Influence of Adiposity in the Blunted Whole-Body Protein Anabolic Response to Insulin With Aging

Stéphanie Chevalier, Réjeanne Gougeon, Nicholas Choong, Marie Lamarche, and José A. Morais

McGill Nutrition and Food Science Centre, McGill University Health Centre-Royal Victoria Hospital, Montreal, Quebec, Canada.

Background. Although insulin resistance of glucose is often reported with aging, that of protein metabolism is still debated. We tested if the insulin sensitivity of protein metabolism parallels that of glucose and is altered with aging.

Methods. Whole-body $^{13}$C-leucine and $^{3}$H-glucose kinetics were measured in the postabsorptive state and during an hyperinsulinemic, euglycemic, isoaminoacidemic clamp in 12 young men (age: 27 ± 1 years; body mass index [BMI]: 23 ± 1 kg/m²), 11 young women (age: 25 ± 1 years; BMI: 21 ± 1 kg/m²), 9 elderly men (age: 70 ± 1 years; BMI: 26 ± 1 kg/m²), and 10 elderly women (age: 69 ± 2 years; BMI: 23 ± 1 kg/m²).

Results. Postabsorptive leucine flux rates adjusted for fat-free mass (FFM) were not different between elderly and young participants. During the clamp, leucine flux and protein synthesis rates increased less in the elderly participants, and protein breakdown decreased equally. Thus, the net anabolic (protein balance) response to hyperinsulinemia was lower in elderly versus young participants ($p = .007$) and was highly correlated with the clamp glucose rate of disposal ($r = -.671$, $p < .001$), indicating insulin resistance of protein concurrent with that of glucose. From regression analysis, FFM explained 73% of the variance in the anabolic response. Age explained an additional 3%, but was accounted for by markers of adiposity. FFM and percent body fat collectively explained 79% of the variance.

Conclusion. Both reduction in absolute FFM and increased adiposity, intrinsic to the aging process, are associated with an altered anabolic action of insulin in stimulating protein synthesis. This alteration may contribute to the progressive muscle loss with aging.

A reduced protein anabolic action of insulin could gradually lead to a decrease in fat-free mass (FFM), a typical and detrimental change in body composition with aging (1). Whether insulin resistance of protein metabolism occurs concurrently with that of glucose in elderly persons is still not established. The gold standard approach to determine insulin resistance of glucose metabolism is the hyperinsulinemic, euglycemic clamp protocol (2). By raising plasma insulin to postprandial levels, endogenous production of glucose is completely suppressed and the glucose infusion rates required to maintain euglycemia are equivalent to the insulin-mediated glucose disposal, a marker of tissue sensitivity to insulin. The challenge in studying protein metabolism along with that of glucose during a hyperinsulinemic clamp has been to prevent the drop in plasma amino acids due to suppression of protein breakdown (3), thus limiting substrate availability for protein synthesis. This is particularly relevant to the recent demonstration of reduced activity of the initiation factor of messenger RNA translation, eIF2B, when plasma amino acids are reduced (4). With the aim of dissecting the role of insulin, hyperaminoacidemia is to be avoided, because amino acids themselves (especially leucine), are able to stimulate protein synthesis through activation of the mammalian target of rapamycin (mTOR) pathway (5). It follows that plasma amino acids should be kept constant to better study the impact of insulin on whole-body protein turnover. When such an approach was used, lower glucose disposal rates and less suppression of whole-body protein breakdown were reported in elderly compared with young persons, at low, but not at higher elevations in plasma insulin concentrations (6). In this study, whole-body protein synthesis was not stimulated at any insulin concentrations.

At the muscle level, amino acids alone stimulate muscle protein synthesis in elderly as well as in young persons (7,8), and it has been found that essential amino acids are responsible for this effect (9). However, muscle protein synthesis increased less in elderly than in young persons during hyperinsulinemia induced by “continuous” oral glucose and amino acid feeding (10). These results suggested the presence of insulin resistance of protein with aging.

Therefore, the present study sought to test if the insulin resistance with aging affects both glucose and whole-body protein, using the hyperinsulinemic, euglycemic, isoaminoacidemic clamp method, in nondiabetic elderly and young men and women. With this methodology, we have previously shown that insulin stimulates protein synthesis in young adults (11) and that adiposity reduces this action (12).

