Effect of Starvation on the Turnover and Metabolic Response to Leucine

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ABSTRACT L-Leucine was administered as a primed continuous 3–4-h infusion in nonobese and obese subjects in the postabsorptive state and for 12 h in obese subjects after a 3-day and 4-wk fast. In nonobese and obese subjects studied in the postabsorptive state, the leucine infusion resulted in a 150–200% rise in plasma leucine above preinfusion levels, a small decrease in plasma glucose, and unchanged levels of plasma insulin and glucagon and blood ketones. Plasma isoleucine (60–70%) and valine (35–40%) declined to a greater extent than other amino acids ($P < 0.001$).

After 3 days and 4 wk of fasting, equimolar infusions of leucine resulted in two- to threefold greater increments in plasma leucine as compared to postabsorptive subjects, a 30–40% decline in other plasma amino acids, and a 25–30% decrease in negative nitrogen balance. Urinary excretion of 3-methylhistidine was however, unchanged. Plasma glucose which declined in 3-day fasted subjects after leucine administration, surprisingly rose by 20 mg/100 ml after 4 wk of fasting. The rise in blood glucose occurred in the absence of changes in plasma glucagon and insulin and in the face of a 15% decline in endogenous glucose production (as measured by infusion of [3-^{3}H]glucose). On the other hand, fractional glucose utilization fell by 30% ($P < 0.001$), thereby accounting for hyperglycemia.

The estimated metabolic clearance rate of leucine fell by 48% after 3 days of fasting whereas the plasma delivery rate of leucine was unchanged, thereby accounting for a 40% rise in plasma leucine during early starvation. After a 4-wk fast, the estimated metabolic clearance rate of leucine declined further to 59% below base line. Plasma leucine nevertheless fell to postabsorptive levels as the plasma delivery rate of leucine decreased 65% below postabsorptive values.

Conclusions: (*a*) Infusion of exogenous leucine in prolonged fasting results in a decline in plasma levels of other amino acids, improvement in nitrogen balance and unchanged excretion of 3-methylhistidine, thus suggesting stimulation of muscle protein synthesis, (*b*) leucine infusion also reduces glucose production and to an even greater extent, glucose consumption, thereby raising blood glucose concentration; and (*c*) the rise in plasma leucine in early starvation results primarily from a decrease in leucine clearance which drops progressively during starvation.

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INTRODUCTION

The branched chain amino acids (leucine, isoleucine, and valine) are principally metabolized in muscle (1–3) where they are the major source of nitrogen (4) and may possibly provide carbon skeletons (5) for alanine formation during fasting. Although muscle tissue is in negative nitrogen balance in the fasting state (6), repletion of muscle nitrogen after protein feeding depends largely on the net uptake of branched chain amino acids (7). Recent data suggest that leucine may have a specific role in stimulating net muscle protein synthesis which is not shared by other amino acids (8–10). Of particular interest in this regard are data indicating that infusion of small amounts of ketoanalogues of the branched chain amino acids in humans spare nitrogen during early starvation (11).

The present study was undertaken to evaluate the effect of physiologic infusions of leucine on amino acid and nitrogen metabolism and on glucose regulation in fasted man. The data were also utilized to calculate leucine turnover in the postabsorptive state and after 3 days and 4 wk of starvation. These data are of interest regarding the mechanism underlying the initial rise and subsequent decline in plasma branched chain amino acids observed during starvation (12).

METHODS

Subjects. Two groups of subjects were studied. The first group consisted of 11 healthy, nonobese volunteers (eight

males and three females) within 20% of ideal body weight (based on 1959 Metropolitan Life Insurance Company Tables). The subjects ranged in age from 21 to 35 yr. They habitually consumed weight-maintaining diets containing a minimum of 200 g of carbohydrate and were taking no drugs. All had negative primary family histories for diabetes mellitus and none had an elevated fasting plasma glucose. The second group consisted of seven healthy obese subjects (one male and six females) who were hospitalized at the Clinical Research Center of the Yale-New Haven Hospital. Each had volunteered to undergo prolonged fasting after failure of dietary treatment. They were 55–189% (117±17%, mean±SE) above ideal body weight and ranged in age from 24 to 48 yr. With the exception of one patient all the obese subjects had normal fasting plasma glucose levels (<100 mg/100 ml) and a normal response to 100 g of oral glucose (13); one subject had a fasting plasma glucose of 120 mg/100 ml. This subject was included inasmuch as his response to leucine did not differ from the other obese subjects. All had normal serum thyroxine levels, and normal renal and liver function. For at least 3 days before study they consumed a 2,500-kcal diet containing 300 g of carbohydrate, and were taking no drugs. The subjects were informed of the nature, purpose, and possible risks of the study before obtaining their written voluntary consent to participate.

During fasting, obese subjects received 2,000 ml (or more) of water, one multivitamin tablet (Theragran, E. R. Squibb & Sons, Princeton, N. J.), 1 mg of folic acid (Folvite, Lederle Laboratories, Division of American Cyanamid Co., Pearl River, N. Y.), and 1.3 g of sodium bicarbonate (sugar free). Five of the subjects intermittently received 20 meq of potassium gluconate (Kaon, Warren-Teed Laboratories, Inc., Columbus, Ohio); however, potassium gluconate was not given for 7 days before, during, or for 3 days after infusion of leucine.

Procedures. The nonobese subjects were studied in the postabsorptive state after a 12–15-h overnight fast. The obese subjects were studied in the postabsorptive state ($n = 5$), after a 3-day fast ($n = 4$), and after a 4-wk fast ($n = 7$). An indwelling catheter was inserted in an antecubital vein for blood sampling and in the contralateral vein for administration of L-leucine. The L-leucine (obtained from Nutritional Biochemicals Corp., Cleveland, Ohio) was prepared as a 2% solution in sterile, pyrogen-free water, and passed through a 22-μm filter apparatus (Millipore Corp., Bedford, Mass.). Each lot was tested for pyrogen (14) and cultured for bacterial and fungal contamination before use.

