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Insulin resistance of muscle protein metabolism in aging

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Abstract

A reduced response of older skeletal muscle to anabolic stimuli may contribute to the development of sarcopenia. We hypothesized that muscle proteins are resistant to the anabolic action of insulin in the elderly. We examined the effects of hyperinsulinemia on muscle protein metabolism in young (25±2 year) and older (68±1 year) healthy subjects using stable isotope tracer techniques. Leg blood flow was higher in the young at baseline and increased during hyperinsulinemia, whereas it did not change in the elderly. Glucose concentrations and muscle uptake were not different between groups at baseline and during hyperinsulinemia. Leg phenylalanine net balance was not different at baseline and significantly increased in both groups with hyperinsulinemia ($P<0.05$) but to a greater extent in the young ($P<0.05$). Muscle protein synthesis increased only in the young during hyperinsulinemia. Muscle protein breakdown did not significantly change in either group, although it tended to decrease in the elderly. Changes in muscle protein synthesis were correlated with changes in leg amino acid delivery ($R=0.89$; $P=0.0001$) and blood flow ($R=0.90$; $P<0.0001$). In conclusion, skeletal muscle protein synthesis is resistant to the anabolic action of insulin in older subjects, which may be an important contributor to the development of sarcopenia.

Keywords

skeletal muscle; hyperinsulinemia; leg blood flow; phenylalanine

Aging is associated with a progressive loss of physical independence, which significantly worsens the quality of life and increases morbidity and mortality. A fundamental cause of and contributor to disability in older people is the involuntary loss of muscle mass and strength (sarcopenia), which eventually reduces function (1-3), thus increasing the risk of falls and vulnerability to injury (4,5). Sarcopenia is most likely a multifactorial disorder (1,6), and recent evidence suggests that a decreased response of muscle protein synthesis to anabolic stimuli may be involved (7-9).

Previous studies had suggested that sarcopenia may be due to a reduced basal rate of muscle protein synthesis (10-14), but we have recently reported in the largest cohort of healthy men to date that despite a decline in muscle mass, basal rates of muscle protein synthesis and net

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balance are not reduced with healthy aging (15). We have also found that aging is not associated with a reduced anabolic response of muscle to amino acids, as they stimulate muscle protein synthesis in the elderly to the same extent as in the young regardless of oral or intravenous administration route (16-20). However, we have recently shown that whereas the addition of carbohydrate to an amino acid meal enhanced the amino acid stimulation of muscle protein synthesis in young subjects (7,21), in older subjects such a combination did not have any additive effect on muscle protein synthesis (7). On the contrary, it blunted the amino acid-induced increase in muscle protein synthesis, thereby blunting the anabolic effect of the meal on muscle proteins (7). Our data have been recently confirmed by another group mimicking the prandial state using the systemic hyperinsulinemic-euglycemic clamp technique in the presence of increased amino acid concentrations (8). These results were intriguing, particularly because the older subjects were healthy, had a normal glucose tolerance, and their whole body glucose turnover, muscle glucose uptake and insulin concentrations were similar to the younger control group (7).

The two major variables introduced with the addition of carbohydrate to the oral amino acid meal that could have affected our previous muscle protein turnover data (7) were increased energy (carbohydrate) and increased insulin (endogenous secretion). Muscle protein synthesis is an energy-consuming process, requiring ~0.7 kcal/g of protein synthesized (~240 kcal per day for an average person) (22). Consequently, if additional energy had any influence on muscle protein synthesis, such an effect should have been positive rather than negative. Insulin is a potent anabolic stimulus for muscle proteins (23-33), and a number of studies have reported that hyperinsulinemia can increase muscle protein synthesis, particularly when muscle amino acid availability is increased (8,23,24,30-33).

Therefore, we hypothesized that muscle protein synthesis in the elderly is resistant to the anabolic action of insulin, and that this effect of aging is independent of a normal glucose tolerance. To test this hypothesis, we exposed the muscles of one leg to hyperinsulinemia by infusing insulin in the femoral artery, with a euglycemic clamp as necessary and measured muscle amino acid and protein turnover with stable isotope methodologies in older and younger healthy, glucose-tolerant volunteers. This design allowed us to expose the leg muscles to postprandial levels of insulin, while preventing large increases in systemic insulin concentrations and the consequent decreases in blood amino acid concentrations (24), thus eliminating the need for exogenous amino acid infusion, which would have represented an important confounder because amino acids can independently stimulate muscle protein synthesis (16,17,20,34).

MATERIALS AND METHODS

Subject characteristics

The Institutional Review Boards of the University of Southern California and the University of Texas Medical Branch approved the study and all subjects gave written, informed consent before participation in the study. Seven healthy young volunteers (age 25±2 yr, weight 64±6 kg, height 1.69±0.04 m, and body mass index-BMI 22±1 kg/m^2, leg volume 8.4±0.6 liters, 4 females and 3 males), and six healthy elderly volunteers (age 68±1 year, weight 85±3 kg, and BMI 26±1 kg/m^2 leg volume 9.7±0.4 liters, 1 female and 5 males) were recruited through newspaper advertisements in Los Angeles, CA, and the Sealy Center on Aging Volunteers Registry of the University of Texas Medical Branch. The eligibility of the volunteers was assessed by clinical history, a physical examination, electrocardiogram, blood count, coagulation profile, plasma electrolytes, fasting blood glucose concentration, oral glucose tolerance test (OGTT), TSH, liver and renal function tests, hepatitis B and C screening, HIV test, and drug screening. Exclusion criteria were heart disease; diabetes or glucose intolerance, as assessed by OGTT (35); other endocrine diseases; obesity; uncontrolled hypertension or

hypertension treated with ACE-inhibitors, calcium channel blockers or β-blockers (for potential interference of these drugs with glucose and/or protein turnover); coagulation disorders; peripheral arterial or venous diseases; cancer; acute or chronic pulmonary diseases; acute or chronic infectious diseases; and allergy to iodides. All subjects were active, although not exercise trained, and living on their own with no limitation in ambulation or history of falls. A DEXA scan was obtained in 10 of the 13 subjects to measure leg muscle mass and body composition.