Methods

Study Design

After 1 week of dietary control, protein and glucose kinetics were assessed using labeled isotope dilution methodology, in both elderly and young participants, during the postabsorptive state. To address whether insulin resistance of protein and glucose was present in aging, kinetics were also measured during an elevation of plasma insulin to levels found postprandially while, at the same time, maintaining both plasma glucose and amino acids at postabsorptive levels and constant (i.e., “clamping” their concentrations) by exogenous infusions. This strategy
allows for isolating the metabolic responses to insulin from those known to arise from changes in plasma amino acid concentrations.

**Participants and Diet**

Elderly (9 men, 10 women) and young (12 men, 11 women) participants were recruited by advertisements in local newspapers (young) and magazines oriented for older adults (elderly). All the elderly participants were healthy, community-dwelling, and independent in instrumental activities of daily living. All participants were screened by medical history, physical examination, and laboratory investigation as previously detailed (13), and were admitted to the McGill University Health Centre–Royal Victoria Hospital’s Clinical Investigation Unit after giving written informed consent. The Human Ethics Review Committee of the hospital approved the protocol. None of the participants was taking medications, other than vitamin supplements, which were stopped the week prior to admission. Young women with regular menstrual cycles were studied during the follicular phase. All participants abstained from their usual physical activities for a week prior to the clamp experiment as they were admitted as inpatients to the Clinical Investigation Unit of the hospital. They were allowed daily walks in the hospital and on its grounds.

During this period, participants received an individualized formula-based isoenergetic diet, according to resting metabolic rate measured by indirect calorimetry (Deltatrac; Sensor Medics, Yorba Linda, CA), multiplied by a physical activity factor of 1.5–1.7 for the elderly participants and 1.7 for the young participants based on qualitative assessment of physical activity. Details of the diet and dietary protocol have been recently described (11). Nitrogen (N) balance studies were conducted during the postabsorptive (without electrolytes; B. Braun Medical, Inc., Irvine, CA) taken at 10-minute intervals. Indirect calorimetry was performed for 20 minutes prior to and during the last 30 minutes of the insulin infusion (18). Glucose turnover was calculated as specified in (18,19) and substrate oxidation as in (18). Leucine kinetics were calculated according to the stochastic model of Matthews and colleagues (20), using plasma α-keto isocaproic acid (α-KIC) as an index of the precursor pool enrichment (reciprocal model). Leucine flux is calculated from the dilution of plasma α-KIC, using the equation $Q = i \frac{(E_i/\text{KIC}) - 1}{1}$, where $Q$ is leucine flux, $i$ is [1-13C]-leucine infusion rate, $E_i$ is the enrichment of [1-13C]leucine, and $\text{KIC}$ is the plasma [13C]α-KIC enrichment. Endogenous leucine rate of appearance (Leu Ra, an index of protein breakdown) is calculated as: $\text{Leu Ra} = Q - I$, where $I$ is the infusion of amino acids; leucine nonoxidative rate of disposal (non-ox Rd, an index of protein synthesis) is calculated as: $\text{Non-ox Rd} = Q - \text{leucine oxidation}$. Leucine oxidation is obtained from $\text{F}^{13}\text{CO}_2/\text{E}_{\text{KIC}}$, where $\text{F}^{13}\text{CO}_2$ is (VCO$_2$ · 13CO$_2$ enrichment)/13CO$_2$ recovery factor.

