Differential Effects of Insulin Resistance on Leucine and Glucose Kinetics in Obesity

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The effects of insulin resistance on glucose and amino acid metabolism were studied in obese nondiabetic women (body mass index [BMI], (32.8 ± 2) and in lean controls. Glucose disposal rate, hepatic glucose production, and leucine carbon flux and oxidation were simultaneously measured during the postabsorptive state and during euglycemic hyperinsulinemia, by means of primed, constant infusions of D-[6,6-$^2H_2$]glucose and L-[1-^{13}C]leucine. Each subject participated in two insulin clamp studies on separate days, at infusion rates of 10 and 40 mU $(m^2 \cdot min)^{-1}$, producing plasma insulin levels of 20 to 25 and 70 to 80 μU/mL, respectively. Fat-free mass (FFM) was calculated from underwater weighing measurements. Insulin-mediated glucose disposal rate was significantly slower in the obese group: 2.05 ± 0.05 versus 3.84 ± 0.18 mg $(kg \cdot min)^{-1}$ in controls during the 10-mU insulin clamp, and 3.80 ± 0.23 versus 9.16 ± 0.47 mg $(kg \cdot min)^{-1}$ during the 40-mU clamp. The insulin-induced decrease in plasma levels of branched chain amino acids was also significantly blunted in the obese group. Baseline leucine flux was similar in lean and obese subjects (78 ± 3 and 71 ± 2 μmol $(kg \cdot h)^{-1}$, respectively), and its decline in response to insulin infusion was also comparable (8% and 10% during the 10-mU/m^2 clamp, and of 17% and 18% during the 40-mU/m^2 clamp in lean and obese, respectively). Basal leucine carbon oxidation (from [^{13}C]leucine and [^{13}C]αketoisocaproate [α-KIC] plasma enrichments) was also similar in lean and obese, and did not change significantly with insulin infusion. A significant correlation (R^2 = .93) was found in both groups between lean body mass and plasma levels of branched chain amino acids. These results indicate that the elevation in plasma branched chain amino acid levels commonly observed in obesity is not necessarily associated with an impaired insulin-mediated amino acid flux, and that other factors, such as body composition, may be also determinants of the hyperaminoacidemia of obesity.

THE ASSOCIATION of obesity with elevated plasma insulin levels has been known for over 20 years.[1] This hyperinsulinemia is usually explained as a compensatory response to insulin resistance, and is manifested by elevated postabsorptive plasma insulin levels, excessive insulin output during the glucose tolerance test, and impaired insulin-mediated glucose utilization during euglycemic clamp studies.[2-4] These manifestations may or may not be associated with impaired glucose tolerance as defined by the standard tests.[5]

Another well-known, although less constant, feature of obesity is hyperaminoacidemia. Elevated postabsorptive plasma levels of the branched chain amino acids and of tyrosine and phenylalanine have been found in a number of studies,[6-8] but not in others,[9,10] and are traditionally interpreted as an additional consequence of insulin resistance. In vitro studies show that insulin enhances cellular amino acid uptake,[11] and in vivo studies in humans demonstrate that insulin inhibits the outflow of amino acids from skeletal muscle that occurs in the postabsorptive state,[12] as well as the overall rate of protein breakdown.[13] Furthermore, the hyperaminoacidemia of obesity resembles that seen in non–insulin-dependent diabetics,[14] supporting the hypothesis of a common mechanism related to impaired insulin action. However, no consistent correlation has been observed between the degree of insulin resistance and the elevation in plasma amino acid levels, and no study has explored the relative impairments of glucose and amino acid metabolism in insulin-resistant obese individuals. The present study used the euglycemic insulin clamp technique and the infusions of labeled leucine to examine glucose and leucine kinetics in response to steady-state hyperinsulinemia in obese and lean control women.

MATERIALS AND METHODS

Subjects

Twelve female volunteers were studied. The obese group consisted of six premenopausal women, who had been overweight for at least 4 years. They had not undergone weight reduction for at least 5 months preceding the study, and were using no medication. They had no history of diabetes or glucose intolerance. The lean control group included six healthy women with no history of excess weight or diabetes, and using no medications. All subjects were screened by a physical examination, and by laboratory tests that included routine blood chemistries, urinalysis, plasma lipoprotein profile, and liver and thyroid function tests. The pre-admission workup included, on a separate day, an oral glucose tolerance test,[5] using a dose of 40 g of glucose/m^2 of body surface area. The study protocol was approved by the Committee on the Use of Humans as Experimental Subjects of MIT and by the Medical Advisory Committee of the MIT Clinical Research Center. All participants gave informed, written consent before entering the study.