Study design

All subjects were instructed to eat their normal diets for the week before participation in the study. The evening before the study, the subjects were admitted to the General Clinical Research Center (GCRC) of the University of Southern California or the University of Texas Medical Branch GCRC. They were given a regular dinner and a snack at 1000, after which they were allowed only water ad libitum. The experimental protocol was identical in both groups, and the study design is shown in Fig. 1. At 0600, polyethylene catheters were inserted into a forearm vein for stable isotope and glucose infusion; in a vein of the contralateral hand for arterialized blood sampling; and into the femoral artery and vein of one leg for blood sampling. The catheter in the femoral artery was also used for the infusion of indocyanine green (ICG) and insulin.

After an initial blood sample was obtained for the measurement of background phenylalanine enrichment and ICG concentration, a primed (2 μmol/kg), continuous infusion of L-[*ring*-$^{2}H_{5}$]phenylalanine or L-[ring-$^{13}C_{6}$]phenylalanine (0.05 μmol·kg^{-1}·min^{-1}) was started (time=0 h).

At 2 h, the first muscle biopsy was taken from the lateral portion of the vastus lateralis muscle of the leg with the femoral catheters inserted, :20 cm above the knee, using a 5-mm Bergström biopsy needle. The tissue was immediately frozen in liquid nitrogen and stored at −80°C until analyzed. Subsequent biopsies were taken from the same incision at different angles so that the muscle biopsies were taken at least 5 cm apart from each other.

Between 4 and 5 h, ICG dye (0.5 mg·ml^{-1}) was infused into the femoral artery and four blood samples were obtained from the femoral vein and the peripheral vein to measure leg blood flow. In addition, four blood samples were obtained from the femoral artery and vein to measure blood free phenylalanine concentrations and enrichments, and blood glucose and insulin concentrations. At 5 h, a second muscle biopsy was taken.

Immediately after the biopsy was taken, the infusion of insulin (0.15 mU·min^{-1}·100 ml of leg^{-1}) directly into the femoral artery was started and continued until the end of the study (8 h). Insulin was infused locally in order to increase the leg insulin concentration to a postprandial level while avoiding excessive systemic hyperinsulinemia. During insulin infusion, 20% dextrose was also infused in the forearm catheter at a variable rate in order to maintain the arterial blood glucose concentration at their preinsulin infusion values (euglycemic clamp).

In both groups, between 7 and 8 h, the measurement of leg blood flow was repeated and blood samples were taken as described for the basal period. At 8 h, before the tracer infusion was stopped, a third muscle biopsy was obtained.

Analytical methods

Concentrations and enrichments of blood phenylalanine were determined on their tertbutyldimethylsilyl derivatives (t-BDMS) using appropriate internal standards (L-[^{15}N] phenylalanine) and gas chromatography/mass-spectrometry (GCMS, 6890 Plus GC, 5973N MSD, 7683 autosampler, Agilent Technologies, Palo Alto, CA), as described previously

(36). Muscle tissue samples were ground, and intracellular free amino acids and muscle proteins were extracted as described previously (36). Intracellular free concentrations and enrichments of phenylalanine were determined by GCMS using appropriate internal standards (L-[^{15}N]phenylalanine (36). Mixed muscle protein-bound phenylalanine enrichment was analyzed by GCMS after protein hydrolysis and amino acid extraction (36), using the external standard curve approach (37).

Plasma insulin concentrations were determined by performing a sandwich enzyme-linked immunosorbent assay (ELISA), which quantified the immobilized antibody-enzyme conjugate by spectrophotometrically measuring horseradish peroxidase enzyme activity with a microplate reader (Bio-Rad, Hercules, CA). Plasma endothelin-1 concentrations were determined using a commercially available ELISA kit (R&D Systems, Minneapolis, MN). This immunoassay is a solid-phase ELISA designed to measure human endothelin-1 in extracted EDTA plasma samples

Serum indocyanine green concentration for the determination of leg blood flow was measured spectrophotometrically (Beckman Coulter, Fullerton, CA) at λ=805 nm (38,39).

Plasma glucose concentration was measured using an automated glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH)

Calculations

The kinetics of muscle phenylalanine were calculated using two different methods: the two-pool model (40) and the three-pool model (41). We elected to use both models because each of them provides unique information regarding muscle amino acid kinetics. Additionally, although the three-pool model provides more detailed information regarding intracellular amino acid kinetics, this is a fairly new method and is used by only a few groups. On the other hand, the two-pool model has been used by a number of research groups, thus allowing for a comparison of our results with data collected by others.

With the two-pool model, the amino acid enrichments and concentrations in the femoral artery and vein are used to estimate muscle protein synthesis and breakdown when using phenylalanine. These parameters are based on the extraction of the labeled amino acid from the femoral artery, the appearance of unlabeled amino acid from the muscle in the femoral vein, and the net arteriovenous difference of the amino acid concentrations, respectively (40). Thus, this model provides data regarding the kinetics of blood amino acids across the leg, with no consideration for intracellular recycling of the amino acids from breakdown to synthesis. In other words, this method allows for the measurement of the effect of our treatments on the net kinetics of *blood* amino acids across the leg, while not offering any insight into the intracellular amino acid kinetics.

The three-pool model is an expansion of the two-pool model and relies not only on the measurement of the amino acid enrichments and concentrations in the femoral artery and vein, but also on the direct measurement of the amino acid enrichment in the free tissue water. This allows for the direct measurement of the amino acid *intracellular* utilization for protein synthesis and release from protein breakdown when using phenylalanine. In addition, it is possible to calculate the rate of phenylalanine transport from the artery into the tissue, and from the tissue into the venous blood.