**Protein and Glucose Kinetic Studies**

Protein and glucose kinetics were studied using labeled isotope dilution methodology during the postabsorptive state followed by a hyperinsulinemic, euglycemic, isoaminoacemic clamp protocol. Primed, continuous infusions of [3-3H]-glucose and [1-13C]-leucine were started 180 minutes after an overnight fast. Euglycemia was maintained with infusion of 20% (w/v) potato starch-derived glucose (Avebe b.a., Foxhol, The Netherlands) and baseline concentrations of plasma individual amino acids with 10% TrophAmine (without electrolytes; B. Braun Medical, Inc., Irvine, CA) by feedback adjustments of infusion rates based on total BCAA concentrations, measured every 5 minutes. Blood and expired air samples were collected every 10 minutes for 40 minutes prior to the insulin infusion, then every 30 minutes until the last 40 minutes, at which time they were again taken at 10-minute intervals. Indirect calorimetry was performed for 20 minutes prior to and during the last 30 minutes of the insulin infusion (18). Glucose turnover was calculated as specified in (18,19) and substrate oxidation as in (18). Leucine kinetics were calculated according to the stochastic model of Matthews and colleagues (20), using plasma α-keto isocaproic acid (α-KIC) as an index of the precursor pool enrichment (reciprocal model). Leucine flux is calculated from the dilution of plasma α-KIC, using the equation $Q = i \frac{(E_i/\text{KIC}) - 1}{1}$, where $Q$ is leucine flux, $i$ is [1-13C]-leucine infusion rate, $E_i$ is the enrichment of [1-13C]leucine, and $\text{KIC}$ is the plasma [13C]α-KIC enrichment. Endogenous leucine rate of appearance (Leu Ra, an index of protein breakdown) is calculated as: $\text{Leu Ra} = Q - I$, where $I$ is the infusion of amino acids; leucine nonoxidative rate of disposal (non-ox Rd, an index of protein synthesis) is calculated as: $\text{Non-ox Rd} = Q - \text{leucine oxidation}$. Leucine oxidation is obtained from $\text{F}^{13}\text{CO}_2/\text{E}_{\text{KIC}}$, where $\text{F}^{13}\text{CO}_2$ is (VCO$_2$ · 13CO$_2$ enrichment)/13CO$_2$ recovery factor.

**Assays**

Plasma glucose was measured by the glucose oxidase method (GM7 Micro-Stat; Analox Instruments USA, Lunenber, MA). Assays for immunoreactive insulin and glucagon, and glucose-specific activity were as in (21). Plasma total BCAA concentrations were measured by a rapid enzymatic, fluorometric assay (11). Individual plasma amino acids were determined by ion-exchange high-performance liquid chromatography (HPLC) with postcolumn ninhydrin detection (23) using a Beckman HPLC System (Beckman Coulter Inc., Fullerton, CA). Plasma free fatty acids were determined using the NEFAC test kit (Wako Chemicals USA, Inc., Richmond, VA). The [13C] enrichment of plasma α-KIC was analyzed by gas chromatography–mass spectrometry (Hewlett-Packard GCMS 5988A; Palo Alto, CA) after derivatization with N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (Regis Technologies Inc., Morton Grove, IL) to yield a tert-butyldimethylsilyl derivative of hydroxyisocaproic acid. Expired air was analyzed for 13CO$_2$ enrichment by isotope ratio mass spectrometry on a Micromass 903D (Vacuum Generators, Winsfor, U.K.).

**Validation Studies of Background Enrichment of Expired 13CO$_2$ and Plasma 13C-KIC, and 13C Bicarbonate Recovery**

We have previously found a 10.1 ± 1.6% dilution in the background enrichment of expired 13CO$_2$ in lean young men, mainly due to infusion of the potato starch–derived glucose with a natural low 13C content (17). Tested in four elderly (two men and two women) and four young women, the percent dilution was not significantly different from that of the lean young men, and leucine oxidation rates were
corrected using the same dilution factor. No dilution effect in plasma $^{13}$C-KIC was found either in young or in elderly participants. The recovery of $^{13}$C from the bicarbonate pool was assessed in the postabsorptive state and during the hyperinsulinemic clamp in four elderly participants and four lean young women and did not differ from that of lean young men (11). Therefore, factors of 0.671 in the postabsorptive state and 0.799 during the clamp period were used in all groups for the calculation of leucine oxidation rates.

### Statistical Analysis

Results are presented as means ± standard error of the mean. A two-factor analysis of variance, with the same main factors. The change in response to the clamp was analyzed by repeated-measures analysis of variance, with the same main factors. When a significant age-by-sex interaction was found, one-factor analyses were performed in separate groups. A regression-based approach was used to compare the metabolic data, to take into account the differences in body composition among the studied groups. Covariate analysis adjusts the mean data for the nonzero intercept of the relationship between the independent variable and FFM, thus removing the effect of differences in FFM among groups. Covariates were included in the model when they were found to have a significant predictive value on the dependent variable, from prior regression analysis (18,24). Adjusted means for FFM + standard error of the mean are presented in the tables below the total units per time, in parentheses. Only the $p$ values of the covariate analysis are reported in the tables. Pearson’s coefficient was used for all correlations and partial correlation when controlling for other variables was required. Significance level was set at $p < .05$. The analyses were performed with SPSS 11.0 for Windows (SPSS, Inc., Chicago, IL).