Experimental Design

The 5-week protocol consisted of two infusion studies separated by 4 weeks. Each infusion was preceded by a 3-day period of controlled dietary intake. During these days, subjects were admit-

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ted to the MIT Clinical Research Center, and consumed all their meals prepared by the CRC metabolic kitchen. These diets provided 1.25 g of protein/kg per day, with 55% of the calories as carbohydrate. All infusion studies were performed during the luteal phase of the menstrual cycle.

Infusion Studies

The infusion protocol, summarized in Fig 1, was started early in the morning after an overnight fast. An 8-in indwelling catheter inserted into a forearm vein was used for all infusions; a second line placed in a retrograde fashion into a dorsal hand vein was used for blood sampling. The hand was placed in a warming box kept at 66°F throughout the study in order to "arterialize" blood.[15] After baseline blood and breath air samples were taken, priming doses of $NaH^{13}CO_3$ (0.09 mg/kg), L-[1-^{13}C]leucine (4.5 μmol/kg) and D-[6,6-$^2H_2$]glucose (22.2 μmol/kg) were given over a period of 2 minutes. Two constant infusions were then started, one of the leucine tracer, at 0.05 μmol $(kg \cdot min)^{-1}$ and another of the glucose tracer, at 0.28 μmol $(kg \cdot min)^{-1}$. These infusions were administered using screw-type infusion pumps (Harvard Apparatus, South Natick, MA), and maintained for the 360 minutes of the study.

Insulin Clamp

After 180 minutes of isotope infusion, an euglycemic insulin clamp was started.[16] This procedure consisted of a primed, constant insulin infusion (Venosulin, Nordisk, Bethesda, MD) at a rate of 10 or 40 mU $(m^2 \cdot min)^{-1}$. A 20% dextrose solution was infused at a variable rate, based on 5-minute measurements of blood glucose, in order to maintain blood glucose levels within 10% of baseline. Both insulin and glucose infusions were administered using high-precision Harvard pumps modified by the addition of an external, 1,000-step controller (Devices for Medicine, Fairfax, VA).

Sampling

During the last hour of the 3-hour baseline period, five simultaneous blood and breath samples were obtained, for measurement of isotopic enrichments and substrate concentrations. Similarly, blood and breath samples were taken during the last hour of the insulin clamp period. Total CO_2 production was measured by open circuit indirect calorimetry during two 30-minute periods, one during the baseline period and another during the last hour of the insulin clamp.

On a separate day, body composition was measured by underwater weighing. The procedure was performed early in the morning,

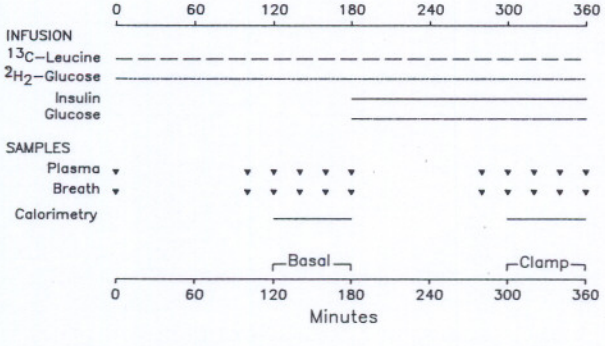

Fig 1. Schedule of the infusion studies. The "basal" period was defined as the 60-minute period from 120 to 180 minutes of the infusion. The euglycemic clamp was started at 180 minutes, immediately after drawing the last sample of the basal period. The "clamp" period was defined as minutes 300 to 360 of the infusion, when insulin-dependent parameters achieved a steady-state.

in the fasting state. Subjects were weighed four times while under water and breathing through a snorkel. The snorkel was connected to a rebreathing bag filled with 100% oxygen, and gas concentration was used to calculate residual lung volume. Percent fat was calculated from total body density.

Isotopes ($NaH^{13}CO_3$ 99% ^{13}C, L-[1-^{13}C]leucine 99% ^{13}C, and D-[6,6-$^2H_2$] glucose 98% atom percent excess [APE]) were purchased in crystalline form from Tracer Technologies, Somerville, MA, and tested for purity, sterility, and nonpyrogenicity by independent laboratories. Infusates were prepared 1 hour before each infusion study, from stock solutions mixed the day before.

Analytical Methods

Plasma amino acids other than tryptophan were determined by ion exchange high-performance liquid chromotography (HPLC); tryptophan was measured fluorometrically using the method of Denckla and Dewey.[17] Plasma insulin and C-peptide levels were determined by a double antibody radioimmunoassay method (IncStar, Stillwater, MN). The interassay coefficient of variation was 8%.