The two-pool and the three-pool models share the following parameters:

$$\text{Amino acid delivery to the leg} = F_{in} = C_A \cdot BF \quad (1)$$

$$\text{Amino acid output from the leg} = F_{out} = C_V \cdot BF \tag{2}$$

$$\text{Leg net balance} = NB = (C_A - C_V) \cdot BF \tag{3}$$

The other kinetic parameters of the two-pool method were calculated as follows:

$$\text{Total rate of appearance} = Total \quad Leg \quad Ra = (C_A \cdot E_A / E_V) \cdot BF \tag{4}$$

$$\text{Release from the leg} = Leg \quad Ra = Total \quad Ra - F_{in} = BF \cdot C_A \left[(E_A / E_V) - 1 \right] \tag{5}$$

$$\text{Rate of disappearance in the leg} = Leg \quad Rd = Ra + NB = BF \cdot \left[(C_A \cdot E_A / E_V) - C_V \right] \tag{6}$$

C_A and C_V are the blood amino acid concentrations in the femoral artery and vein, respectively. E_A and E_V are the amino acid enrichments, expressed as tracer/tracee ratio, in the femoral arterial and venous blood, respectively. BF is leg blood flow as calculated from the steady state ICG concentration values in the femoral and wrist veins, as described previously (38,39). Data were expressed per 100 ml of leg volume.

The specific parameters of the three-pool model were calculated as follows:

$$\text{Muscle inward transport} = F_{M,A} = \{ [C_V \cdot (E_M - E_V) / (E_A - E_M)] + C_A \} \cdot BF \tag{7}$$

$$\text{Muscle outward transport} = F_{V,M} = \{ [C_V \cdot (E_M - E_V) / (E_A - E_M)] + C_V \} \cdot BF \tag{8}$$

$$\text{Arteriovenous shunting} = F_{V,A} = F_{in} - F_{M,A} \tag{9}$$

$$\text{Intracellular rate of appearance} = F_{M,0} = F_{M,A} \cdot \left[(E_A / E_M) - 1 \right] \tag{10}$$

$$\text{Intracellular rate of disappearance} = F_{0,M} = F_{M,0} + NB \tag{11}$$

E_M is the amino acid enrichment, expressed as tracer/tracee ratio, in the muscle.

Additionally, we calculated the intracellular amino acid availability as the sum of transport into the muscle $F_{M,A}$ and the intracellular rate of appearance $F_{M,O}$.

$$\text{Intracellular amino acid availability} = F_{M,A} + F_{M,O} \tag{12}$$

By using phenylalanine kinetics, it is also possible to calculate the efficiency of amino acid utilization for muscle protein synthesis and the amount of phenylalanine deriving from breakdown that is recycled back into synthesis without appearing in the blood as follows:

$$\text{Efficiency of phenylalanine utilization for protein synthesis} = F_{O,M} / \left(F_{M,A} + F_{M,O}\right) \tag{13}$$

$$\text{Intracellular phenylalanine recycling} = F_{0,M} - Leg \quad Rd = F_{M,0} - Leg \quad Ra \tag{14}$$

We also calculated the fractional synthetic rate (FSR) of mixed muscle proteins by measuring the incorporation rate of the phenylalanine tracer into the proteins ($\Delta E_P/t$) and using the precursor product model to calculate the synthesis rate as follows:

$$\text{FSR} = \left(\Delta E_p / t\right) / \left[\left(E_{M(1)} + E_{M(2)}\right)/2\right] \cdot 60 \cdot 100 \tag{15}$$

where $E_{M(1)}$ and $E_{M(2)}$ are the enrichments of phenylalanine in the free intracellular pool in two sequential muscle samples.

Leg glucose utilization was calculated as net glucose uptake across the leg:

$$\text{Leg glucose uptake} = (C_A - C_V) \cdot BF \tag{16}$$

To determine the degree of muscle tissue exposure to insulin, we calculated the insulin delivery rate to the leg. This is because a small portion of the insulin directly infused in the leg was recycled through the systemic circulation back into the leg, thus increasing the amount of insulin delivered to the muscle. Additionally, changes in leg blood flow can significantly affect insulin concentration when the exogenous infusion is constant, and for this reason, insulin concentration alone may not reflect the actual insulin availability for the muscle tissue. The arterial insulin concentration was not measurable during insulin infusion because the infusion was administered through the arterial catheter; therefore, insulin delivery to the leg was estimated by calculating the insulin outflow from the femoral vein multiplying the insulin concentration in the femoral vein (Ins_{FV}) by the blood flow:

$$\text{Insulin delivery} = Ins_{FV} \cdot BF \tag{17}$$

Although this method may slightly underestimate the insulin delivery rate because some insulin is taken up by the muscle cells after binding the insulin receptor and will not return in the venous blood, for the reasons listed above, we found it preferable to rely only on the calculated insulin dose as assessed at the time of infusion.

Statistical analysis

Statistical analyses were performed using the JMP ver. 4.0.5 statistical software (SAS Institute, Cary, NC). The effects of insulin on the response variables during the basal and insulin infusion periods were compared using ANOVA for repeated measures, the main effects being subject, group (young, old) and time (basal, insulin). Post hoc comparisons were carried out using the *t* test with Bonferroni's correction. Pairwise correlations were carried out using the Pearson product-moment correlation. Stepwise multiple regression analysis was carried out using the

step-forward method. Differences were considered significant at $P < 0.05$. Data are expressed as the mean ± SE.

RESULTS

Leg muscle mass and indexes of muscle mass and volume

Leg muscle mass, as measured in 6 young and 4 older subjects, was not different between young and older subjects (7287±889 g and 9077±99 g, respectively). Similarly, the leg muscle mass index, calculated by dividing the leg muscle mass by the squared height in meters to account for the effect of total body size on muscle mass (16), was not different between young and older subjects (2591±206 g/m^2, and 2751±22 g/m^2, respectively). Leg volume correlated with leg muscle mass (r=0.62, P=0.0565), as described previously in a larger number of subjects (15) and was therefore used as a surrogate measure of muscle mass in all subjects. Leg volume (8.4±0.6 liters, and 9.7±0.4 liters) and the leg volume index, calculated by dividing leg volume by the squared height (2.83±0.13 liters/m^2 and 2.97±0.08 liters/m^2), were not different between young and older subjects, respectively.