### RESULTS

#### Participant Characteristics

The participant characteristics are presented in Table 1. There were significant age-related differences in body composition in both sexes, namely an increased adiposity and lower FFM compared with the young participants. Typical sex differences were also seen: greater height, weight, and FFM in men, and higher adiposity in women, but with lesser waist circumference. Energy intake was lower in women compared with men as total per day, but not different when adjusted for FFM. Protein intake was controlled per FFM and therefore not different among the groups. Body weight was stable prior to the study and maintained during the inpatient stay. N balance was slightly positive in young women but was not different between elderly and young participants or between men and women. Fasting plasma glucose was higher in the elderly than in the young participants and in men than in women, and all concentrations were below 6.9 mmol/L. Plasma glucose 2 hours after a 75 g oral glucose tolerance test was also higher in elderly participants, although none reached the diagnostic criteria for diabetes. Eight individuals in the elderly group and none in young had impaired glucose tolerance (25).

#### Metabolic Responses to the Hyperinsulinemic Clamp

Fasting plasma insulin concentrations were higher in elderly men than in young men and in elderly women (Table 2). During the clamp, insulin increased more to higher values in the elderly than in young participants. Endogenous production of glucose was completely suppressed with hyperinsulinemia in all groups (data not presented). There was no significant overall age or sex effect on clamp glucose Rd, but significant age-by-sex interactions in clamp glucose Rd, as it tended to be lower only in the elderly men. There was an age effect ($p = .05$) on the insulin sensitivity index adjusted for FFM. Serum free fatty acid concentrations were

### Table 1. Participant Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Young Men ($N = 12$)</th>
<th>Elderly Men ($N = 9$)</th>
<th>Young Women ($N = 11$)</th>
<th>Elderly Women ($N = 10$)</th>
<th>Age Effect ($p$ Value)</th>
<th>Sex Effect ($p$ Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>27.2 ± 1.0</td>
<td>69.7 ± 1.4</td>
<td>24.9 ± 1.3</td>
<td>69.1 ± 1.5</td>
<td>&lt;.001</td>
<td>—</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178.9 ± 1.6</td>
<td>172.9 ± 2.4</td>
<td>160.7 ± 1.9</td>
<td>157.6 ± 1.5</td>
<td>.018</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>73.6 ± 3.4</td>
<td>77.2 ± 4.5</td>
<td>55.1 ± 1.5</td>
<td>57.5 ± 2.3</td>
<td>—</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.0 ± 1.0</td>
<td>25.6 ± 0.9</td>
<td>21.3 ± 0.6</td>
<td>23.1 ± 0.8</td>
<td>.013</td>
<td>.019</td>
</tr>
<tr>
<td>FFM, kg</td>
<td>60.9 ± 1.5</td>
<td>54.3 ± 1.9</td>
<td>40.7 ± 0.6</td>
<td>35.9 ± 0.9</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Muscle mass, kg</td>
<td>32.5 ± 0.8</td>
<td>28.9 ± 1.1</td>
<td>19.4 ± 0.5</td>
<td>16.4 ± 0.7</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>16.3 ± 2.1</td>
<td>28.7 ± 2.2</td>
<td>25.7 ± 2.1</td>
<td>37.1 ± 1.5</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>81.9 ± 2.4</td>
<td>95.5 ± 4.0</td>
<td>69.1 ± 1.7</td>
<td>78.6 ± 2.4</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.86 ± 0.01</td>
<td>0.96 ± 0.03</td>
<td>0.74 ± 0.01</td>
<td>0.82 ± 0.02</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Energy intake, kcal/d</td>
<td>2833 ± 89</td>
<td>2436 ± 84</td>
<td>2096 ± 44</td>
<td>1832 ± 70</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Protein intake, g/d</td>
<td>2252 ± 80</td>
<td>2157 ± 58</td>
<td>2444 ± 60</td>
<td>2398 ± 81</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>N balance, g/d</td>
<td>107 ± 4</td>
<td>93 ± 3</td>
<td>74 ± 2</td>
<td>68 ± 2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>4.9 ± 0.6</td>
<td>5.3 ± 0.9</td>
<td>4.8 ± 0.8</td>
<td>5.0 ± 0.6</td>
<td>&lt;.001</td>
<td>.014</td>
</tr>
<tr>
<td>OGTT 2h, mmol/L</td>
<td>4.8 ± 0.4</td>
<td>8.3 ± 1.0</td>
<td>5.5 ± 0.5</td>
<td>7.9 ± 0.7</td>
<td>&lt;.001</td>
<td>—</td>
</tr>
</tbody>
</table>

Notes: Means ± standard errors of the mean. Results were analyzed by a two-factor analysis of variance. For energy and protein intake, fat-free mass (FFM) was included as a covariate. (Adjusted values are shown in parentheses). No sex-by-age interaction was found significant.