The leucine solution was administered as a primed-continuous infusion via a portable peristaltic pump (Extracorporeal Medical Specialties, Inc., King of Prussia, Pa.). The priming dose was given over the initial 25-min period at twice the continuous infusion dose. The continuous infusion dose in both nonobese and obese subjects, 75 μmol/m^2 body surface area per min, was calculated to raise plasma leucine concentration to levels comparable to those observed after protein feeding (7). Leucine was administered to postabsorptive nonobese and obese subjects for 3 and 4 h, respectively and for 12 h (between 9 a.m. and 9 p.m.) during fasting. Subjects undergoing prolonged (4 wk) fasting received 12-h leucine infusions on 2 consecutive days. In two of the nonobese control subjects leucine was infused respectively, at five (15, 40, 75, 85, and 225 μmol/m^2 per min) and four (15, 45, 75, and 150 μmol/m^2 per min) infusion doses and an additional obese female subject (aged 42 yr) received four (25, 50, 100, and 180 μmol/m^2 per min) leucine infusion doses to determine if leucine removal is linear over the range of concentrations studied.

After the subjects had been resting in the recumbent position for a minimum of 30 min, at least two base-line blood samples were drawn at 15-min intervals before the infusion (control values represent the mean of the preinfusion measurements); additional blood samples were drawn at 30-, 60-, or 180-min intervals until completion of the infusion.

To determine the effects of leucine on protein catabolism during starvation, total nitrogen and 3-methylhistidine excretion were measured before, during, and after prolonged (12 h) leucine infusion (total dose was 12–20 g/day) in 3-day and 4-wk fasted subjects. Urine was collected in refrigerated containers for 24-h periods (9.00 a.m.–9:00 a.m.) beginning 2–3 days before leucine administration (preinfusion period), during the 1- or 2-day infusion period, and for 2 days after leucine administration. Nitrogen balance was determined as the difference between the grams of nitrogen administered as leucine (0.107 g N/g leucine) and the urinary nitrogen excretion. Values for each subject's daily nitrogen balance and 3-methylhistidine excretion during and after the leucine infusions were compared with the mean of the preinfusion values. Urine was also collected before, during, and after the completion of the leucine infusions to determine the effect of the infusions on urinary amino acid excretion.

To examine the mechanism responsible for leucine-induced hyperglycemia in 4-wk fasted subjects, [3-^{3}H]-glucose (New England Nuclear, Boston, Mass.) was administered as a primed continuous infusion to four patients during the 1st day of leucine administration. The labeled glucose was administered as an initial intravenous priming dose (25 μCi) followed immediately by a continuous intravenous infusion at a rate of 0.25 μCi/min. In each of these studies the specific activity of the [3-^{3}H]glucose in blood had reached a plateau before infusion of leucine. The [3-^{3}H]glucose infusion was then continued for the initial 6 h of leucine administration to evaluate changes in glucose production and utilization (15).

Analyses and calculations. Plasma glucose was measured by the glucose oxidase technique (16). The methods used for the determination of plasma immunoreactive insulin, plasma immunoreactive glucagon (using Unger antibody 30K), blood ketones, and plasma acidic and neutral amino acids have already been described (17, 18). Total nitrogen in urine was determined in duplicate by the standard Kjeldahl technique and urinary creatinine by the picric acid method (19). 3-Methylhistidine in urine was measured by the column chromatographic method (20). Plasma [3-^{3}H]glucose radioactivity was determined by standard liquid scintillation counting procedures after radioactive water was removed by overnight evaporation in a vacuum oven at 70°C.

Rates of endogenous glucose production and utilization were calculated in the steady state before leucine administration and during nonsteady-state conditions by the equations of Steele et al. (21) in their derivative form. The value of 0.65 of the initial glucose pool size (pool fraction) was used as the rapidly mixing compartment of the glucose pool to compensate for nonuniform mixing within the entire glucose pool (22, 23). All calculations were performed with a program written in BASIC on a Hewlett-Packard desk computer (Hewlett-Packard Co., Palo Alto, Calif.). Glucose clearance (a measure of fractional glucose turnover) was calculated by dividing the glucose utilization rate by plasma glucose concentration.

The metabolic clearance rate of leucine (MCR_L),[1] the volume of plasma completely and irreversibly cleared of leucine per minute, was calculated according to the formula

[1] *Abbreviations used in this paper*: MCR_L, metabolic clearance rate of leucine; PDR_L, plasma delivery rate of leucine.

(24): MCR_L = (leucine infusion rate) ÷ (total plasma leucine concentration at equilibrium − endogenous leucine concentration). In this calculation it was assumed that: (*a*) exogenous L-leucine metabolism is indistinguishable from that of endogenous leucine; (*b*) leucine removal is linear over the range of concentrations studied (see Results); and (*c*) endogenous leucine production is not completely suppressed by exogenous leucine administration. Inasmuch as the measurements of plasma leucine employed in this study do not distinguish between exogenous and endogenous leucine, the contribution of endogenously produced leucine was estimated from changes in plasma isoleucine levels. It was assumed that the percent change in isoleucine concentration during leucine administration reflected the changes in endogenous leucine concentration. Recent studies from our laboratory have, in fact, shown that during infusion of somatostatin (25) or insulin and glucose,[2] the time-course and percentage change in leucine and isoleucine concentrations are virtually identical and differ from all other amino acids. Since the contribution of endogenous leucine to total plasma leucine during the infusion was estimated rather than directly measured the term estimated MCR_L is utilized in the text. The plasma delivery rate of leucine (PDR_L), the quantity of leucine entering plasma each minute, was estimated in the postabsorptive state and in 3-day and 4-wk fasted subjects according to the formula: PDR_L = (basal plasma leucine concentration)·(estimated MCR_L).

Statistical analyses were performed with the Student's *t* test (the paired *t* test was used when applicable) and linear regression analysis (26). Data in the text are presented as the mean±SE.