Glucose and insulin

Table 1 shows the blood glucose and insulin concentrations and kinetics across the leg. Arterial glucose concentrations were not different between young and older subjects in the basal period and during insulin infusion, and they slightly, but significantly, decreased during insulin infusion. In addition, the insulin and glucose infusion rates and leg glucose uptake during insulin infusion were not different between young and old. Systemic and femoral vein insulin concentrations were higher in the old (P<0.05) at baseline and increased significantly in both groups (P<0.0001) with insulin infusion. However, systemic and femoral vein insulin concentrations showed a larger increase in the older subjects (P<0.05). This was likely due to differences in blood flow (see below), since the insulin delivery to the leg was not different between groups either at baseline or during insulin infusion.

Blood flow and endothelin-1 concentrations

Blood flow (ml·min^{-1}·100 ml of leg^{-1}) across the leg was significantly lower (P<0.01) in the older subjects both at rest (young: 4.0±0.4, old: 2.6±0.2) and during hyperinsulinemia (young: 6.7±1.3, old: 2.7±0.2), and it tended to increase during insulin infusion only in the young subjects (P=0.10). Endothelin-1 concentrations (pg/ml) in the femoral vein were higher (P<0.05) in the older subjects than in the young, both at baseline (young: 1.54±0.52, old: 3.53 ±0.72) and during insulin infusion (young: 0.71±0.28, old: 2.46±0.39), and slightly but significantly decreased with insulin infusion (P<0.05).

Phenylalanine concentrations and enrichments

Phenylalanine blood and intramuscular concentrations and enrichments are shown in Table 2. The arterial concentrations of phenylalanine were not different at baseline, and they were slightly, but significantly, decreased (P<0.01) with insulin in young and older subjects with no differences between groups. Phenylalanine venous concentrations were not different at baseline and decreased significantly in both groups during insulin infusion (P<0.0001), with a larger decrease in the young (P<0.05). Phenylalanine intracellular concentrations were significantly higher (P<0.01) in the older subjects both at baseline and during hyperinsulinemia, and they significantly decreased with insulin (P<0.05) in both groups with no treatment-by-group interaction.

Phenylalanine enrichments in the femoral artery were significantly higher in the older subjects (P<0.05), both at baseline and during insulin infusion, and significantly increased (P<0.001)

with insulin infusion in both groups, with no time-by-group interaction. Phenylalanine enrichments in the femoral vein were not different between groups at baseline and significantly increased ($P<0.01$) during insulin infusion in both groups, with no differences between groups. Free phenylalanine enrichments in the muscle tissue were not different between groups and did not change with insulin infusion in either group.

Muscle amino acid and protein kinetics

Phenylalanine kinetics across the leg during basal conditions and the hyperinsulinemic clamp are shown in Table 3. Phenylalanine delivery to and release from the leg were significantly higher ($P<0.05$) in the young at baseline and during insulin. Additionally, phenylalanine delivery to the leg tended to increase in the young only, but it did not reach significance ($P=0.10$). Phenylalanine transport in the muscle tissue was not different between groups at baseline, and it tended to decrease during hyperinsulinemia in the older subjects ($P=0.09$). The transport of phenylalanine from the muscle to the venous blood was not different between groups at baseline and decreased with insulin in both groups with no differences between groups ($P<0.05$). Phenylalanine arteriovenous shunting was significantly lower ($P<0.05$) in the older subjects at baseline and during insulin. Also, phenylalanine shunting increased significantly both in young and older subjects during insulin infusion.

Phenylalanine disappearance from the blood (Rd) and utilization for protein synthesis ($F_{0,M}$) were not different between groups at baseline, but they significantly increased ($P<0.05$) only in the young during hyperinsulinemia. The fractional synthetic rate of muscle protein synthesis assessed using the precursor-product technique also confirmed this finding (Fig. 2). Phenylalanine release from proteolysis and Ra were not different at baseline and did not change with insulin. Phenylalanine net balance was not different between groups at baseline, and it increased in both groups ($P<0.01$). However, phenylalanine net balance became positive only in the young ($P<0.05$), indicating that a switch from net muscle protein catabolism to net muscle protein anabolism occurred only in the young (Fig. 3).

Phenylalanine intracellular availability decreased only in the elderly, because of the decline in phenylalanine transport into the muscle tissue. The efficiency of phenylalanine utilization for protein synthesis was significantly ($P<0.05$) lower in the young at baseline (old: 33 ± 4%, young: 20±2%), and it increased ($P<0.01$) with insulin in both groups (old: 40±4%; young: 42 ±4%) with no differences between groups.

Correlations

Blood flow was significantly and negatively correlated with endothelin-1 concentrations during insulin infusion ($R=-0.6132$, $P<0.05$), but not at baseline ($R=-0.4954$, $P=0.12$). There was a significant linear and positive relationship between the insulin-induced changes in amino acid delivery and the changes in muscle protein synthesis in both young and older subjects ($R=0.89$, $P=0.0001$). Similarly, there was a significant linear and positive correlation between changes in intracellular amino acid availability and the change in muscle protein synthesis ($R=0.94$, $P<0.0001$). In addition, there was a significant positive relationship between the insulin-induced changes in blood flow and the changes in muscle protein synthesis ($R=0.90$, $P<0.0001$) and between changes in insulin delivery to the leg and muscle protein synthesis ($R=0.57$, $P=0.042$). Interestingly, there were no significant relationships between the changes in muscle protein synthesis and changes in phenylalanine concentration ($R=-0.17$, $P=0.58$) or insulin concentrations ($R=0.44$, $P=0.13$). Leg volume, an index of leg muscle mass, was not correlated with changes in muscle protein synthesis.

