-1}$ were added to the glucose infusion rates for the 6 and 10 mU(m^2 · min)$^{-1}$ studies, respectively, as the hepatic contribution to total glucose disposal (16). Glycosuria did not occur during these clamp studies. Steady state insulin concentrations were calculated as a mean of value from 190 to 360 min. All infusions were given using Harvard screw type infusion pumps (Harvard Instruments, Millis, MA). This technique has recently been validated to produce a stable insulin stimulus at low pump infusion rates (17).

Analytical methods. Isotope enrichment of plasma leucine, alanine, glucose, and α-KIC were determined by selected ion monitoring gas chromatography/mass spectrometry as previously described (4, 6, 7). The isotope enrichment of all achieved a new plateau during the insulin infusion (Fig. 1). Expired air samples were analyzed for $^{13}CO_2$ enrichment in a dual collector isotope ratio mass spectrometer (Nuclide Corp., State College, PA). Plasma obtained at 0, 180, and 360 min were also analyzed for plasma 3-MeHis concentration, using a modification of the method by Wassner et al. (18).

Plasma glucose concentrations were measured by the glucose oxidase method on a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, CA). Plasma insulin levels and amino acid concentrations were determined as previously described (4).

Statistical analyses. Differences in leucine flux, leucine oxidation, alanine flux, rates of alanine *de novo* synthesis, and amino acid concentrations between the basal and the insulin clamp periods at the four insulin infusion rates were analyzed by two-way analysis of variance. Insulin concentrations required for half-maximal responses for glucose disposal and the decline in plasma leucine flux were determined using nonlinear regression (Statistical Analysis System, Cary, NC; nonlinear regression) fitting the data to the exponential model $Y = a(e^{-bI})$.

All other comparisons were made by using *t* test. All data are expressed as mean±SE of the mean.

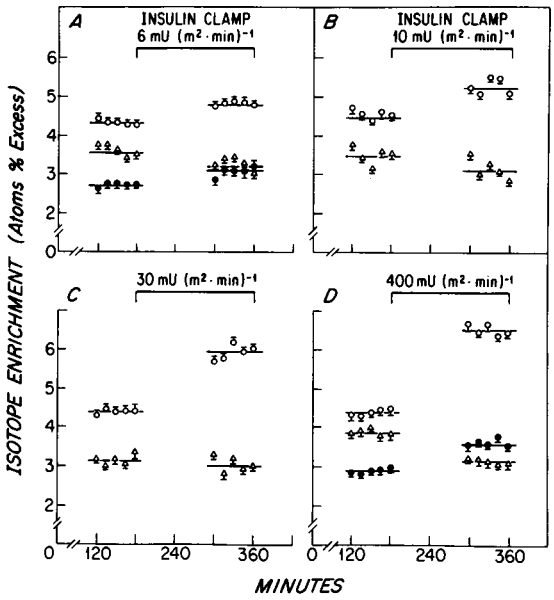

Figure 1. Isotope enrichments of plasma leucine (LEU) (○, mean±SE), α-ketoisocaproate (α-KIC) (●), and alanine (ALA) (△) during basal (120–180 min) and euglycemic insulin clamp (300–360 min) periods. Plateau values (mean±SE) during basal and clamp periods, respectively, were: (*A*) LEU, 4.33±0.05 and 4.85±0.04; α-KIC, 2.72±0.02 and 3.11±0.06; ALA, 3.61±0.06 and 3.31±0.06. (*B*) LEU, 4.55±0.07 and 5.32±0.10; ALA, 3.53±0.08 and 3.16±0.15. (*C*) LEU, 4.40±0.04 and 5.95±0.11; ALA, 3.16±0.06 and 3.07±0.09. (*D*) LEU, 4.41±0.05 and 6.53±0.05; α-KIC, 2.93±0.03 and 3.63±0.05; ALA, 3.86±0.06 and 3.21±0.03.