RESULTS

Response to leucine infusion in postabsorptive, nonobese subjects

Fig. 1 demonstrates the changes in plasma leucine, glucose, insulin, and glucagon produced by the infusion of leucine in normal subjects. Plasma leucine (128±9 μM) increased rapidly, reaching a plateau between 60 and 120 min. Mean concentrations at equilibrium (353±19 μM) were 150–200% above control values and similar to the levels observed after protein ingestion (7). There was a small (4–6 mg/100 ml), but significant ($P < 0.01$) decrease in plasma glucose after 1 h: the mean maximal decrement averaged 6±1 mg/100 ml. Plasma insulin (18±1 μU/ml) and glucagon (84±16 pg/ml) remained unchanged throughout the 3-h study period. Similarly, blood ketones (0.07 ±0.02 mM) were not altered by leucine administration.

The effect of the leucine infusion on plasma amino acid concentrations in nonobese subjects is shown in Table I. The branched chain amino acids, isoleucine, and valine fell progressively during the 3-h study period. The decline in plasma isoleucine (59±4%, $P < 0.001$) and valine (33±4%, $P < 0.001$) exceeded that of all other amino acids. Small reductions (10–30%)

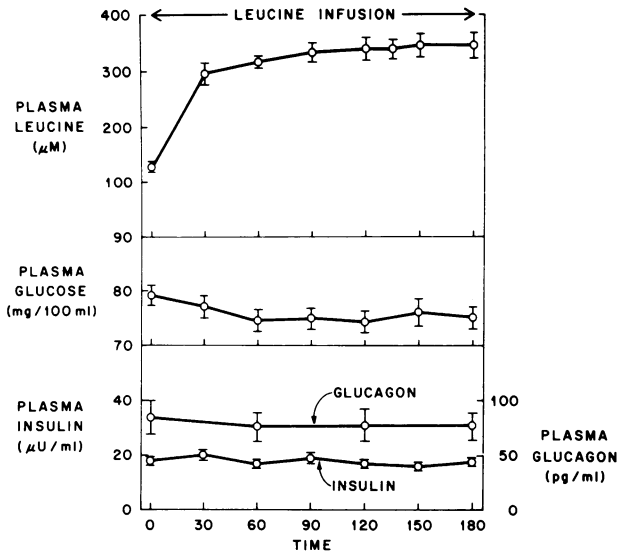

FIGURE 1 Effect of leucine infusion (3 h) on plasma leucine, glucose, insulin and glucagon concentrations (mean±SE) in postabsorptive nonobese subjects. The basal data (0 time) represent the mean of two observations at 15-min intervals.

in plasma threonine ($P < 0.001$), serine ($P < 0.001$), glycine ($P < 0.02$), cystine ($P < 0.02$), methionine ($P < 0.01$), tyrosine ($P < 0.001$), and phenylalanine ($P < 0.001$) were also observed at 3 h. Measurement of urinary amino acids in three control subjects indicated no significant urinary losses of leucine during the infusion (<0.1% of the administered dose) and no change in the excretion of other amino acids.

Response to leucine infusion in postabsorptive, obese subjects

In the obese group, the basal leucine concentration (150±16 μM) and the plateau leucine concentration achieved during the leucine infusion (420±24 μM) were slightly, but not significantly, increased above those observed in the nonobese group (128±9 and 353±19 μM, respectively). The time required to reach a stable plateau (120–150 min) was also slightly increased in the obese group (Figs. 1 and 2). As in the nonobese group, the obese subjects demonstrated a small decline in plasma glucose (10±5 mg/100 ml) whereas plasma insulin (28±7 μU/ml) and glucagon (64±7 pg/ml) concentrations were unchanged during leucine administration.

The plasma amino acid response to leucine infusion in postabsorptive obese subjects was comparable to that observed in nonobese controls. As in the nonobese group, plasma isoleucine (69±3%, $P < 0.001$) and valine (43±2%, $P < 0.005$) decreased to a greater extent than the other amino acids.

[2] DeFronzo, R. A., R. S. Sherwin, and P. Felig. Unpublished observations.

TABLE I

*Plasma Concentrations of Amino Acids during a 3-h Intravenous Infusion of Leucine in Nonobese Subjects in the Postabsorptive State**

Amino acid	0‡	30 min	60 min	120 min	150 min	180 min
			μM			
Leucine	128±9	295±19¶	318±11¶	350±17¶	358±20¶	357±23¶
Isoleucine	65±4	59±6	46±5¶	33±4¶	29±4¶	25±4¶
Valine	220±11	208±10	184±13¶	165±12¶	153±12¶	143±11¶
Taurine	35±2	36±3	35±2	35±2	34±2	35±4
Threonine	142±10	142±9	128±9¶	127±9¶	121±8¶	119±9¶
Serine	110±7	109±8	101±6‖	98±7	94±6¶	94±6¶
Proline	188±24	198±19	173±22	166±18§	170±21§	163±22
Citrulline	31±5	32±4	25±5	27±4	25±3	25±5
Glycine	239±15	242±25	220±13‖	224±17	218±14‖	215±13§
Alanine	365±24	370±22	344±24	353±32	339±28	331±31
α-Aminobutyrate	24±5	24±5	23±5	22±5	22±5	22±7
Cystine	101±9	101±9	88±6‖	86±6§	84±6§	84±7§
Methionine	25±1	24±1	23±2	20±1¶	20±1‖	19±2‖
Tyrosine	54±3	52±3	46±2¶	43±1¶	39±2¶	38±2¶
Phenylalanine	49±2	50±3	43±3¶	38±3¶	38±2¶	38±3¶

* Data presented as mean±SE. *P* values refer to significance of difference from preinfusion values (paired *t* test). Only values which differ significantly are indicated.

‡ Control values represent the mean of two observations on each subject preceding leucine administration.

§ Indicates $P < 0.05$.