Plasma [1-^{13}C]leucine and [^{13}C]αketoisocaproate (α-KIC) enrichments were determined by chemical ionization selected ion monitoring, in a Hewlett Packard Model 5790 GC-MS instrument (Palo Alto, CA). *N*-heptafluorobutyryl isobutyl ester derivatives of leucine and quinoxalinol-trimethyl silyl derivatives of α-KIC were prepared as previously described.[18] Plasma samples for [6,6-$^2H_2$]glucose determinations were prepared as pentaacetate derivatives[19] and analyzed by electron impact ionization.

^{13}C enrichment in breath air CO_2 was measured by isotope ratio mass spectrometry after double cryogenic extraction, using a VG Isogas Sira 10 instrument. Results are expressed as atom percent excess relative to postabsorptive baseline.

Plasma glucose was measured by the glucose oxidase method, using a Beckman Glucolyzer (Beckman Instruments, Fullerton, CA).

Calculations

Glucose disposal rate was calculated using Steele's equation for non–steady-state conditions.[20] This equation is based on a monocompartmental, constant volume model; some of its assumptions have recently been criticized based on its ability to produce physiologically uninterpretable negative values for hepatic glucose output.[21,22] Nevertheless, this equation appeared adequate for the type of matched comparisons made in the present study. Our calculations produced a slightly negative value of −1.016 in only one case. As suggested by some investigators, negative values with the Steele equation appear to be more common at higher rates of insulin infusion.[23]

Insulin clearance rate was calculated as: $I_c = I_i/(I_p - I_b)$, where I_i is the insulin infusion rate [μU $(m^2 \cdot min)^{-1}$], I_p is the mean plasma insulin concentration during the last hour of the clamp period, and I_b is the baseline plasma insulin level.

Leucine flux was calculated from the tracer infusion rate and the mean plasma isotopic enrichment during the basal and clamp 1-hour periods.[24] Leucine oxidation was calculated using data on total CO_2 production and breath $^{13}CO_2$ enrichments measured during these same periods. The $^{13}CO_2$ enrichment in breath air caused by the natural abundance of [^{13}C]glucose in the dextrose solution used during the insulin clamp was measured in preliminary studies. Two subjects underwent euglycemic hyperinsulinemic clamps at 10 and 40 mU $(m^2 \cdot min)^{-1}$, without infusion of [1-^{13}C]leucine, and breath $^{13}CO_2$ enrichments were measured at frequent intervals. At the higher insulin infusion rate, and with glucose infusions above 5 mg $(kg \cdot min)^{-1}$, breath $^{13}CO_2$ enrich-

ments were 0.0015 and 0.0016 APE. No significant isotopic enrichments above background were detected with dextrose infusion rates below 5 mg $(kg \cdot min)^{-1}$. Thus, a correction for natural ^{13}C abundance was introduced only when the infusion rate during the actual studies was higher than 5 mg $(kg \cdot min)^{-1}$, which occurred in all lean controls and in one obese during the 40-mU/m^2 clamp level. A number of studies at our center, using the same batch of dextrose solution, have obtained comparable natural ^{13}C enrichments.[25,26] Our correction assumes that, at the same rate of glucose disposal (and thus of dextrose infusion), the glucose oxidative fraction is similar in lean and obese, as demonstrated in previous studies.[27] In addition, we measured the percent recovery of ^{13}C from administered $Na^{13}CO_3$ in eight of the 12 subjects (five controls and three obese). Mean recoveries were 74% in lean subjects and 72% in the obese. A single recovery value of 75% was used for all calculations. Previous studies in our center have shown that the insulin infusion itself has no effect on the fractional ^{13}C recovery.[26]

Leucine carbon flux was also estimated using plasma α-KIC isotopic enrichment, which is presumably a better indicator on the intracellular leucine pool.[28] Data on leucine kinetics are expressed per kilogram of fat-free mass (FFM), assuming that this body compartment better reflects the size of the tissue pool where leucine is primarily metabolized.[29]

Statistical Analysis

Glucose and amino acid responses to insulin in each group were compared by two-way ANOVA. Baseline comparisons were done using a two-tailed *t* test. Linear regression was used to correlate some variables as described below. Data are expressed as mean ± SEM, and statistical significance was assessed at the 95% confidence interval. The CLINFO database system and the SAS statistical package were used.