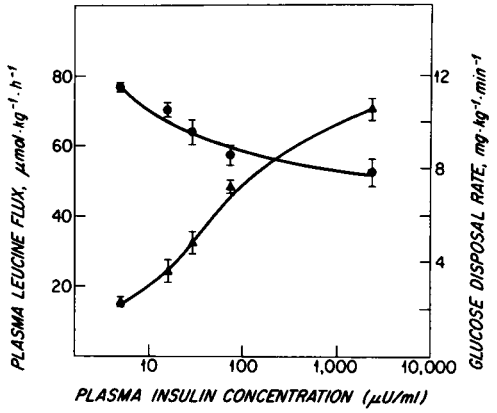

Figure 2. Effect of insulin on plasma leucine flux (— ● —) and glucose uptake (— ▲ —). The changes in leucine flux and glucose uptake show a dose-response effect to insulin ($P < 0.01$ for both).

Results

Insulin levels and glucose metabolism. Basal serum insulin levels (5.4±0.5 μU/ml), basal plasma glucose levels (91±1 mg/dl), and mean plasma glucose concentrations during the 6, 10, 30, and 400 mU(m²·min)⁻¹ insulin infusions (93±2, 92±2, 89±2, and 91±1 mg/dl, respectively) were similar in all studies. The insulin infusions resulted in stable, infusion rate-dependent hyperinsulinemia. Steady state insulin levels were 16.4±0.8, 29.1±2.7, 75.3±5.0, and 2,407±56 μU/ml for the 6, 10, 30, and 400 mU(m²·min)⁻¹ studies, respectively. In response to increasing steady state levels of insulin, there was a dose-dependent increase in glucose disposal rate (Table I, Fig. 2) ($P < 0.01$).

Leucine metabolism. Plasma leucine levels declined in an insulin dose-dependent fashion (Table I, Fig. 2). Basal plasma leucine flux was 77±1 μmol(kg·h)⁻¹ and decreased in an insulin dose-dependent manner to 70±2, 64±3, 57±3, and 52±4 μmol(kg·h)⁻¹ in the 6, 10, 30, and 400 mU(m²·min)⁻¹ studies, respectively ($P < 0.01$). The basal rate of L was 12±1.0 μmol(kg·h)⁻¹ and appeared to increase at the lowest rate of insulin infusion, but remained unchanged relative to the basal value during the other three studies at the higher insulin infusion rates (Table I). The changes in KIC were similar to those found for L (Table I). Plasma 3-MeHis levels (basal, 5.8±0.3 μM) declines paralleled the changes in leucine flux (Fig. 3).

Glucose disposal/leucine flux. Based on the dose-response relationships observed above for leucine flux and glucose disposal, calculations were made of the insulin concentration necessary for ½ maximal changes in glucose disposal, plasma leucine levels, and leucine flux. These were 33±5, 34.6±7, and 31.7±6 μU/ml, respectively. The differences are not statistically significant.

Alanine metabolism. Basal alanine flux was similar in repeat studies in each subject averaging 236±13 μmol(kg·h)⁻¹. Under these conditions, it was estimated that 150±13 μmol(kg·h)⁻¹, or 64% of alanine flux, was due to *de novo* alanine synthesis with the remainder arising from proteolysis. During the insulin infusion studies, total alanine flux did not change significantly (Table I). However, because leucine flux declined, which indicates a decreased inflow of amino acids via protein breakdown, *de novo* alanine synthesis increased, especially at the three highest levels of insulin administration. There was no significant dose-response effect, however.

Discussion

This study characterizes the acute effects of changes in circulating insulin on leucine and alanine kinetics in man. The present findings extend earlier observations on the influence of insulin on plasma amino acid levels (1–3) and indicate that insulin decreases leucine flux in a dose-dependent manner due to a reduction in protein breakdown.

The important relationship between insulin and leucine metabolism is well established (1–3). In man, insulin accelerates the disposal of an exogenous load of leucine (19) and hyperinsulinemia inhibits the release of leucine from muscle (20). In vitro studies have demonstrated that insulin enhances the uptake of leucine by muscle (21), adipose tissue (22), and liver and kidney (23), and that insulin promotes protein synthesis (24, 25).