‖ Indicates $P < 0.01$.

¶ Indicates $P < 0.001$.

Response to prolonged leucine infusion during starvation

Circulating substrates and hormones. As expected (12), after a 3-day fast, plasma leucine concentration rose by 40% ($P < 0.05$) above postabsorptive levels. Infusion of leucine resulted in elevations in plasma leucine which were 85–90% greater than those observed in the postabsorptive state ($P < 0.005$). Furthermore, the time required to reach a stable plateau (6 h) was prolonged two- to threefold (Fig. 2). After prolonged (4 wk) fasting, base-line leucine concentration (139±7 μM) had, as expected (12), returned to levels no different from postabsorptive values (P = NS). However, the leucine infusion again produced a markedly greater rise in plasma leucine concentration than observed in the postabsorptive state (Fig. 2). At equilibrium (6–12 h), plasma leucine levels were more than twofold greater than those observed before fasting (930±39 vs. 420±24 μM in the postabsorptive state, $P < 0.001$).

The plasma glucose response to leucine is shown in Fig. 3. As in the postabsorptive state, the leucine infusion in 3-day fasted subjects resulted in a small (10±4 mg/100 ml) decline in plasma glucose concentration whereas plasma insulin (21±5 μU/ml) and glucagon (92±10 pg/ml) remained stable. In marked contrast, in prolonged (4 wk) fasted subjects the leucine infusion produced a 15–20-mg/100 ml rise ($P < 0.001$) in plasma glucose (Fig. 3). The mean maximal increment in plasma glucose was 20±3 mg/100 ml. This hyperglycemic response to leucine occurred in the absence of changes in insulin and glucagon levels. Plasma insulin and glucagon which were 14±3 μU/ml and

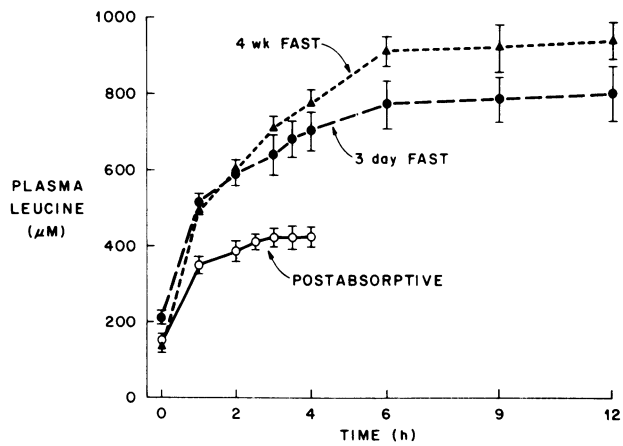

FIGURE 2 Plasma leucine concentrations during the infusion of exogenous leucine (75 μmol/m² per min) to obese subjects in the postabsorptive state (4 h) and after 3 days and 4 wk of starvation (12 h). The rise in plasma leucine after starvation was two- to threefold greater than that observed in the postabsorptive state ($P < 0.005$–<0.001).

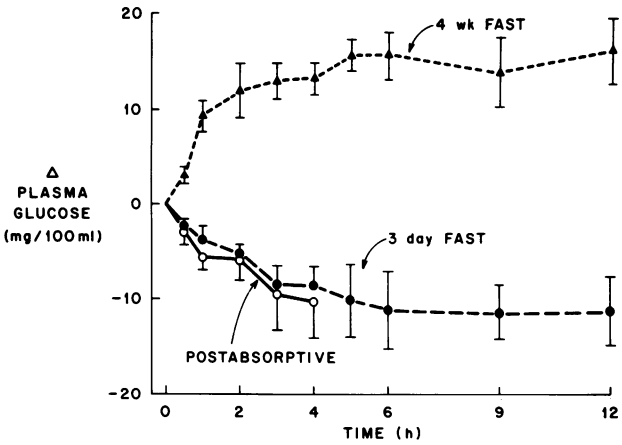

FIGURE 3 Changes in plasma glucose in response to the infusion of leucine in obese subjects in the postabsorptive state and after 3 days and 4 wk of starvation. Plasma glucose declined to a similar extent in postabsorptive and 3-day fasted subjects. In contrast, in 4-wk fasted subjects the leucine infusion produced a prompt increase in plasma glucose ($P < 0.001$).

80±11 pg/ml, respectively, remained unchanged through the leucine infusion (final values: insulin, 15±3 μU/ml; glucagon, 88±10 pg/ml).

The response of circulating amino acids to leucine infusion in fasted subjects is summarized in Table II. During starvation prolonged infusion of leucine produced reductions in virtually all amino acids other than leucine. The amino acid response was comparable in both 3-day and 4-wk fasted subjects. As in the postabsorptive state, the other branched chain amino acids, isoleucine (60–70%, $P < 0.001$) and valine (50–55%, $P < 0.001$), exhibited the most pronounced reductions in plasma concentration. However, declines exceeding 20% were also demonstrable for threonine, serine, proline, alanine, α-aminobutyrate, methionine, tryosine, and phenylalanine ($P < 0.05$–0.001). Total acidic and neutral amino acids, in 3-day (1,565 μM) and 4-wk (1,681 μM) fasted subjects were reduced by 32 and 37%, respectively, at the completion of the 12 h infusion. On the other hand, the leucine infusion produced no consistent changes in the urinary excretion of these amino acids. Changes in plasma amino acids in 3-day fasted subjects occurred in the absence of significant changes in blood ketones. Blood ketones in the 4-wk fasted group (6.0±0.7 mM) rose slightly to 6.5±0.8 mM, but only after 12 h ($P < 0.05$).