RESULTS

Relevant characteristics of the two subject groups are shown in Table 1. Significant differences between groups were observed in body weight, percent fat, and FFM. The mean age of the obese group was also significantly different from that of lean controls, but, as mentioned above, all of the women were premenopausal and undergoing regular menses. All subjects remained healthy throughout the study. The mean difference in body weight between the first and second infusion was 0.4 kg in the obese and 0.08 kg in the lean group.

Table 1. Demographic and Body Composition Data of the Groups Studied

	Lean	Obese
Age (yr)	22 ± 1	37 ± 2*
Weight (kg)	56.2 ± 2.0	87.4 ± 7.0*
Height (cm)	162.6 ± 2.7	162.8 ± 2.7
Surface area (m^2)	1.60 ± 0.04	1.92 ± 0.08*
Desirable weight (kg)†	57.2 ± 1.9	57.3 ± 1.9
Body mass index	21.2 ± 0.4	32.8 ± 2.0*
Obesity index	0.99 ± 0.02	1.52 ± 0.09*
FFM (kg)‡	42.6 ± 1.1	51.1 ± 3.3*
Body fat (%)	24 ± 1	41 ± 1*

NOTE. Values are mean ± SEM, six subjects per group.

*$P < .05$.

†Desirable weight based on medium size body frame, Metropolitan Life Insurance tables, 1959.

‡From hydrostatic weighing.

Table 2. Plasma Insulin and C-Peptide Levels During the Basal (120 to 180 Minutes) and Clamp (300 to 360 Minutes) Periods of the Two Insulin Clamp Studies

	Basal	10 mU	40 mU
Insulin (μU/mL)			
Lean	6 ± 0.5	20 ± 0.8[b]	70 ± 1.7[c]
Obese	14 ± 1.3[a]	24 ± 1.8[b]	80 ± 2.9[d]
C-peptide (ng/mL)			
Lean	1.21 ± 0.1	0.73 ± 0.07[b]	0.63 ± 0.04[b]
Obese	1.94 ± 0.22[a]	1.25 ± 0.05[c]	1.05 ± 0.09[d]

NOTE. Within each variable, groups not sharing the same superscript letter are significantly different, $P < .05$.

Postabsorptive plasma insulin and C-peptide levels were significantly higher in the obese (Table 2). All subjects had normal fasting blood glucose and oral glucose tolerance tests (OGTT), but the obese group attained a significantly higher plasma insulin peak during the OGTT (89 ± 9 μU/mL, compared with 45 ± 12 μU/mL in controls). Likewise, peak plasma C-peptide levels during the OGTT were higher in the obese (9.02 ng/mL *v* 4.59 ng/mL).

Postabsorptive plasma levels of the large neutral amino acids valine, leucine, isoleucine, phenylalanine, and tyrosine were significantly higher in the obese (Table 3).

Glucose Metabolism

Blood glucose was maintained within 6% or less of baseline values in all insulin clamp studies. During the steady-state period (300 to 360 minutes) of the 10-mU $(m^2 \cdot min)^{-1}$ insulin clamp, mean plasma glucose was 87.2 ± 0.3 in lean and 89.7 ± 0.3 mg/dL in the obese subjects. At similar period of the 40-mU $(m^2 \cdot min)^{-1}$ clamp, values were 86.3 ± 0.7 and 87.0 ± 0.8 mg/dL in lean and obese, respectively. Plasma insulin levels at steady-state during the 10-mU clamp were 20 ± 0.8 μU/mL in lean and 24 ± 1.8 μU/mL in the obese. The 40-mU clamp increased plasma insulin to a steady-state level of 70 ± 1.7 and 80 ± 2.9 μU/mL in lean and obese, respectively (Table 2). Insulin clearance rate was similar in both groups, and was significantly lower during the 40-mU clamp [(mean of 602 mL $(m^2 \cdot min)^{-1}$)] than during the 10-mU clamp [(831 mL $(m^2 \cdot min)^{-1}$].