*Significantly different from zero.

BMI = body mass index; OGTT 2h = plasma glucose 2 hours after an oral glucose tolerance test; — = not significant.
equally suppressed with hyperinsulinemia (data not presented). Resting energy expenditure adjusted for FFM was higher in women at baseline, but there was no sex or age effect on resting energy expenditure adjusted for FFM, during the clamp, or as a change in response to the clamp. At baseline, npRQ was lower in elderly men compared with young men, but not different between young and elderly women. Nonprotein RQ increased in all participants, to higher values in elderly than in young participants and to elderly men, but not different between young and elderly women. There was a small but significant increase in leucine and in IAA, and a decrease in DAA, concentrations than did men. There was a small but significant decrease in lipids oxidations adjusted for FFM.

Amino Acid and Glucose Infusions

The total amino acid infusion rates required to maintain individual baseline levels were lower in the elderly than in the young participants (Figure 1A). When the amino acid infusion rates were adjusted for FFM and postabsorptive BCAA concentrations, as both were independent predictors, they remained significantly lower in the elderly at \( p = .003 \). No sex effect was noted when these adjustments were made. Total glucose infusion rates (Figure 1B) were lower in the elderly participants, but not when they were adjusted for FFM. There was an age-by-sex interaction \( (p = .004) \) on the glucose infusion rates adjusted for FFM, being lower only in the elderly men compared with the young men, \( p = .024 \).

Plasma Amino Acids Concentrations

At baseline, leucine concentrations were higher in the elderly than in the young participants (Table 3). Women had lower plasma leucine, BCAA, indispensable amino acids (IAA), dispensable amino acids (DAA), and total AA concentrations than did men. There was a small but significant increase in leucine and in IAA, and a decrease in DAA, during the clamp. Of note is that these quantitatively small increments in AA did not correlate with any leucine kinetic responses to the hyperinsulinemic clamp. There were no differences among groups in the percent change in AA concentrations during the clamp.

Leucine Kinetics

Leucine kinetics are presented at baseline and during hyperinsulinemia in Table 4, and the responses to the clamp are illustrated in Figure 2. Comparisons are based on means...
adjusted for FFM, shown in parentheses. In the postabsorptive state, there were no age or gender effects on total leucine flux rates. However, oxidation rates were lower, and synthesis rates higher, in the elderly men than in the young men, resulting in a less negative net balance in the former. These differences were not seen between young and elderly women. During the hyperinsulinemic clamp, rates of leucine flux, oxidation, and protein synthesis increased, whereas protein breakdown rates decreased in all, to values not different among the groups. Net balance was switched from negative (protein loss) to positive (protein accretion) values, which were lower in the elderly participants than in the young participants and lower in the women than in the men. Leucine infusion rates were not different among groups, but, as for total AA infusion rates, they were significantly lower than those of the young when adjusted for the target postabsorptive leucine concentrations (adjusted means; \( p < .001 \)). With aging, the changes in leucine flux, protein synthesis, and net balance in response to the clamp were lower (Figure 2). When leucine kinetics were also corrected for the higher change in plasma insulin in the elderly participants, the lesser responses to the clamp remained significant.

The sensitivity of the protein-metabolic response to insulin is defined as the change in net balance, or the anabolic response to hyperinsulinemia (as glucose sensitivity is defined as its rate of disposal during a clamp). As illustrated in Figure 3, both indices were positively correlated with \( r = 0.671, p < .001 \). The anabolic response was also correlated with the insulin sensitivity index \( (r = 0.657, p < .001) \), as were amino acid and glucose infusion rates \( (r = 0.598, p < .001) \).