Nitrogen balance. The effect of leucine administration (12 h) on nitrogen balance in obese subjects fasted 3 days and 4 wk is shown in Fig. 4. Nitrogen balance during early starvation was improved 23% by leucine administration. Negative nitrogen balance decreased from 11.0±1.5 g/day on day 2 of fasting to 8.4±1.8 g/day ($P < 0.2$) when leucine was given on day 3 and then increased again to 11.0±1.5 g/day ($P < 0.02$) on the day after the infusion. In contrast, urinary creatinine excretion remained unchanged during the 5-day study period. In the subjects studied after a 4-wk fast, negative nitrogen balance remained stable during the 3-day preinfusion control period (mean 5.0±0.2 g/day), and was reduced by 25–30% ($P < 0.005$) on the days leucine was given (3.6±0.2 g/day on day 1 and 3.8±0.2 g/day on day 2). On the 2 postinfusion days negative nitrogen balance returned to preinfusion control levels. In contrast to the changes in nitrogen balance, urinary excretion of 3-methylhistidine (46±11 μmol/day preinfusion) was not altered by the leucine infusions (46±12 during and 41±14 postleucine). Similarly, urinary creatinine excretion was no different during the preinfusion (1.06 ±0.06 g/day), infusion (1.32±0.12 g/day), and postinfusion (1.23±0.12 g/day) periods.

Glucose kinetics. To determine the mechanism of the rise in plasma glucose accompanying infusion of leucine during prolonged starvation, four 4-wk fasted subjects received [3-^{3}H]glucose before and for 6 h after leucine administration. Changes in plasma glucose, glucose production, glucose utilization, and glucose clearance are shown in Fig. 5. Plasma glucose (67±4 mg/100 ml preinfusion) increased within 30–60 min, reaching values 15–20 mg/100 ml above baseline levels (83±3 mg/100 ml at 6 h). The rise in plasma glucose occurred in the face of a small (15%) but significant decline in endogenous glucose production ($P < 0.01$). Total glucose utilization (76±9 mg/min preinfusion) fell by 15–25%, ($P < 0.025$), despite the elevation of plasma glucose concentration. Glucose clearance (133±14 ml/min preinfusion) consequently declined by 30% ($P < 0.001$) and remained suppressed throughout the period of leucine administration.

Leucine kinetics. Fig. 6 demonstrates the plateau plasma concentrations of leucine in two nonobese and one obese subject who received varying infusion doses of leucine. In each subject, there was a direct linear correlation between the leucine infusion rate and plasma leucine concentration ($r = 0.998$, 0.997, and 0.999, $P < 0.001$). Since these studies indicated that the leucine removal system was linear over the range of leucine concentrations observed in postabsorptive as well as fasted subjects, it was possible to calculate the MCR_L and PDR_L. The mean estimated MCR_L and PDR_L in nonobese, postabsorptive subjects was 265±11 ml/m^2 body surface area per min and 33.1±2.3 μmol/m^2 body surface area per min, respectively. These values correspond to a daily (24 h) basal PDR_L of 95.1±8.7 mmol/day.

The estimated MCR_L in postabsorptive obese subjects was reduced by 20% (212±14 ml/m^2 per min) as compared to the nonobese control group ($P < 0.02$). In contrast, estimated PDR_L values in the postabsorp-

TABLE II

*Plasma Concentrations of Amino Acids during Intravenous Infusion of Leucine in Fasting Obese Subjects**

Amino acid	Condition	0‡	1 h	2 h	3 h	4 h	6 h	9 h	12 h
						μM			
Leucine	3D‡	212±17	516±26**	588±36**	635±55**	700±53**	771±66**	784±63**	801±74**
	4W§	139±17	483±22**	596±21**	707±29**	772±38**	910±35**	926±68**	943±47**
Isoleucine	3D	107±10	99±10	79±9**	61±8**	54±6**	44±4**	40±1**	42±2**
	4W	76±9	67±9**	55±9**	40±6**	34±5**	26±4**	24±3**	26±5**
Valine	3D	365±24	354±35‖	305±30**	276±35**	253±30**	216±31**	187±20**	182±24**
	4W	181±14	169±14‖	145±11¶	123±12**	112±11**	95±7**	85±7**	82±6**
Taurine	3D	34±6	29±3	29±3	32±3	29±2	30±2	34±3	36±6
	4W	49±3	42±4**	42±3¶	42±3‖	40±3¶	40±3¶	41±4‖	40±3¶
Threonine	3D	117±7	114±10	97±8**	92±4¶	87±3¶	81±2‖	73±6**	70±5**
	4W	228±29	221±29	209±31**	200±28¶	204±30‖	193±30**	168±30**	164±24**
Serine	3D	97±14	91±14	81±13¶	77±10¶	74±10¶	69±10¶	64±9¶	63±7**
	4W	99±7	93±8	86±8**	80±6¶	80±9¶	73±7**	71±6¶	68±4**
Proline	3D	150±36	117±40	131±37¶	111±26	100±34‖	102±26‖	98±27‖	85±23‖
	4W	185±22	167±19‖	171±15	156±37	129±11‖	138±23**	136±18**	112±10¶
Citrulline	3D	15±6	12±5	12±4	12±5	14±4	16±5	12±5	13±6
	4W	23±6	27±8	28±8	29±4	21±4	33±11	25±5	25±5
Glycine	3D	188±15	174±20	148±21¶	170±19	146±18¶	147±16‖	137±17¶	137±12‖
	4W	306±39	290±45	260±41¶	275±42‖	271±43	279±45‖	268±46¶	265±34**
Alanine	3D	294±33	264±31‖	251±26‖	253±19	221±23**	210±17¶	200±20¶	189±24¶
	4W	162±9	153±11‖	140±10¶	131±9**	127±9**	134±8¶	123±9¶	124±7**
α-Aminobutyrate	3D	50±2	52±3	47±1	42±1¶	44±2	36±4‖	32±2**	26±1**
	4W	40±3	40±3	43±2	36±3‖	34±4¶	34±2‖	28±1¶	29±3¶
Cystine	3D	131±18	127±15	135±22	121±21	119±14	113±14	108±10	124±18
	4W	92±6	89±7	90±7	87±7	89±6	80±6¶	82±8	77±6‖
Methionine	3D	25±1	23±2	20±1**	17±1¶	17±1	16±1‖	16±1‖	16±1‖
	4W	25±1	23±1	23±3	18±2‖	17±2‖	12±2**	11±1**	15±1¶
Tyrosine	3D	63±4	58±4‖	52±3¶	47±2¶	43±1¶	41±2¶	39±2¶	42±1¶
	4W	51±3	49±3	37±2‖	34±3**	31±2**	28±2**	26±2**	27±2**
Phenylalanine	3D	61±4	57±4¶	51±3¶	48±2‖	43±1‖	45±1¶	45±2‖	49±1‖
	4W	48±3	43±2‖	33±1‖	28±3**	26±2**	25±2**	28±2**	28±3¶