The relationships between changes in muscle protein synthesis as measured with the phenylalanine tracer ($\Delta F_{0,M}$) and concomitant changes in several potentially relevant variables

(blood flow phenylalanine concentrations, delivery, transport and intracellular availability; insulin concentration and delivery) and leg volume, used as an index of muscle mass, were analyzed using the stepwise regression technique. In the final model, $\Delta F_{0,M}$ correlated with the changes in blood flow (*BF*, $P<0.0001$), intracellular phenylalanine availability (ΔIC_{aa}, $P=0.0092$), and, to a lesser extent, phenylalanine inward transport ($\Delta F_{M,A}$, $P=0.16$). Changes in endothelin-1 did not enter in the model. The final regression equation was the following:

$$\Delta F_{0,M}=0.71+0.43 \cdot \Delta BF+3.03 \cdot \Delta IC_{aa} - 1.01 \cdot \Delta F_{M,A}$$

The R-square for this multiple regression model was 0.93.

DISCUSSION

Our results indicate that muscle protein synthesis is resistant to the anabolic action of insulin in healthy, nondiabetic older humans. Specifically, muscle protein synthesis significantly increased during hyperinsulinemia in the young, whereas there was no effect in the older volunteers. Because muscle protein breakdown did not significantly change in either young or older subjects, the net muscle protein balance became positive only in the young, indicating that a switch from muscle protein catabolism to anabolism occurred only in the younger subjects. Our current results strongly suggest that this age-associated insulin resistance of muscle proteins is the primary reason for the reduced muscle anabolic response to feeding. Indeed, evidence is now accumulating indicating that the anabolic response of skeletal muscle proteins to mixed feeding is reduced with aging both in humans (7) and animals (42,43), despite the fact that amino acids alone can normally stimulate protein synthesis in older muscle (16-19). Interestingly, the current results were obtained in very healthy older subjects with no evidence of muscle loss. Additionally, in these subjects, muscle mass did not correlate with the response of muscle protein synthesis to insulin. This suggests that a reduced muscle protein response to insulin, and consequently to feeding, precedes the development of sarcopenia, and may therefore play a primary role in the development of sarcopenia.

The response of muscle protein synthesis to insulin in our subjects was strongly and positively associated with insulin-induced changes in amino acid delivery to the muscle, intracellular amino acid availability and blood flow, whereas no associations were found with insulin or amino acid concentrations. This suggests that the effects of insulin on human muscle protein synthesis are not only due to a direct stimulation of initiation of mRNA translation, well documented in animals (44-46) and more recently in humans (8), but also to indirect changes, both in muscle perfusion and amino acid availability. The vascular tone is modulated by endothelium-derived vasodilators and vasoconstrictors, the most important being nitric oxide (NO) and endothelin-1, respectively (47). Insulin-induced increases in muscle perfusion and capillary recruitment are mediated by increased NO production (48,49), which reduces endothelin-1 production (50). The insulin vasodilation is independent of its effect on glucose turnover, although it can positively modulate insulin-stimulated glucose uptake (51,52). In our experiment, insulin increased leg blood flow in the young, whereas no changes were detected in the older subjects, confirming previous reports showing that the vasodilatory response to insulin declines with aging (53). The lack of vasodilatory response to insulin in our older subjects was plausibly mediated by the higher endothelin-1 concentrations, because increased endothelin-1 activity has been shown to prevent vasodilation to insulin in insulin-resistant individuals via both a direct vasoconstrictor effect and an indirect negative effect on NO availability (54,55). The differences in the vasodilatory response to insulin were not sufficient to impair glucose uptake in these glucose-tolerant subjects; yet they appeared to be a strong predictor of the response of muscle protein synthesis and anabolism to insulin. Nonetheless, blood flow was not the only predictor of the protein synthetic response to insulin. Because

insulin-stimulated glucose uptake was not different between groups, we could conclude that healthy aging induces a selective insulin resistance of muscle proteins regardless of glucose tolerance and is probably due, at least in part, to an impaired response of blood vessels to the dilatory effects of insulin.

Our findings may also provide a much-needed explanation for the apparently contradictory results previously reported in the literature regarding the mechanisms leading to insulin-induced muscle protein anabolism in humans. Studies in which muscle protein synthesis was stimulated by insulin also reported an increased amino acid delivery to the examined muscle region (23,24,30-33). Conversely, most studies reporting no change in muscle protein synthesis and a decrease in breakdown during insulin infusion also reported a decrease or no change in amino acid delivery to the muscle (25-27,29,33). Since these experiments had been performed in young healthy subjects, insulin-induced vasodilation generally occurred. Thus, differences in amino acid delivery were largely due to differences in amino acid concentrations, which depended upon the modality of insulin infusion (systemic or local) and/or the concomitant infusion of exogenous amino acids. Systemic insulin infusion decreases amino acid concentrations (25,29,33,56,57) unless amino acids are replaced by exogenous infusion (8, 25,27,30,32,33), whereas local insulin infusion allows for the exposure of muscle to high insulin levels, while avoiding major declines in amino acid concentration (24,26).

Another interesting finding was that insulin had no effect on muscle protein breakdown in the two groups. This may seem to be at odds with previous research; however, we have recently shown that when blood amino acid availability is maintained during a clamp, muscle protein breakdown is not inhibited as it is when a systemic insulin infusion lowers amino acid concentrations (58). In the current study, blood phenylalanine concentrations decreased by only 9% in the young and 8% in the elderly during the insulin infusion, whereas a systemic insulin infusion reduces blood amino acid concentrations by 25−40% (25,29,33,56-58). Therefore, the role of amino acid availability during hyperinsulinemia in regulating muscle protein breakdown is an area in need of future research.