Stepwise linear regression analysis was performed to identify predictors of the anabolic response (Table 5). The largest proportion of the variance (73%) was explained by FFM. Age explained an additional 3% of the variance, but when any index of adiposity (be it percent body fat, waist or hip circumference, waist/hip ratio, or BMI) was entered in the model, age did not retain its significant predictive value and was excluded. Thus, 79% of the variance of the anabolic response to hyperinsulinemia was explained collectively by FFM and percent body fat, with FFM having a positive impact and percent body fat, a negative one.

**DISCUSSION**

The present study showed a lesser whole-body anabolic response to hyperinsulinemia with aging. The blunted anabolic response in elderly participants was mediated by the failure of insulin to stimulate protein synthesis to the same extent as in the young, as protein breakdown was suppressed equally. The lesser sensitivity of the protein response was associated with that of glucose and was largely explained by a lower FFM and a greater proportion of adiposity, both typical age-related differences in body composition. These findings are consistent with previous results obtained from hyperinsulinemic euglycemic, isoaminoacidemic clamp studies showing impairment of the anabolic action of insulin associated with excess adiposity (12).

Less suppression of protein breakdown in elderly than in young participants has been reported at low (90–123 pmol/L), but not higher (196–256 pmol/L) plasma insulin elevations (6). That study used a similar approach to our study, but whole-body protein synthesis was not stimulated at either level of insulin. However, these insulin levels were below those commonly seen after ingestion of a mixed meal. In the present study, the higher plasma insulin concentrations achieved (565–695 pmol/L), chosen to reflect postprandial levels, may have been sufficient to mask any possible altering effect of aging on protein breakdown, but allowed for the demonstration of a blunted response in the stimulation of protein synthesis.

Whether the age-related insulin resistance is attributed to intrinsic defects in protein synthesis due to aging per se, apart from the changes in body composition, has yet to be defined. Data from a large number of hyperinsulinemic, euglycemic clamp experiments done in 20 European centers has shown the insulin resistance of glucose in elderly per-
Table 3. Plasma Amino Acid Concentrations (μmol/L) in Young and Elderly Men and Women at Baseline and During the Hyperinsulinemic Clamp

<table>
<thead>
<tr>
<th>Plasma Amino Acids</th>
<th>Young Men ( (N = 12) )</th>
<th>Elderly Men ( (N = 9) )</th>
<th>Young Women ( (N = 11) )</th>
<th>Elderly Women ( (N = 10) )</th>
<th>Age Effect ( (p \text{ Value}) )</th>
<th>Sex Effect ( (p \text{ Value}) )</th>
<th>Interaction ( (p \text{ Value}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>130 ± 4</td>
<td>144 ± 3</td>
<td>110 ± 3</td>
<td>119 ± 5</td>
<td>.014</td>
<td>&lt;.001</td>
<td>—</td>
</tr>
<tr>
<td>Clamp*</td>
<td>146 ± 5</td>
<td>153 ± 9</td>
<td>122 ± 5</td>
<td>124 ± 4</td>
<td>—</td>
<td>&lt;.001</td>
<td>—</td>
</tr>
<tr>
<td>% change</td>
<td>12 ± 3</td>
<td>6 ± 3</td>
<td>11 ± 3</td>
<td>5 ± 3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total BCAA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>406 ± 13</td>
<td>439 ± 17</td>
<td>350 ± 10</td>
<td>359 ± 14</td>
<td>—</td>
<td>&lt;.001</td>
<td>—</td>
</tr>
<tr>
<td>Clamp*</td>
<td>412 ± 13</td>
<td>441 ± 23</td>
<td>357 ± 12</td>
<td>356 ± 11</td>
<td>—</td>
<td>&lt;.001</td>
<td>—</td>
</tr>
<tr>
<td>% change</td>
<td>2 ± 2</td>
<td>0 ± 2</td>
<td>2 ± 2</td>
<td>-1 ± 2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total IAA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>929 ± 28</td>
<td>952 ± 28</td>
<td>805 ± 29</td>
<td>809 ± 23</td>
<td>—</td>
<td>&lt;.001</td>
<td>—</td>
</tr>
<tr>
<td>Clamp*</td>
<td>948 ± 20</td>
<td>990 ± 34</td>
<td>852 ± 38</td>
<td>853 ± 21</td>
<td>—</td>
<td>&lt;.001</td>
<td>—</td>
</tr>
<tr>
<td>% change</td>
<td>3 ± 2</td>
<td>4 ± 1</td>
<td>6 ± 3</td>
<td>6 ± 2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total DAA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1585 ± 48</td>
<td>1556 ± 47</td>
<td>1360 ± 54</td>
<td>1526 ± 40</td>
<td>—</td>
<td>.012</td>
<td>—</td>
</tr>
<tr>
<td>Clamp*</td>
<td>1456 ± 24</td>
<td>1465 ± 52</td>
<td>1315 ± 62</td>
<td>1465 ± 45</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>% change</td>
<td>-8 ± 2</td>
<td>-6 ± 2</td>
<td>-3 ± 2</td>
<td>-4 ± 2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>2514 ± 72</td>
<td>2500 ± 63</td>
<td>2165 ± 63</td>
<td>2335 ± 53</td>
<td>—</td>
<td>&lt;.001</td>
<td>—</td>
</tr>
<tr>
<td>Clamp</td>
<td>2404 ± 41</td>
<td>2455 ± 70</td>
<td>2167 ± 82</td>
<td>2318 ± 55</td>
<td>—</td>
<td>.005</td>
<td>—</td>
</tr>
<tr>
<td>% change</td>
<td>-4 ± 2</td>
<td>-2 ± 1</td>
<td>0 ± 3</td>
<td>1 ± 1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Notes: Values are presented as means ± standard errors of the mean (in μmol/L). Results were analyzed by a two-factor analysis of variance (ANOVA) at basal and clamp periods. Change in response to the clamp was analyzed by a two-factor repeated-measures ANOVA on absolute data; % change is shown for ease of comparison.