* Data presented as mean±SE. Control values represent the mean of two observations on each subject preceding leucine administration. *P* values refer to significance of difference from preinfusion values (paired *t* test). Only values which differ significantly are indicated.

‡ Indicates studies after 3 days of fasting.

§ Indicates studies after prolonged fasting (4 wk).

‖ Indicates $P < 0.05$.

¶ Indicates $P < 0.01$.

** Indicates $P < 0.001$.

tive state were comparable in obese (34.6±6.6 μmol/m² per min) and nonobese subjects (33.1±2.3 μmol/m² per min, P = NS).

Changes in plasma leucine, estimated MCR_L and PDR_L during starvation are illustrated in Fig. 7. During prolonged fasting, plasma leucine (150±16 μM) initially rose as expected (12), to 212±17 μM at 3 days of starvation ($P < 0.05$). As fasting continued for 4 wk, plasma leucine, as expected (12), returned to levels comparable to the postabsorptive state 139±17 μM P = NS). In contrast, estimated MCR_L was reduced by 48% after 3 days of fasting (110±11 ml/m² per min, $P < 0.001$) and declined further to values 59% below postabsorptive levels (86±4 ml/m² per min, $P < 0.001$) when fasting was continued for 4 wk. Estimated PDR_L was not significantly altered after 3 days of starvation (24.5±3.6 vs. 34.6±6.6 μmol/m² per min in the postabsorptive state, $P > 0.1$) but was

reduced by 65% below postabsorptive levels (12.1±1.8 μmol/m^2 per min, $P < 0.01$) during prolonged (4 wk) starvation.

DISCUSSION

The current data demonstrate that infusions of exogenous leucine during brief and prolonged fasting in doses which raise plasma leucine levels to values comparable to those observed during protein feeding (7, 27), result in a decline in plasma concentration of other amino acids and a decrease in negative nitrogen balance. These changes in amino acid and nitrogen metabolism occurred in the absence of changes in plasma insulin, glucagon, or blood ketones. Furthermore, the reduction in net nitrogen loss was not accompanied by a decrease in urinary excretion of 3-methylhistidine. Excretion of 3-methylhistidine has been used as an index of muscle protein degradation since histidine is methylated only after its incorporation into protein and is not utilized for protein synthesis (20). These findings thus suggest that the decrease in circulating amino acids as well as the fall in net nitrogen loss are due at least in part to stimulation of muscle protein synthesis rather than inhibition of muscle protein catabolism by leucine. Of interest in this regard are recent observations demonstrating that leucine or a mixture of three branched chain amino acids increased [^{14}C]lysine and [^{14}C]acetate

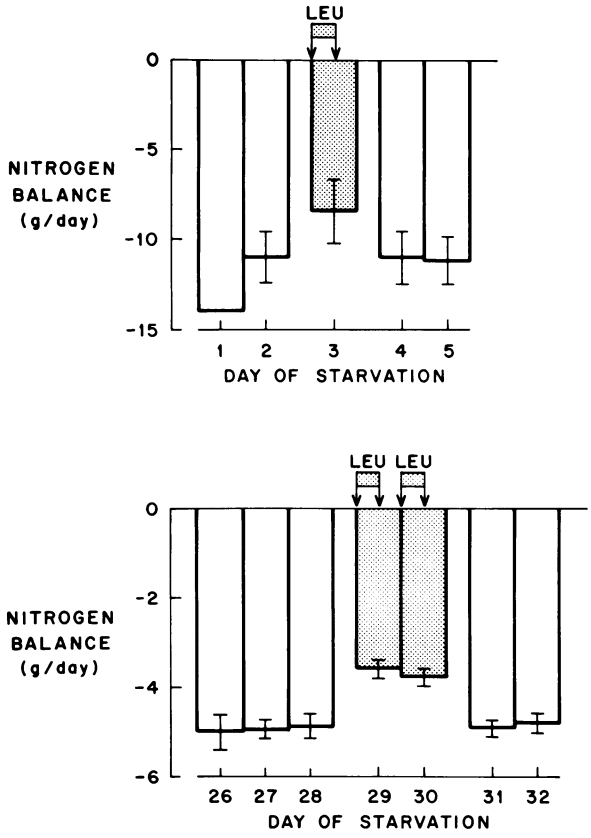

FIGURE 4 Effect of prolonged leucine infusion on daily nitrogen balance (mean±SE) in short-term and prolonged fasted subjects. Exogenous leucine was administered as 12-h infusions (9:00 a.m.–9:00 p.m.) on the 3rd day of starvation and on each of 2 consecutive days (29 and 30) during prolonged starvation. Negative nitrogen balance was significantly reduced by leucine infusion during short-term ($P < 0.02$) and prolonged ($P < 0.005$) starvation as compared to the preinfusion and postinfusion periods. Urinary 3-methylhistidine and creatinine excretion, however, was not altered by leucine administration.