At the cellular level, the mechanisms responsible for the reduced protein synthetic response to the direct and indirect effects of insulin in older human muscle are still not known. A recent paper reported that when human muscles were exposed to hyperinsulinemia with concomitant hyperaminoacidemia, a condition resembling the postprandial state, there was an increased phosphorylation of PKB, mTOR, and 4E-BP1 both in young and older subjects. However, as opposed to the younger controls, p70S6K phosphorylation did not increase in the older subjects and consequently muscle protein synthesis did not increase in this group (8). Thus, it is possible that the regulation of mRNA translation and/or the components involved in the intracellular signaling cascades regulating protein synthesis may be involved. Nonetheless, it is important to underscore that in this case, the baseline p70S6K phosphorylation was much higher the in older subjects than in the younger controls and that the concomitant amino acid infusion probably affected the phosphorylation of mTOR and 4E-BP1 (59). Thus, future studies are needed to better characterize the cellular changes in the response of muscle proteins to insulin that occur with aging.

In summary, our data show that muscle protein synthesis does not increase when older human muscle is exposed to local hyperinsulinemia, as it does in young subjects. Furthermore, the overall protein anabolic response to insulin is significantly reduced with age. In addition, there was no relationship between the change in amino acid concentration and the change in protein synthesis, but there was a very significant positive relationship with the changes in blood flow and amino acid availability. This suggests that a reduced vasodilatory response of older human muscle to insulin may play an important role in the reduced anabolic action of insulin in aging by decreasing nutrient flow and availability. The validity of our results is confirmed by the

concordance of the data obtained with the three different methods that we used to measure muscle protein synthesis with the phenylalanine tracer (i.e., two-pool model, three-pool model, and precursor-product model). We conclude that protein metabolism is resistant to the anabolic action of insulin in older human skeletal muscle. Future studies are required to identify the cellular mechanisms responsible for the insulin resistance of muscle protein synthesis in aging muscle and to determine the effects of manipulations of the vascular tone on the response of muscle proteins to insulin.

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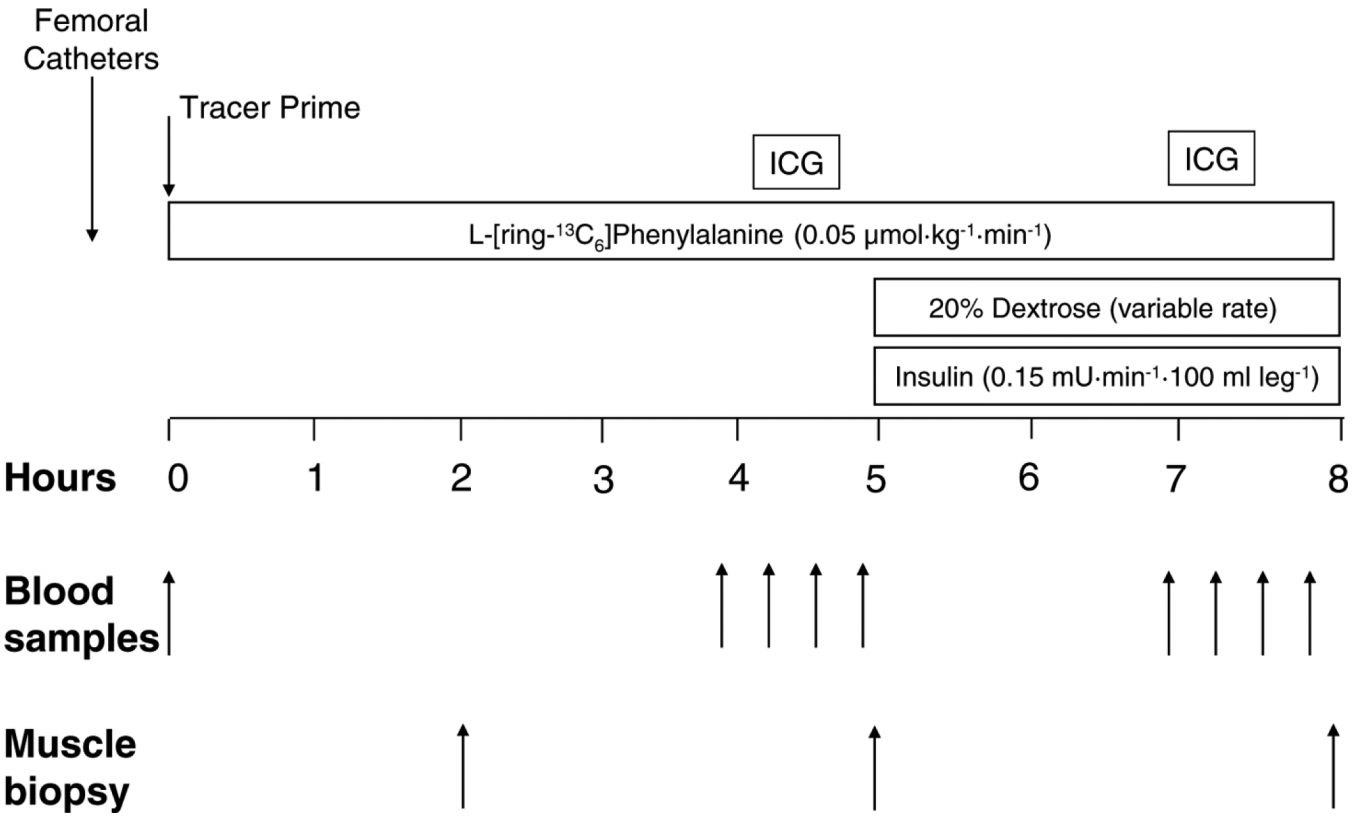

