TRANSLATIONAL PHYSIOLOGY

No major sex differences in muscle protein synthesis rates in the postabsorptive state and during hyperinsulinemia-hyperaminoacidemia in middle-aged adults

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Smith GI, Atherton P, Reeds DN, Mohammed BS, Jaffery H, Rankin D, Rennie MJ, Mittendorfer B. No major sex differences in muscle protein synthesis rates in the postabsorptive state and during hyperinsulinemia-hyperaminoacidemia in middle-aged adults. J Appl Physiol 107: 1308–1315, 2009. First published July 30, 2009; doi:10.1152/japplphysiol.00348.2009.—Men have more muscle than women, but most studies evaluating sex differences in muscle protein metabolism have been unable to discern sexual dimorphism in basal muscle protein turnover rates in young and middle-aged adults. We hypothesized that the anabolic response to nutritional stimuli (i.e., amino acids and insulin) would be greater in young/middle-aged men than women. We therefore measured the rates of muscle protein synthesis (MPS) in 16 healthy individuals [8 men and 8 women, matched for age (mean ± SE: 37.7 ± 1.5 yr) and body mass index (25.2 ± 0.7 kg/m²)] after an overnight fast (plasma insulin ~5 µU/ml and plasma phenylalanine ~60 µM) and during a hyperinsulinemic-hyperaminoacidemic-euglycemic clamp (plasma insulin ~28 µU/ml; plasma phenylalanine ~110 µM; plasma glucose ~5.4 mM). The rates of MPS were not different between men and women (ANOVA main effect for sex; P = 0.49). During the clamp, the rate of MPS increased by ~50% (P = 0.003) without any difference in the increases from basal values between men and women (+0.019 ± 0.004 vs. +0.018 ± 0.010%/h, respectively; P = 0.93). There were also no differences between men and women in the basal concentrations of muscle phosphorylated AktSer⁴⁷₃, AktThr³⁸⁹, mTORSer²⁴⁴⁸, and p70s6kThr³⁸⁹ or in the hyperinsulinemia-hyperaminoacidemia-induced increases in phosphorylation of those signaling elements (P ≥ 0.25). We conclude that there are no major differences in the rate of MPS and its intracellular control during basal conditions and during hyperinsulinemia-hyperaminoacidemia between young and middle-aged adult men and women.

WOMEN have less lean body (mostly muscle) mass and more body fat than men (9, 23). The differences in adult body composition are usually attributed to differences in the sex-hormone milieu. Testosterone is anabolic in muscle (15), and this is usually thought to be mediated by its transcriptional activity and eventual effect on muscle protein synthesis (MPS), although there are reports of inhibition of muscle protein breakdown by testosterone (5, 6, 35). Conversely, female sex steroids inhibit MPS and muscle growth in rodents (7, 34). Nonetheless, the rate of MPS has been reported to be the same during the follicular and luteal phase of the menstrual cycle in young women despite marked differences in plasma estradiol and progesterone concentrations (21), and the basal rate of MPS has been reported to be identical in men and women (11, 16, 26) or, contrary to the obvious assumption, slightly greater in women than in men (13, 32). Except for our recent study in older adults (32), previous studies focusing on sex differences in muscle protein metabolism evaluated only the basal rate of MPS. In 65- to 80-yr-old adults, we demonstrated significant sexual dimorphism in response to mixed meal ingestion (32) with a more pronounced anabolic response, both in terms of MPS and anabolic signaling, in men than in women. Consequently, we hypothesized that the anabolic response to nutritional stimuli (i.e., amino acids and insulin) would also be greater in younger men compared with younger women. We therefore measured the fractional rate of MPS during basal postabsorptive conditions and during hyperinsulinemia-hyperaminoacidemia-euglycemia by using stable isotope-labeled amino acid tracer techniques in young and middle-aged men and women; we also measured concentrations of total muscle RNA and protein to gauge the protein synthetic capacity. Furthermore, to gain some insight into the underlying cellular processes responsible for the anticipated differences, we measured the concentrations of activated (phosphorylated) elements of intracellular signaling pathways involved in the regulation of MPS [Akt; ribosomal protein S6 protein kinase (p70s6k); mammalian target of rapamycin (mTOR); extracellular signaling-regulated 1 and 2 mitogen-activated protein kinase (Erk 1/2 MAPK); and eukaryotic elongation factor 2 (eEF2)] (10, 29).