In the present study, the flux (rate of entry/exit) of leucine in plasma was estimated using stable isotope tracer dilution. A precise estimation of in vivo protein synthesis and degradation rates cannot be made from plasma leucine enrichments alone. However, changes in the plasma appearance rate of leucine should mirror changes in leucine released from protein because leucine is a nutritionally indispensable (essential) amino acid, and the principal net source of unlabeled leucine in the post-absorptive state must be from body protein pools. Hence, the observed insulin dose-dependent decline in plasma leucine flux

Table I. Effect of Euglycemic Insulin Infusion on Glucose, Leucine, and Alanine Metabolism

	Basal	Insulin infusion rate ($mU[m^2 \cdot min]^{-1}$) 6	10	30	400
Insulin ($\mu U/ml$)	5.4±0.5	16.4±0.8	29.1±2.7	75.3±5.0	2407±56
Glucose disposal ($mg[kg \cdot min]^{-1}$)	2.3±0.1	3.6±0.5‡	4.8±0.5‡	7.2±0.2‡	10.5±0.5‡
Plasma leucine level (μM)	120±2	97±4‡	88±6‡	60±3‡	44±4‡
Plasma leucine flux ($\mu mol[kg \cdot h]^{-1}$)	77±1	70±2‡	64±3‡	57±3‡	52±4‡
L§ ($\mu mol[kg \cdot h]^{-1}$)	12±1	16±1*	14±1	11±1	12±3
KIC‖ ($\mu mol[kg \cdot h]^{-1}$)	19±2	24±2*	—	—	22±2
Plasma alanine level (μM)	277±14	293±21	281±4	265±14	246±14
Plasma alanine flux ($\mu mol[kg \cdot h]^{-1}$)	206±13	246±25	256±21	277±31	245±17
Alanine *de novo* synthesis ($\mu mol[kg \cdot h]^{-1}$)	150±13	168±23	185±21*	213±29*	187±15*

*, $P < 0.05$, basal *vs.* clamp. ‡, $P < 0.01$, insulin dose-dependent changes. §, Estimated using circulating [1-^{13}C]leucine as the precursor enrichment for oxidation. ‖, Estimated using circulating [1-^{13}C]α-ketoisocaproic acid as the precursor enrichment for oxidation.

should reflect a decreased rate of plasma leucine inflow from body protein stores, i.e., diminished proteolysis. This interpretation is further supported by the parallel changes in plasma 3-MeHis concentrations in response to different insulin levels since this amino acid is derived from the breakdown of myofibrillar proteins (26). It is important to remember that the relationship between plasma 3-MeHis concentrations and the absolute rate of myofibrillar protein breakdown has not been established and is likely to be complex. However, the parallel changes serve as an additional qualitative index of the in vivo effect of insulin on proteolysis. These findings are supported by recent work by Tomas et al. (27) who noted that changes in the fractional degradation rates of myofibrillar protein appear to be relatively more attenuated by insulin than rates of synthesis in growing diabetic rats treated with corticosterone.

The present data support previous work in man demonstrating that insulin-mediated suppression of proteolysis is an integral part of protein anabolism in the postabsorptive state and following meals (28, 29). In addition, these findings in healthy young men are consistent with those of Gertner et al. (30) who demonstrated normalization of leucine kinetics in Type I diabetics during subcutaneous insulin infusion therapy, presumably as a result of reduction in high rates of protein breakdown. The data in this study are additionally compatible with results of insulin infusions in dogs that show a diminished release of leucine from both liver and hind limb tissues (31).

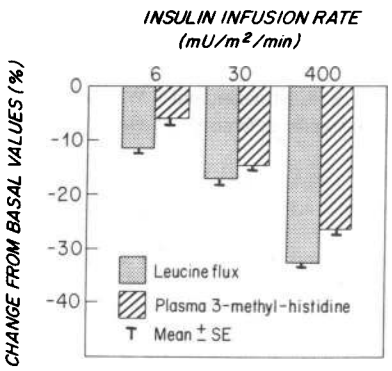

Figure 3. Changes in leucine flux and plasma 3-methyl-histidine (3-MeHis) in response to insulin. The percent declines in 3-MeHis parallel the changes in leucine flux and are insulin dose-dependent ($P < 0.05$).

In contrast, Eriksson et al. (19) have suggested that leucine disposal is independent of plasma insulin concentrations. However, their protocol employed glucose and leucine infusions to stimulate pancreatic insulin release and the resultant hyperglycemia and elevated plasma leucine levels created a less controlled insulin stimulus than the present studies.