The glucose infusion rate required to achieve euglycemia was significantly lower in the obese group. During the last hour of the 10-mU clamp, the mean glucose infusion rate was 1.76 ± 0.2 and 2.7 ± 0.15 mg $(kg \cdot min)^{-1}$ in obese and lean, respectively. During the 40-mU clamp, respective values were 4.21 ± 0.46 and 9.35 ± 0.52 mg $(kg \cdot min)^{-1}$. Glucose disposal rate was also significantly lower in the obese, both in the baseline period and during the plateau phases of 10- and 40-mU insulin clamps (Fig 2). Endogenous glucose production was suppressed by 62% in the lean subjects and by 51% in the obese during the 10-mU clamp. The 40-mU clamp completely suppressed endogenous glucose output in both groups. Other estimates of glucose utilization not shown, such as glucose metabolic rate and

Table 3. Postabsorptive Plasma Levels of Large Neutral Amino Acids

	Lean	Obese
Valine	187.3 ± 5.5	259.0 ± 15.4*
Leucine	101.0 ± 3.7	130.3 ± 10.4*
Isoleucine	51.4 ± 1.6	71.4 ± 3.8*
Phenylalanine	51.6 ± 3.2	60.3 ± 2.2*
Tyrosine	47.3 ± 6.9	70.1 ± 5.7*
Tryptophan	48.6 ± 1.1	44.1 ± 2.1
Methionine	21.4 ± 1.2	23.2 ± 1.4
Trp/LNAA ratio†	0.112 ± 0.006	0.074 ± 0.004*

NOTE. Values are μmol/L, mean ± SEM.

*Groups differ, Student's *t* test, $P < .05$.

†Plasma Trp divided by the summed concentration of valine, leucine, isoleucine, phenylalanine and tryosine.

the glucose disposal/plasma insulin ratio, were also consistent with the impaired insulin-mediated glucose utilization in the obese.

Amino Acid Metabolism

Plasma branched chain amino acid levels declined significantly in response to insulin infusion. This decrease was seen in both subject groups, but it was less marked in the obese (Table 4). There was a less pronounced but still statistically significant insulin-mediated decline in the plasma levels of aromatic amino acids. For most amino acids, values obtained from arterialized blood at 120 to 180 minutes (baseline infusion period) were lower than those obtained early in the morning from venous blood. Thus, comparisons of the insulin-induced changes in plasma amino acid levels were made between values obtained from arterialized blood at the 120 to 180 and 300 to 360 infusion periods (Table 4).

Leucine flux in the postabsorptive state was slightly lower in the obese, but this difference disappeared when values were expressed per kilogram of lean body mass (Fig 3). The insulin clamp produced a significant decline in leucine flux in both groups, but had no effect on leucine oxidation. Estimates of leucine carbon flux using plasma α-KIC isotopic enrichments[28] showed a response pattern similar to that provided by the leucine enrichment data. The plasma α-KIC/leucine enrichment ratio remained constant during the baseline and insulin clamp periods, with no significant differences between lean and obese (Fig 4).

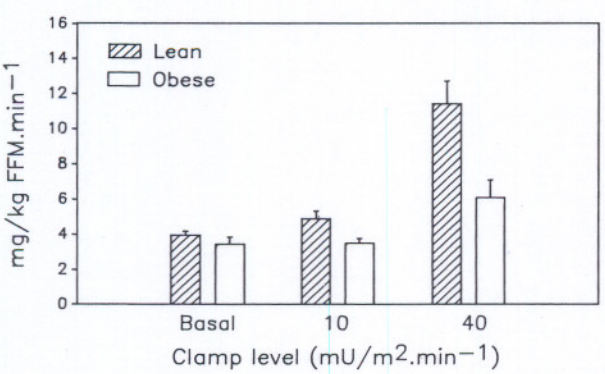

Fig 2. Glucose disposal rate during the 10- and 40-mU $(m^2 \cdot min)^{-1}$ insulin clamp periods, expressed per kilogram of FFM. Mean values for lean and obese differ at basal and at both clamp levels, $P < .05$. Basal and clamp periods as defined in Fig 1.

DISCUSSION

The present study compared the effects of insulin resistance on parameters of glucose and amino acid metabolism in nondiabetic women with moderate obesity. Our results show that the insulin resistance of obesity, although causing a significant impairment in glucose disposal rate, does not affect leucine flux or oxidation under conditions of euglycemic hyperinsulinemia.

Since insulin administration lowers plasma amino acid concentrations in normal individuals,[30] the hyperaminoacidemia of obesity has been interpreted as another manifestation of the insulin resistance associated with obesity. A decreased insulin response would blunt the insulin-mediated amino acid uptake into peripheral tissues, and amino acids would therefore accumulate in the plasma compartment. Indeed, the amino acids usually elevated in obesity are those whose plasma levels are more sensitive to insulin: the branched chain amino acids, and, to a lesser extent, tyrosine and phenylalanine. Conversely, plasma tryptophan levels change very little in response to insulin, presumably because of the competitive effect of its binding to albumin.[31]

Table 4. Mean Plasma Amino Acid Levels During the Basal (120 to 180 Minutes) and Clamp (300 to 360 Minutes) Periods of the Insulin Clamp Studies