METHODS

Subjects. Eight men and eight age- and body mass index (BMI)-matched premenopausal women participated in this study (Table 1). All subjects were considered to be in good health after completing a comprehensive medical evaluation, which included a history and physical examination and standard blood tests. None of the subjects engaged in regular physical activities (i.e., they exercised ≤1.5 h/wk) or took medications (including hormonal contraceptives) that could interfere with muscle protein metabolism, and none reported excessive alcohol intake or consumed tobacco products. Written informed
A hyperinsulinemic-hyperaminoacidemic-euglycemic clamp was started for 7 h. Four hours after the start of the tracer infusions, 

\[ ^{2}H_{5}\text{phenylalanine} \]

and production during the clamp procedure, the 

increased plasma amino acid availability and reduced hepatic glucose concentration of

\[ 0.80 \text{ mg/dL} \]

ring 

\[ ^{2}H_{5}\text{phenylalanine} (\text{priming dose 2.8 g/kg FFM, infusion rate 0.08 g/kmol kg FFM/min}) \] and \[ [6,6,6^{2}H_{3}\text{glucose} (\text{priming dose 18 g/kmol kg wt, infusion rate 0.22 g/kmol kg body wt^{-1}min^{-1}}) \]

both purchased from Cambridge Isotope Laboratories (Andover, MA), were started and maintained for 7 h. Four hours after the start of the tracer infusions, a hyperinsulinemic-hyperaminoacidemic-euglycemic clamp was started and maintained for 3 h. Human insulin (Novolin R, Novo Nordisk, Princeton, NJ) was infused at a rate of 20 IU/m^2 body surface area (BSA)-min^{-1} (initiated with a priming dose of 80 IU/m^2 BSA-min^{-1} for 5 min and then 40 IU/m^2 BSA-min^{-1} for additional 5 min). Plasma amino acid availability was increased by providing an intravenous amino acid mixture (Travasol 10%, Baxter, Deerfield, IL) infused at a rate of 105 mg amino acids/kg FFM^{-1}h^{-1} (priming dose 35 mg amino acids/kg FFM). Euglycemia (blood glucose concentration of 5.5 mmol/L) was maintained during the clamp procedure by variable rate infusion of 20% dextrose (Baxter) enriched to 2.5% with [6,6,6^{2}H_{3}]glucose. To adjust for the increased plasma amino acid availability and reduced hepatic glucose production during the clamp procedure, the 

\[ [6,6,6^{2}H_{3}\text{glucose} \] and \[ [6,6,6^{2}H_{3}\text{glucose} \] infusion rates were increased to 0.12 g/kmol kg FFM^{-1}min^{-1} (phenylalanine) and decreased to 0.11 g/kmol kg body wt^{-1}min^{-1} (glucose), respectively.

Blood samples (4 ml) were obtained before beginning the tracer infusion and then at 30, 60, 90, 180, 210, 220, 230, 240, 270, 300, 330, 360, 390, 400, 410, and 420 min to determine the labeling of phenylalanine and glucose in plasma and plasma substrate and hormone concentrations. Additional blood (~1 ml) was obtained every 10 min during the clamp procedure to monitor plasma glucose concentration. Muscle tissue (~50–100 mg) was obtained under local anesthesia (lidocaine, 2%) from the quadriceps femoris using a Tilley-Henkel forceps (4) at 1 and 4 h to determine the basal rate of MPS (labeled amino acid incorporation into muscle protein; see Calculations) and the basal concentrations of phosphorylated elements of intramuscular signal transduction proteins (Akt; mTOR; p70s6k; Erk 1/Erk 2 MAPK; and eEF2) involved in the regulation of MPS. A third muscle biopsy was obtained at 7 h (i.e., 3 h after starting the clamp procedure) to determine both the rate of MPS and the intracellular signaling responses to hyperinsulinemia-hyperaminoacidemia. The second and third biopsies were obtained from the same incision on the leg contralateral to that biopsied first; the forceps was directed in proximal and distal direction so that the two biopsies were collected ~5–10 cm apart.