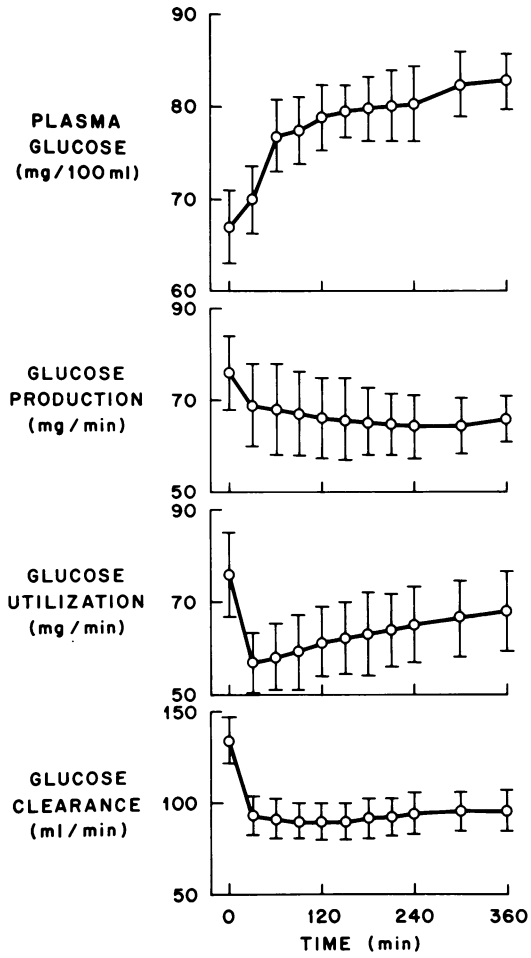

FIGURE 5 Effect of leucine infusion on plasma glucose concentration, endogenous glucose production, glucose utilization, and clearance in prolonged (4 wk) fasted subjects. Plasma glucose increased despite a 15% fall in glucose production ($P < 0.01$). In contrast, glucose utilization ($P < 0.025$) and glucose clearance ($P < 0.001$) declined, thereby accounting for the elevation in plasma glucose concentration. Plasma insulin and glucagon were unchanged throughout the study.

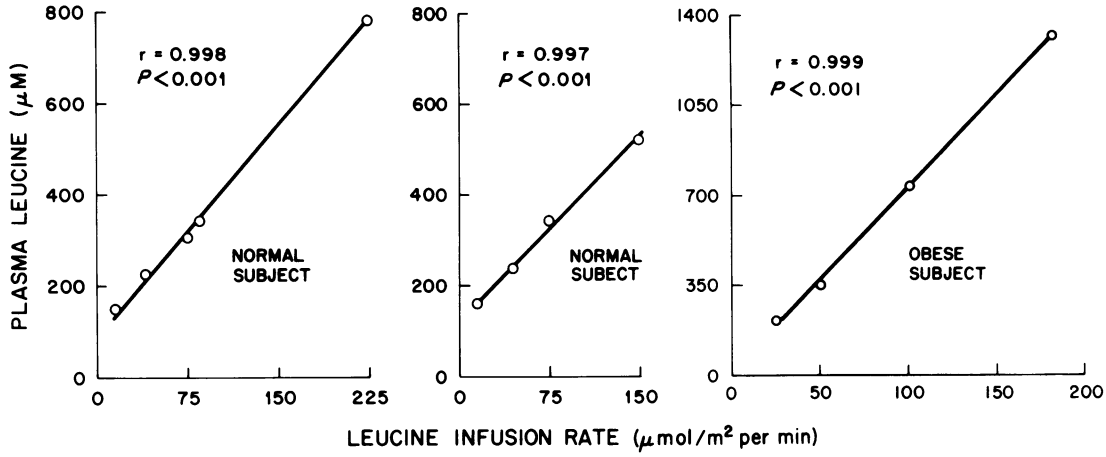

FIGURE 6 Changes in plateau plasma leucine concentration in relation to the leucine infusion rate in two nonobese and one obese subject who received varying infusion doses of exogenous leucine.

incorporation into diaphragm muscle from fed and fasted rats incubated with and without insulin (9). All other amino acids given singly or in combination had no stimulatory effect (8, 9). Furthermore, injection of leucine into fasted rats significantly increased muscle polysome content, suggesting stimulation of protein synthesis (10). The current findings are thus compatible with previous in vitro studies and suggest that leucine availability may be a regulatory factor in muscle protein synthesis in man. However, these studies do not exclude the possibility that other amino acids may have a similar protein-sparing effect.

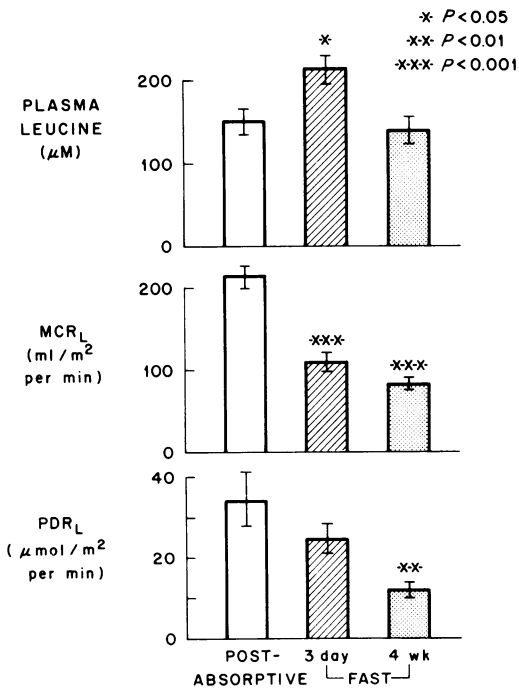

FIGURE 7 The effects of short-term (3 day) and prolonged (4 wk) fasting on plasma leucine concentration and the estimated MCR_L and the PDR_L of leucine in obese subjects. P values represent the significance of differences in plasma leucine, estimated MCR_L and PDR_L as compared to the postabsorptive state.