	Basal	10 mU	40 mU
Valine			
Lean	175 ± 7.6	136 ± 6.0[b]	107 ± 4.3[d]
Obese	211 ± 15.0[a]	172 ± 11[c]	149 ± 13.7[e]
Leucine			
Lean	117 ± 4.2	88 ± 5.4[b]	56 ± 4.9[d]
Obese	131 ± 7.4[a]	104 ± 4.0[c]	77 ± 6.1[e]
Isoleucine			
Lean	54 ± 3.1	34 ± 2.9[b]	18 ± 3.8[d]
Obese	63 ± 5.8[a]	42 ± 3.6[c]	30 ± 4.3[e]
Phenylalanine			
Lean	48 ± 4.2	41 ± 3.3[b]	37 ± 3.9[b]
Obese	55 ± 2.9[a]	48 ± 4.1[c]	41 ± 7.4[d]
Tyrosine			
Lean	41 ± 2.7	33 ± 3.3[b]	26 ± 3.6[d]
Obese	55 ± 3.2[a]	44 ± 3.7[c]	36 ± 4.2[e]
Tryptophan			
Lean	43 ± 2.7	42 ± 1.8	35 ± 3.1[a]
Obese	39 ± 1.6	40 ± 2.3	34 ± 3.7[a]
Methionine			
Lean	19 ± 2.0	15 ± 1.6[a]	12 ± 2.3[b]
Obese	17 ± 1.8	14 ± 2.1[a]	11 ± 3.0[b]
Trp/LNAA			
Lean	0.098 ± 0.005	0.129 ± 0.004[b]	0.142 ± 0.007[d]
Obese	0.080 ± 0.006[a]	0.102 ± 0.004[c]	0.104 ± 0.005[c]

NOTE. Values are in μmol/L, mean ± SEM of four separate measurements from "arterialized" blood samples. Data from basal period represent the average of two separate measurements. Within each variable, values not sharing the same superscript letter are significantly different, two-way ANOVA ($P < .05$).

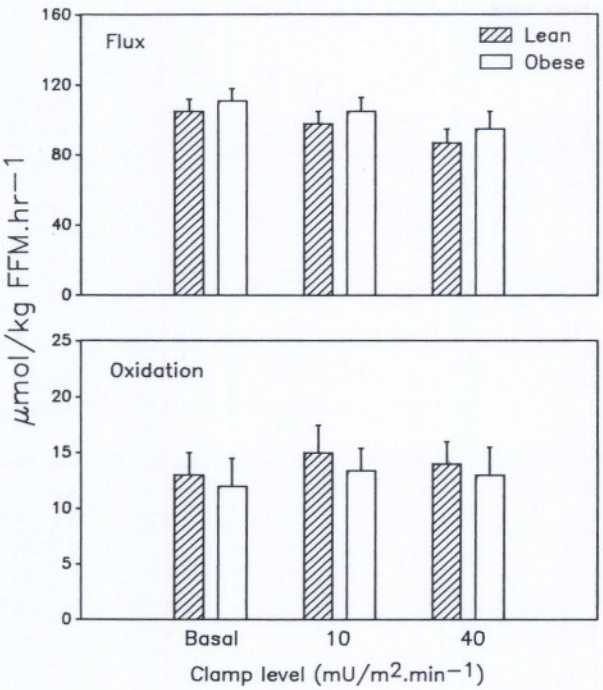

Fig 3. Leucine flux and oxidation during the 10- and 40-mU $(m^2 \cdot min)^{-1}$ clamp periods. Flux and oxidation calculated using the mean of four plasma and breath isotopic enrichment over 1-hour periods, as described in Fig 1. There are no statistically significant differences between mean values of lean and obese groups.

Several studies have described plasma amino acid concentrations in nondiabetic obese.[6,8-10,14,32-34] In some, postabsorptive plasma large neutral amino acid levels were found to be significantly elevated in the obese compared with those in lean controls.[6,8,14] But in others, postabsorptive levels[9,10,34] and the decrease in branched chain amino acids during the OGTT were found to be normal.[34] A study measuring the decline in branched chain amino acid levels during a constant insulin infusion found a blunted response in the obese.[9] In another study that reported normal plasma valine clearance in the obese, a 4-g dose of valine was infused over 5 minutes, increasing the plasma concentration of this amino acid to over 2,000 μmol/L, and thus making uncertain the implications of this finding for the disposal of physiological amino acid concentrations.[33] In part, these differences may be related to the heterogeneity of the obese groups studied with respect to the magnitude of the weight excess and the gender of the subjects. Our study found significant elevations of postabsorptive plasma levels of the branched chain amino acids and of phenylalanine and tyrosine in the obese, as well as a blunted response to euglycemic hyperinsulinemia (Table 4).