*Clamp effect, \( p < .001 \).

1Different from men of same age, \( p = .006 \).

BCAA = branched-chain amino acids; IAA = indispensable amino acids; DAA = dispensable amino acids; AA = amino acids.
Obvious. At baseline, these two groups were matched for lower insulin sensitivity index, and a more blunted protein anabolic response to insulin. Thus, insulin resistance of the elderly men was more pronounced than in young men, as indicated by the lower insulin sensitivity index and the blunted anabolic response to insulin (33). The elderly men were more insulin resistant than were the young adults, suggesting accumulation of visceral adipose tissue, which has been linked with insulin resistance (29,34) more than with other fat depots. In addition, the decrease in serum free testosterone levels with age could also contribute to the blunted anabolic response (34).

We opted to include gender as a main, additional factor to aging in our statistical analyses because we have demonstrated it to have effects on the anabolic responses to insulin. The elderly men were more insulin resistant than were the women with respect to both glucose and protein metabolism: Elderly men had higher fasting insulin and plasma glucose, lower glucose infusion rates during the clamp, lower insulin sensitivity index, and a more blunted protein anabolic response to insulin. Thus, insulin resistance of protein was present concomitantly with that of glucose. A single explanation for a gender difference with aging is not obvious. At baseline, these two groups were matched for BMI and glucose tolerance by an oral glucose tolerance test, and were selected to include a range of physical activity levels. But, although percent body fat was higher in elderly women, elderly men had higher waist circumference suggesting accumulation of visceral adipose tissue, which has been linked with insulin resistance (29,34) more than with other fat depots. In addition, the decrease in serum free testosterone levels with age could also contribute to the blunted anabolic response (34).

The fact that, in the whole cohort studied, the rate of glucose disposal during the clamp correlated with the protein anabolic response reinforces that insulin resistance of glucose is accompanied by, and perhaps linked with, that of protein. This observation suggests that insulin resistance may occur at the early steps of the insulin-signaling pathway, shared by both glucose (for its cellular uptake) and by amino acids (for initiation of messenger RNA translation).

**Summary**

This study showed that the whole-body anabolic response to insulin was blunted in elderly compared with young men.
participants and was associated with the lesser insulin sensitivity of glucose. Although muscle mass is reduced with aging, this reduction did not fully explain the lower response seen in elderly participants. Increased relative adiposity combined with lower FFM, both changes in body composition intrinsic to the aging process, explained a large part of the lesser sensitivity of protein metabolism to insulin. Our results also indicated that the lesser anabolic response resulted from a blunted action of insulin in stimulating protein synthesis rather than in suppressing protein breakdown. Because amino acids can stimulate protein synthesis, elderly persons might benefit from an increased dietary protein intake to compensate for their insulin resistance.

**REFERENCES**


Received May 25, 2005
Accepted September 14, 2005
Decision Editor: Luigi Ferrucci, MD, PhD