Figure 1.
Study design

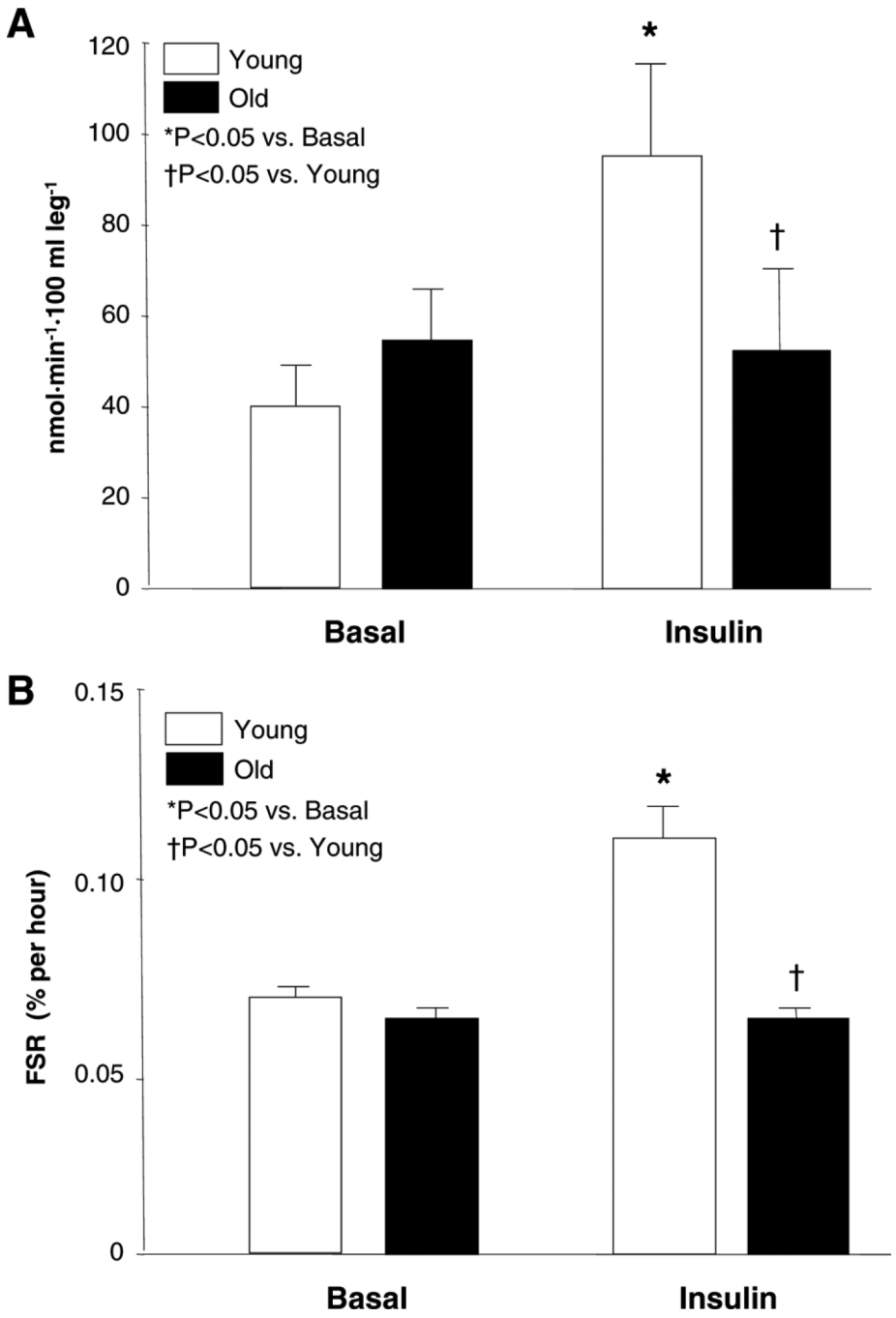

Figure 2.
Muscle protein synthesis measured using both (***A***) a three-pool model technique and (***B***) a direct incorporation technique (FSR) in the basal state and during local hyperinsulinemia in young and older subjects.