Sample processing and analyses. One milliliter of blood was collected in prechilled tubes containing heparin, plasma was separated immediately by centrifugation, and glucose concentration was measured immediately. The remaining blood (~3 ml) was collected in prechilled tubes containing EDTA; plasma was separated by centrifugation within 30 min of collection and then stored at ~80°C until final analyses. Muscle samples were rinsed in ice-cold saline immediately after collection, cleared of visible fat and connective tissue, frozen in liquid nitrogen, and stored at ~80°C until final analysis.

Plasma glucose concentration was measured on an automated glucose analyzer (Yellow Spring Instruments, Yellow Springs, OH). Plasma insulin concentration was determined by radioimmunoassay (Linco Research, St. Louis, MO). ELISA kits purchased from Immuno-Biological Laboratories (IBL-America, Minneapolis, MN) were used to determine the concentrations of testosterone, progesterone, 17β-estradiol, and sex hormone binding globulin (SHBG) in plasma.

To determine the labeling of plasma glucose, plasma proteins were precipitated with ice-cold acetone, and hexane was used to extract plasma lipids. The aqueous phase, containing glucose, was dried by speed-vac centrifugation (Savant Instruments, Farmingdale, NY), glucose was derivatized with heptfluorobutyric acid, and the tracer-to-trace ratio (TTR) was determined by using gas-chromatography/mass-spectrometry (GC-MS, Hewlett-Packard MSD 5973 system with capillary column) as previously described (24).

To determine plasma concentrations of leucine [thought to be a major regulator of MPS (30)] and phenylalanine and the labeling of plasma phenylalanine, known amounts of nor-leucine and [1-^{13}C]phenylalanine were added to an aliquot of each plasma sample, plasma proteins were precipitated, and the supernatant, containing free amino acids, was collected to prepare the t-butylidimethylsilyl (t-BDMS) derivative of phenylalanine to determine their TTRs by GC-MS (MSD 5973 System, Hewlett-Packard) (27, 33). To determine phenylalanine labeling in muscle proteins and in tissue fluid, samples (~20 mg) were homogenized in 1 ml trichloroacetic acid solution (3% wt/vol), proteins precipitated by centrifugation, and the supernatant, containing free amino acids, collected. The pellet containing muscle proteins was washed and then hydrolyzed in 6 N HCl at 110°C for 24 h. Amino acids in the protein hydrolysate and supernatant samples were purified on cation-exchange columns (Dowex 50W-X8–200, Bio-Rad Laboratories, Richmond, CA), and the t-BDMS derivative of phenylalanine prepared to determine its TTR by GC-MS (MSD 5973 System, Hewlett-Packard) (27, 33). The extent of phenylalanine labeling in blood, muscle tissue fluid, and muscle protein was calculated based on the simultaneously measured TTR of standards of known isotope labeling.

Western analysis was used to measure the phosphorylation of Akt, mTOR, p70s6k, Erk 1/Erk 2 MAPK, and eEF2. Briefly, frozen muscle tissue (~20 mg) was rapidly homogenized in ice-cold buffer [50 mM Tris·HCl, pH 7.5, 1 mM EDTA, 1 mM EGTA, 10 mM glycerophosphate, 50 mM NaF, 0.1% Triton X, 0.1% 2-mercaptoethanol, 1 complete protease inhibitor tablet (Roche Diagnostics, Burgess Hill, UK)] at 10 μl/mg tissue. Proteins were extracted by shaking for 15 min at 4°C, and samples were then centrifuged at 13,000 g for 10 min at 4°C and the supernatant containing the proteins was collected. The