The reported correlations between plasma amino acid levels and insulin concentrations have also been inconsistent. Some epidemiological data suggest a weak correlation between obesity, amino acid and insulin levels.[35] The study of Felig et al[6] also reported a significant correlation between plasma insulin level and the concentration of each of the amino acids found elevated in the obese: valine, leucine, isoleucine, tyrosine, and phenylalanine. However, such correlations were not found in other studies,[8,9,34] including the one reported here.

We combined a labeled leucine infusion with the euglycemic insulin clamp technique to assess simultaneously the insulin-dependent changes in leucine and glucose kinetics. Our data show that obese women with clear impairment in insulin-dependent glucose disposal nevertheless show a similar decline in plasma leucine flux when compared with lean controls. The mean basal leucine flux in our obese group, 70 ± 2 μmol/kg · h, was similar to that reported by Staten et al in a group of six obese women.[36] Another study reported higher leucine fluxes in five obese women compared with five lean men,[37] raising the possibility of sex-related differences.

Simultaneous comparisons of insulin's actions on glucose and amino acid kinetics have been made for normal men[26,38] and elderly subjects,[39] but not previously, to our knowledge, in obese subjects. Previous studies on amino acid kinetics in obesity[40-43] have focused on the effects of weight reduction diets on whole body protein turnover, whereas our goal was to compare the relative effects of insulin resistance on glucose and amino acid kinetics in obese subjects consuming a stable dietary intake. Data from studies measuring glucose and amino acid kinetics during euglycemic hyperinsulinemia in healthy men show that the plasma insulin concentration needed to produce half-maximal decline in plasma large neutral amino acid levels is similar to that needed for half-maximal glucose disposal,[26] suggesting that a decreased responsiveness to insulin could conceivably affect both substrates to a similar degree. However, our finding of a differential effect of insulin resistance on

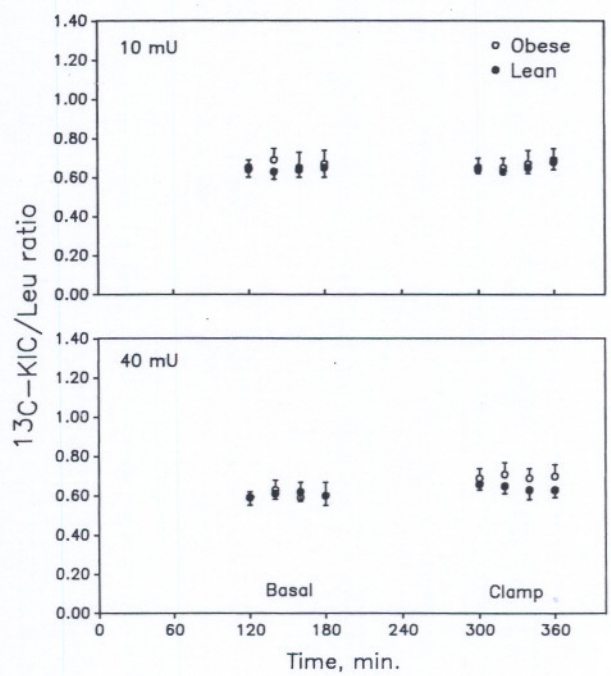

Fig 4. Plasma enrichment ratio of [1-^{13}C]-α-KIC to [1-^{13}C]leucine during the basal and clamp periods.

glucose and amino acid metabolism is consistent with similar findings in type II diabetics[36] and in the elderly.[39]

Postabsorptive leucine oxidation in obese subjects was similar to lean controls, a finding consistent with previous reports.[37] The lack of effect of hyperinsulinemia on leucine oxidation in both lean and obese groups is at variance with some studies,[44] but a similar lack of response has been reported in healthy men[26] and in the elderly.[39] A study measuring [^{13}C]leucine oxidation after a 150-g oral glucose dose reported no change in the mean leucine oxidation of three male and three female obese subjects.[45] That response was obtained under non–steady-state conditions of significantly higher plasma glucose and insulin levels in the obese group, whereas in our study leucine kinetics were measured while both plasma glucose and insulin were kept at similar levels in lean and obese. Although the study of Nair et al did not report the changes in other glucoregulatory hormones, it is likely that the changes in leucine kinetics measured under those conditions reflect the combined effect of several hormones.