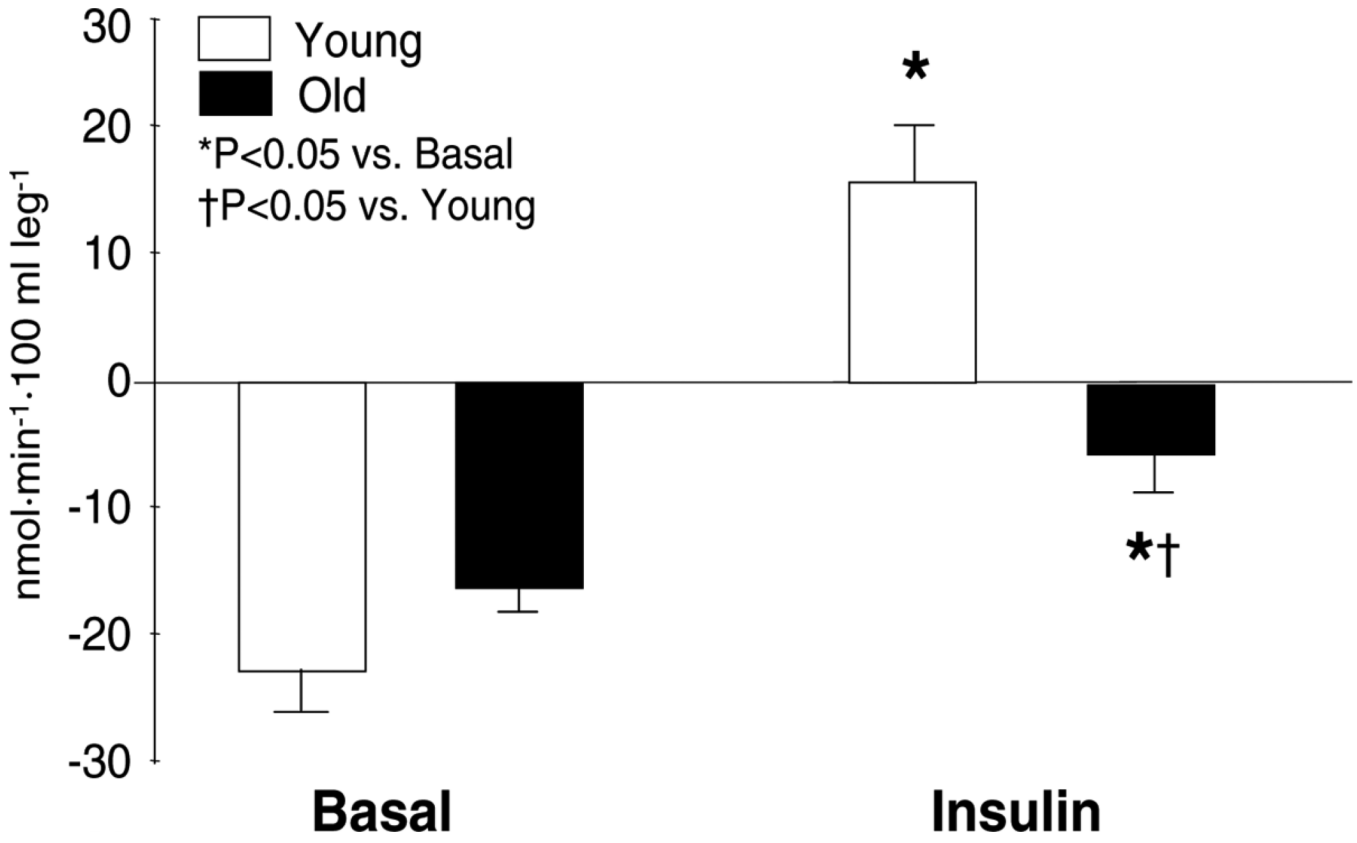

Figure 3.
Phenylalanine net balance across the leg in the basal state and during local hyperinsulinemia in young and older subjects.

Table 1

Plasma glucose and insulin concentrations and kinetics and infusion rates in young and older subjects in the basal state and during insulin infusion in the femoral artery of one leg

	Young Adults		Older Adults	
	Basal	**Insulin**	**Basal**	**Insulin**
Insulin infusion rate ($\mu U \cdot min^{-1} \cdot 100\ ml\ leg^{-1}$)	–	162 ± 23	–	190 ± 17
Glucose infusion rate ($\mu mol \cdot kg \cdot^{-1} min^{-1}$)	–	11.0 ± 0.7	–	10.5 ± 1.0
Systemic insulin concentration (μU/ml)	5.3 ± 0.2	9.7 ± 0.3*	8.5 ± 1.2†	23.7 ± 2.8*†
Femoral vein insulin concentration (μU/ml)	5.3 ± 0.2	40.3 ± 1.0*	8.5 ± 1.2†	83.4 ± 6.2*†
Arterial glucose concentration (mmol/l)	4.9 ± 0.1	4.8 ± 0.2*	4.7 ± 0.2	4.4 ± 0.2*
Muscle glucose uptake ($mmol \cdot min^{-1} \cdot 100\ ml\ leg^{-1}$)	–	1.8 ± 0.3	–	1.5 ± 0.4
Insulin delivery to the leg ($\mu U \cdot min^{-1} \cdot 100\ ml\ leg^{-1}$)	21 ± 3	270 ± 52*	22 ± 3	218 ± 11*

*$P < 0.05$ vs. basal.

†$P < 0.05$ vs. young adults.

Table 2

Concentrations and enrichments of free phenylalanine in the femoral artery and vein, and in the muscle tissue of young and older subjects in the basal state and during insulin infusion in the femoral artery of one leg

	Young Adults		Older Adults	
	Basal	**Insulin**	**Basal**	**Insulin**
Concentrations		**(μmol/l)**		
Artery	55 ± 2	50 ± 2*	60 ± 2	55 ± 2*
Vein	60 ± 2	48 ± 2*	66 ± 2	56 ± 1*†
Muscle	89 ± 9	82 ± 6*	135 ± 10†	99 ± 11*†
Enrichments		**(*tracer/tracee ratio*)**		
Artery	0.069 ± 0.001	0.078 ± 0.002*	0.089 ± 0.007†	0.099 ± 0.007*†
Vein	0.056 ± 0.002	0.067 ± 0.003*	0.065 ± 0.006	0.077 ± 0.006*
Muscle	0.047 ± 0.003	0.050 ± 0.003	0.052 ± 0.004	0.057 ± 0.005

*$P < 0.05$ vs. Basal

†$P < 0.05$ vs. Young Adults.

Table 3

Phenylalanine kinetics across the leg in young and older adults in the basal state and during insulin infusion in the femoral artery of one leg

	Young Adults		Older Adults	
	Basal	Insulin	Basal	Insulin
	(nmol·min^{-1}·100 ml leg^{-1})			
F_{in} – Delivery to the leg	221 ± 25	338 ± 65	156 ± 12†	145 ± 8†
F_{out} – Release from the leg	243 ± 29	322 ± 63	171 ± 13†	149 ± 1†
F_{ma} – Transport into muscle free pool	144 ± 35	158 ± 41	113 ± 20	75 ± 11
F_{vm} – Transport from muscle free pool	164 ± 37	142 ± 38*	128 ± 21	79 ± 11*
F_{va} – Arteriovenous shunting	77 ± 28	180 ± 39*	43 ± 11†	70 ± 12*†
F_{om} – Utilization for protein synthesis	40 ± 8	93 ± 21*	58 ± 8	57 ± 12†
F_{mo} – Release from proteolysis	63 ± 13	78 ± 19	73 ± 9	61 ± 14
Rd – Disappearance from the blood	35 ± 8	71 ± 19*	48 ± 9	40 ± 7†
Ra – Release in blood from proteolysis	57 ± 13	56 ± 16	63 ± 10	44 ± 9
IC_{av} – Intracellular availability	225 ± 41	235 ± 57	186 ± 27	136 ± 21*†
NB – Net balance across the leg	−22 ± 5	15 ± 4*	−15 ± 2	−4 ± 2*†

$^{*}P < 0.05$ vs. Basal

$^{\dagger}P < 0.05$ vs. Young Adults.