The decline in leucine flux observed in the present studies may also result from an alteration in protein synthesis, i.e., an increased rate of leucine incorporated into protein. Insulin is known to promote protein synthesis and a number of mechanisms for its effect have been recently described. Buse et al. (32) described a translational inhibitor of protein synthesis whose activity was lessened by the addition of insulin and amino acids to the preparation. Harmon et al. (33) demonstrated that insulin acutely regulates protein synthesis in skeletal muscle at the level of polypeptide chain initiation. In the present experiments, the supply of amino acids available for protein synthesis was limited by the diminished rate of protein breakdown. This emphasizes the need to further examine the quantitative nature of the interaction of leucine metabolism and insulin levels when the supply of leucine and other amino acids is not reduced.

The development of a dose-response relationship in the present study permits determination of insulin levels necessary for half-maximal decline in leucine flux. The determined value, 31.7±6 μU/ml, is similar to the insulin concentration required for half-maximal glucose disposal (33±5 μU/ml). Our insulin value for half-maximal glucose disposal is somewhat lower than those reported by previous authors, probably for methodologic reasons (34–36). Rizza et al. (34) employed 2-h insulin infusions which may not result in stable plateaus of glucose disposal and thus may underestimate glucose disposal at low insulin infusion rates. For example, the present 180-min studies resulted in stable plateaus of glucose disposal of 4.8 $mg(kg \cdot min)^{-1}$ with insulin levels of 29.1±2.7 μU/ml. Data from the 120-min insulin infusions of Rizza et al. (34), resulting in comparable insulin concentrations of 26±2 μU/ml, produced glucose disposal rates of 2.0±0.3 $mg(kg \cdot min)^{-1}$, which are 40% of values in the present study. Using 120–180-min glucose infusion rates will thus produce lower half-maximal insulin values. In the study by Rowe et al. (35), correction for hepatic glucose was not done, introducing a systematic bias towards higher half-maximal insulin

values than reported here. The insulin sensitivity of leucine and glucose kinetics thus appears to be less than that for inhibition of lipolysis and increased potassium disposal (37).

There is the possibility of an effect of exogenous glucose on leucine metabolism separate from that of insulin in these studies. While high glucose infusion rates result in decreased net outflow of amino acids from the human forearm and leg (38), the resultant hyperglycemia creates a significant insulin response. The present study suggests that the changes in amino acid levels detected are more closely dependent on the degree of hyperinsulinemia.

These studies also identify insulin-mediated stimulation of *de novo* alanine synthesis. Prior studies of alanine metabolism in man have focused on alanine's role in gluconeogenesis (39), its role in nitrogen transport out of muscle (40), and its relationship to the metabolism of the BCAA (40, 41). From our earlier studies we suggested that the stimulation of *de novo* alanine synthesis was enhanced in the presence of hyperglycemia in healthy individuals (4). However, in insulin-dependent diabetics, the stimulation of alanine *de novo* synthesis by hyperglycemia apparently does not occur when the availability of insulin is limited (42). The present data extend these findings by demonstrating that, in the presence of hyperinsulinemia with euglycemia, alanine *de novo* synthesis is increased to maintain unchanging alanine levels as the introduction of alanine into the amino acid pools produced by endogenous proteolysis is reduced.

The study design does not permit determination of the tissue source of the newly synthesized alanine. DeFronzo et al. (43) found no net change in the release of alanine by splanchnic and leg tissues during euglycemic insulin clamp studies. However, Miller et al. (44) recently reported that the kidney is a significant producer of alanine in the postabsorptive state. Studies involving regional exchange of substrates are difficult to compare with those utilizing an isotopic methodology, but the results are not necessarily in conflict. It is possible that renal or other tissues may be responsible for the enhanced synthesis of alanine with maintenance of plasma alanine levels in our studies.

In summary, these data demonstrate a previously undocumented dose-dependent relationship between insulin and whole body leucine metabolism in man. This suggests that insulin serves to restrain endogenous protein breakdown following an overnight fast and may help to explain why the poorly controlled diabetic shows enhanced proteolysis (45). In addition, the data support findings that alanine *de novo* synthesis is enhanced by glucose, particularly when associated with adequate levels of insulin. Finally, protein breakdown was progressively reduced by increased levels of insulin.

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