There are issues related to the insulin clamp and tracer infusion techniques that should be considered when comparing glucose and amino acid kinetics as measured in the present study. First, during the euglycemic clamp glucose-consuming cells are offered unlimited amounts of glucose from exogenous sources, and it is under these conditions that their response to insulin is measured. Conversely, the insulin-induced amino acid uptake during the euglycemic clamp would be measured under conditions of limited amino acid availability, since no exogenous amino acids are given. But data obtained in normal men receiving amino acid infusions during clamp studies suggest that substrate availability and insulin have synergistic but distinct effects on protein turnover, the former stimulating mainly net protein synthesis, and the latter acting primarily by inhibiting protein breakdown.[44,46] A second caveat in our measurements may stem from the reported substrate competition between glucose and amino acids.[47,48] It is possible that the decreased glucose uptake of the obese subjects allows for an enhanced amino acid uptake, relative to controls. However, we found no correlation between glucose disposal rate and leucine flux in either group.

The Role of Body Composition

Studies on whole body glucose metabolism in humans underscore the quantitative importance of body composition. The data support the notion that most of the insulin-induced changes in glucose disposal rate observed during euglycemic clamp studies are due to changes in skeletal muscle glucose uptake. Similarly, studies measuring the incorporation of [1-^{13}C]leucine into muscle proteins during systemic [1-^{13}C]leucine infusion show that feeding increases protein synthesis by 100% in skeletal muscle, but by only 40% in the rest of the body.[29] In our study, leucine flux and oxidation were similar in lean and obese subjects when expressed per kilogram of FFM (Fig 3).

Fat distribution in the obese individual has also been shown to correlate with some of the metabolic derangements of obesity. Glucose intolerance, hypertension, diabetes, and hyperlipidemia have been shown to be more prevalent in obese subjects with upper-body adiposity.[49-51] Although we did not set a waist-hip ratio threshold for our admission criteria, all our obese subjects were, by clinical impression, of the lower adiposity type, and all had also normal glucose tolerance test and blood pressure.

Whether the size of the lean mass compartment also affects plasma amino acid levels is unclear. A study by Holm et al[52] found a significant correlation between lean body mass and the summed plasma concentration of valine, leucine, isoleucine, phenylalanine, and tyrosine, the amino acids usually elevated in obesity. This correlation between lean mass and plasma amino acids was maintained even after a period of intensive physical training, which lowered plasma insulin levels in the overweight group, showing that the plasma amino acid levels followed more closely the changes in lean body mass than plasma insulin. In our study, the correlation between branched chain amino acids and FFM had a R^2 of .933 (Fig 5). Stepwise regression analysis using phenylalanine, tyrosine, and tryptophan, along with each of the branched chain amino acids, demonstrated that the correlation was primarily determined by the latter amino acid group. The data in Fig 5 suggest that obese persons with lower FFM relative to total excess weight may have plasma branched chain amino acid levels within the normal range, in spite of having elevated plasma insulin levels and markedly impaired glucose disposal rates during clamp studies. These data also emphasize the dissociation between plasma amino acid levels and insulin resistance as measured by glucose disposal. For example, the mean basal glucose disposal rate of the three obese women with higher branched chain amino acid levels was 1.92 mg (kg · min)$^{-1}$, very similar to that of the three women with the lower branched chain levels: 1.77 mg (kg · min)$^{-1}$. These findings may help explain some of the discrepancies among studies of plasma amino acid levels in obesity. Further, they suggest that the increase in lean body mass associated with obesity (which can comprise as much as 40% of the excess weight), as well as dietary regimens

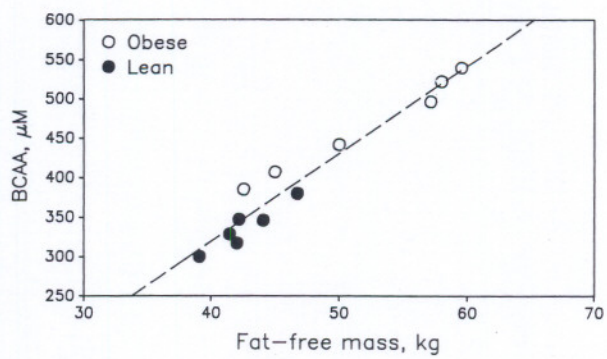

Fig 5. Correlation between the summed concentration of the branched chain amino acids valine, leucine, and isoleucine (BCAA) and the amount of fat-free mass determined by underwater weighing. The coefficient of determination (R^2) was .933.

designed to preserve lean mass, can be important factors in determining the hyperaminoacidemia of obesity.

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