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Body mass index, kg/m²</th>
<th>Body mass, kg*</th>
<th>Fat-free mass, kg*</th>
<th>Appendicular muscle mass, kg*</th>
<th>SHBG, nmol/l</th>
<th>Testosterone, ng/ml</th>
<th>Progesterone, ng/ml</th>
<th>17β-Estradiol, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>38±2</td>
<td>25.5±1.0</td>
<td>78.5±4.5</td>
<td>62.1±2.8</td>
<td>165±19</td>
<td>5.22±0.46</td>
<td>0.40±0.07</td>
<td>25.4±1.8</td>
</tr>
<tr>
<td>Women</td>
<td>37±2</td>
<td>25.0±0.9</td>
<td>68.4±1.8</td>
<td>46.2±2.0</td>
<td>309±33</td>
<td>0.43±0.09</td>
<td>4.61±1.57</td>
<td>56.5±10.9</td>
</tr>
</tbody>
</table>

Values are means ± SE. SHBG: sex-hormone binding globulin. *Measured by dual-energy X-ray absorptiometry as described in Experimental protocol.
protein concentration in the supernatant was determined by the Bradford method with a commercial reagent (B6916, Sigma-Aldrich, St. Louis, MO) and adjusted to 3 mg/ml in 3× Laemmli buffer. Fifty microliters of protein from each sample was loaded onto 12% XT-Bis Tris gels, separated by SDS PAGE, and transferred on ice at 100 V for 45 min to methanol-prewetted 0.2-μm PVDF membranes. Blots were then incubated sequentially with 5% (wt/vol) nonfat milk and primary antibodies (anti-rabbit anti-β-actin, anti-eEF2Thr56, Erk 1/Erk 2 MAPKTyr202/204, and pan-actin (loading control) purchased from New England Biolabs. Membranes were developed using Immobillon Western Chemiluminescent HRP substrate (Millipore, Billerica, MA), and the protein bands were visualized and quantified by densitometry on a Chemidoc XRS (Bio-Rad Laboratories, Hercules, CA) ensuring no pixel saturation. Data were expressed in relation to GADPH.

To determine the total RNA-to-protein ratio in muscle, an index of the capacity for protein synthesis, an aliquot of the total RNA concentration was measured after removal of the DNA interphase and protein concentration was measured after complete removal of the upper phase following phase separation; protein concentration was measured using Immobillon Western Chemiluminescent HRP substrate (Millipore, Billerica, MA), and the protein bands were visualized and quantified by densitometry on a Chemidoc XRS (Bio-Rad Laboratories, Hercules, CA) ensuring no pixel saturation. Data were expressed in relation to GADPH.

**RESULTS**

Subjects’ age and body composition. Men and women were matched for age and BMI (Table 1). As expected, total body weight, FFMM, and muscle mass were less in women than in men; plasma SHBG, estradiol, and progesterone concentrations were ~2-fold (SHBG and estradiol) to 10-fold (progesterone) greater (P < 0.02) in women than in men and plasma testosterone concentration were ~12-fold greater (P < 0.001) in men than in women (Table 1).

**Plasma glucose, insulin, leucine, and phenylalanine concentrations.** Plasma glucose and insulin concentrations were not different between men and women in the basal state; during the clamp, plasma glucose was successfully maintained at ~5.4 mM and insulin concentrations rose by ~4-fold both in men and women (Table 2). There were no statistically significant differences in plasma leucine or phenylalanine concentrations between men and women; although plasma leucine concentration tended to be greater in men (Table 2). During the clamp, plasma leucine concentration increased by ~30% from basal values (P < 0.001) and plasma phenylalanine concentration approximately doubled (P < 0.001) in each group (Table 2).