The current observations are also of interest with respect to the protein-sparing effect of isotonic mixtures of amino acids (28) and α-ketoanalogues of essential amino acids (29). The hyperaminoacidemia associated with the infusion of isotonic mixtures of amino acids involves two- to threefold increments in the plasma levels of only the branched chain amino acids (30). In addition, nitrogen conservation has been observed when the α-ketoanalogues of the branched chain amino acids were infused alone (11). It is of interest that the infusion rates of branched chain amino acids and of their α-ketoanalogues employed in those studies were similar to the doses employed in the current study (11, 28).

In addition to its effects on nitrogen and amino acid metabolism, leucine administration after prolonged (4 wk) starvation surprisingly induced a 15–20-mg/100 ml rise in plasma glucose (Fig. 3). In contrast, in postabsorptive and 3-day fasted subjects, equimolar infusions of leucine had a mild hypoglycemic effect. The rise in blood glucose observed in prolonged fasting could not be ascribed to utilization of leucine as a gluconeogenic substrate since leucine is not a precursor of glucose (31). The glucose turnover studies in fact indicate that the elevation in plasma glucose associated with leucine administration was not due to increased glucose production but was solely a consequence of reduced glucose utilization. Glucose clearance declined by 30% during the leucine infusion ($P < 0.001$) and glucose production fell by 15%

($P < 0.01$). This reduction in glucose output may be attributed to reduced substrate availability (total amino acids and alanine fell by 35 and 24%, respectively), a direct inhibitory effect by leucine on hepatic gluconeogenesis (32), or an undetected rise in portal insulin concentration. The fall in glucose utilization, on the other hand, suggests that leucine may decrease and/or replace glucose uptake as a fuel during prolonged starvation. The calculated initial reduction in glucose uptake (100–110 μmol/min) during leucine administration was in fact, similar to the leucine infusion dose. Of interest in this regard are recent data indicating that glucose oxidation by brain slices is reduced in vitro in the presence of 1.0 mM leucine (33).

The changes in nitrogen and glucose homeostasis induced by leucine infusion occurred in the absence of changes in insulin and glucagon concentration. Although previous studies have demonstrated that leucine has little effect on glucagon secretion (34), bolus injections and oral ingestion of this amino acid augment insulin secretion in man (35, 36). However, blood leucine levels achieved in postabsorptive subjects under those conditions markedly exceed those obtained with the continuous infusion doses employed in the current study (37). Although growth hormone and cortisol were not measured in this study, other reports have shown that increased levels of growth hormone or cortisol in prolonged starvation are without effect on total nitrogen balance (38, 39). Furthermore, augmented growth hormone secretion could not account for the rapid changes in glucose homeostasis observed in the current study (38).

Of particular interest were the effects of starvation on leucine turnover. Equivalent intravenous infusions of leucine resulted in greater increments in plasma leucine concentration during fasting than in the postabsorptive state. The estimated metabolic clearance rate of leucine was reduced by 48 and 59% after 3 days and 4 wk of starvation, respectively (Fig. 7).

It should be noted that the calculations employed in determining MCR_L assume partial suppression of endogenous leucine production during exogenous leucine administration. Changes in endogenously derived plasma leucine were estimated from alterations in plasma isoleucine concentration since the blood levels of these amino acids characteristically parallel each other under a variety of metabolic conditions (see Methods). However, even if this assumption is not valid, the effect of starvation on MCR_L would not be appreciably altered. If endogenous leucine production was unaffected by the leucine infusion, MCR_L 289±25 ml/m² per min in the postabsorptive state) remains significantly reduced after 3 days (134±16 ml/m² per min, $P < 0.005$) and 4 wk (96±6 ml/m² per min, $P < 0.001$) of starvation. On the other hand, if endogenous leucine production were completely suppressed by the infusion, a fall in MCR_L during fasting is still observed (postabsorptive 182±12, 3-day fasted 98±9 ($P < 0.001$), and 4-wk fasted 81±4 ml/m² per min, $P < 0.001$). Finally, even if one assumes that endogenous leucine production were totally suppressed by the infusion in the postabsorptive state but not in fasted subjects, the metabolic clearance rate (182±12 ml/m² per min in the postabsorptive state) declines significantly after 3 days (134±16, $P < 0.05$) and 4 wk (96±6, $P < 0.001$) of starvation.

Although the current study examines plasma leucine clearance in man, previous studies dealing with the influence of fasting on leucine catabolism have generally measured in vitro, leucine transport, and degradative enzymes in the rat. Branched chain amino acid transport and transamination have been reported as unchanged or increased by starvation in the rat (40–42). In addition, in vitro and in vivo studies indicate accelerated branched chain amino acid oxidation in starved rats (41, 43–45). Whether alterations in any of these processes can account for the observed decline in leucine clearance during starvation remains unestablished since there are no current data regarding the rate-limiting step in overall, total body disposal of branched chain amino acids in man.

With respect to the mechanism of reduced leucine turnover in starvation, recent studies have demonstrated decreased leucine clearance in diabetes and restoration of leucine clearance to normal by administration of insulin (46). A possible role for insulin in mediating the starvation induced alterations in leucine disposal is in keeping with the 25 and 50% decline in plasma insulin observed in 3-day and 4-wk fasted subjects, respectively. However it is unlikely that the marked reduction in leucine turnover observed in starvation can be accounted for solely on the basis of insulin deficiency inasmuch as starvation is associated with a twofold greater fall in estimated MCR_L than that observed in insulin-deficient diabetics (46).

The absence of changes in the estimated PDR_L during early starvation suggests that the elevations in plasma leucine induced by 3 days of starvation are a consequence of decreased amino acid removal rather than augmented tissue release. Studies of net balance of amino acids across human forearm have also failed to show a consistent change in leucine output after 3 days of fasting (12, 47). As starvation continued for 4 wk, PDR_L markedly declined (Fig. 7) thus accounting for the return of plasma leucine to base line, postabsorptive levels, despite the continued fall in estimated MCR_L. The fall in PDR_L with prolonged fasting is consistent with earlier data demonstrating decreased net release of branched chain amino acids from forearm tissues in prolonged (4–6 wk) starvation (12).

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