**Glucose kinetics.** Basal glucose Ra was not different in men and women (12.7 ± 0.7 vs. 14.2 ± 0.6 μmol·kg⁻¹·min⁻¹, respectively; P = 0.12). During the clamp, glucose Ra decreased by ~70% to 3.9 ± 0.3 μmol·kg⁻¹·min⁻¹ in men and 4.1 ± 0.7 μmol·kg⁻¹·min⁻¹ in women (P = 0.75). Glucose Rd during the clamp was ~25% greater in women than in men (39.9 ± 3.7 vs. 32.1 ± 2.7 μmol·kg⁻¹·min⁻¹, respectively; P = 0.057).

**Free phenylalanine labeling in plasma and muscle tissue.** Plasma phenylalanine labeling was at steady state during evaluation of the muscle protein FSR, both in the basal state and during the hyperinsulinemic-hyperaminoacidemic-euglycemic clamp; i.e., the phenylalanine TTR (grand mean ± SE) was 0.092 ± 0.003 and 0.103 ± 0.002 at 60 and 240 min after the start of the tracer infusion, respectively, and 0.103 ± 0.003 and 0.098 ± 0.002 at 30 and 180 min after the start of the amino acid and insulin infusion, respectively. During basal conditions, the labeling of phenylalanine in plasma was greater (P < 0.01) in men than in women.

### Table 2. Plasma glucose, insulin, leucine, and phenylalanine concentrations during basal postabsorptive conditions, and during the hyperinsulinemic-hyperaminoacidemic-euglycemic clamp procedure

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Clamp</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>4.9 ± 0.1²</td>
<td>5.4 ± 0.1*²</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>5.5 ± 1.3²</td>
<td>27.3 ± 2.1*²</td>
</tr>
<tr>
<td>Leucine, μM</td>
<td>120 ± 7²</td>
<td>162 ± 12*²</td>
</tr>
<tr>
<td>Phenylalanine, μM</td>
<td>57 ± 7²</td>
<td>100 ± 13*²</td>
</tr>
</tbody>
</table>

Values are means ± SE *Value significantly different from corresponding value during basal conditions (P < 0.001).
women, whereas the labeling of free phenylalanine in muscle was greater ($P < 0.01$) in women than in men (Table 3). Consequently, the muscle-to-plasma free phenylalanine labeling ratio was greater ($P < 0.01$) in women than in men. These differences between the sexes disappeared during the amino acid insulin infusion (Table 3).

MPS. There were no differences between men and women in the muscle RNA concentration ($0.34 \pm 0.05$ vs. $0.37 \pm 0.05 \mu g\text{RNA/mg muscle wet wt}$, respectively; $P = 0.66$), the muscle alkali soluble protein concentration ($113 \pm 24$ vs. $101 \pm 17 \mu g\text{protein/mg muscle wet wt}$, respectively; $P = 0.72$), or the capacity for MPS (i.e., total RNA-to-protein ratio in muscle: $4.6 \pm 1.3$ vs. $4.5 \pm 0.9 \mu g\text{RNA/mg protein}$, respectively; $P = 0.95$). There were also no differences between men and women in the basal FSR when calculated with free muscle tissue phenylalanine labeling as the precursor pool labeling (Fig. 1); the minimum and maximum basal muscle protein FSR values in men and women were $0.021$ and $0.025%/h$, respectively (min) and $0.066$ and $0.062%/h$ (max), respectively. During the amino acid/insulin infusion, the FSR increased by $\sim 50\%$ ($P = 0.003$) in both men and women with no difference in the increase between them ($+0.019 \pm 0.004%/h \text{vs. } +0.018 \pm 0.010%/h$, respectively; $P = 0.93$). Hence, the translational efficiency was not different between men and women, either during basal conditions $[0.010 (0.006, 0.019) \text{vs. } 0.008 (0.006, 0.021) \text{mg protein-}\mu\text{g RNA}^{-1}\cdot\text{h}^{-1}$, respectively; $P = 0.82$] or during the amino acid/insulin infusion $[0.013 (0.008, 0.041) \text{vs. } 0.016 (0.008, 0.025) \text{mg protein-}\mu\text{g RNA}^{-1}\cdot\text{h}^{-1}$, respectively; $P = 0.73$).

Neither the plasma estradiol or progesterone concentrations nor the estradiol-to-progesterone concentration ratio in plasma correlated with the muscle protein FSR in women (all $P \geq 0.29$).

Importantly, when muscle FSR values were calculated using plasma phenylalanine labeling as a surrogate for the precursor pool labeling to allow a direct comparison of our results with those obtained by Henderson et al. (13), who unlike others (11, 16, 26) before reported a difference between men and women in the basal rate of muscle protein synthesis, the calculation yielded basal rates of MPS that were apparently greater in women than in men; the FSR values obtained ($0.031 \pm 0.004$ vs. $0.021 \pm 0.003%/h$, respectively; $P = 0.05$) were very close to those reported by Henderson et al (13). The use of the plasma labeling as the precursor pool did not affect the magnitude of the anabolic responses or reveal any differences in the two groups during the insulin and amino acid infusion ($P = 0.63$).

Phosphorylation of signaling transduction proteins in muscle. In the postabsorptive state, the concentrations of both phosphorylated Akt$^{Thr308}$ and Akt$^{Ser473}$ and phosphorylated mTOR$^{Ser2448}$ and p70S6K$^{Thr389}$ in muscle were not different in men and women (Fig. 2). Hyperinsulinemia-hyperaminoacidemia increased the concentrations of these phosphorylated signaling elements (all $P \leq 0.05$) in both men and women without a difference in the response ($P \geq 0.25$) between men and women (Fig. 2). Phosphorylated Erk 1/2 MAPK$^{Tyr202/204}$ in muscle was $\sim 50–100\%$ greater ($P = 0.051$) in women than in men both during postabsorptive conditions and during amino acid/insulin infusion; amino acid and insulin infusion had no effect ($P = 0.14$) on the concentration of phosphorylated Erk 1/2 MAPK$^{Tyr202/204}$ (Fig. 3). The concentration of phosphorylated eEF2$^{Thr56}$ in muscle was $\sim 20\%$ less in women than in men both during postabsorptive conditions and during amino acid/insulin infusion, but the difference did not reach statistical significance ($P = 0.11$); amino acid/insulin infusion had no effect ($P = 0.60$) on the concentration of phosphorylated eEF2$^{Thr56}$ (Fig. 3).

**DISCUSSION**

In the present study we did not discover major differences between the rates of MPS in men and women, either during basal postabsorptive conditions or during hyperinsulinemia-hyperaminoacidemia. Furthermore we were unable to detect major differences in the capacity for MPS, as RNA-to-protein ratio, or in the concentration of phosphorylated elements of the Akt-mTOR pathway regulating translation.

Our results regarding the rates of MPS in men and women are in agreement with all previous studies (11, 13, 16, 26) in which sex differences in basal muscle protein turnover were evaluated in young to middle aged men and women. All (11,

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**Table 3. Free phenylalanine tracer-to-tracee ratios in plasma and muscle tissue water during basal postabsorptive conditions and during the hyperinsulinemic-hyperaminoacidemic clamp procedure**

<table>
<thead>
<tr>
<th></th>
<th>Men Basal</th>
<th>Men Clamp</th>
<th>Women Basal</th>
<th>Women Clamp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmal</td>
<td>0.111±0.0026</td>
<td>0.0958±0.0013†</td>
<td>0.1045±0.0018*</td>
<td>0.0979±0.0027†</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.0615±0.0023</td>
<td>0.0741±0.0030†</td>
<td>0.0759±0.0027*</td>
<td>0.0757±0.0014</td>
</tr>
<tr>
<td>Muscle/plasma</td>
<td>0.5535±0.0122</td>
<td>0.7726±0.0244†</td>
<td>0.7259±0.0217*</td>
<td>0.7751±0.0153†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Value significantly different from corresponding value in men ($P < 0.01$). †Value significantly different from corresponding basal value ($P < 0.05$).
sufficiently small probability of Type I error (0.05), we
sufficient power (ences of the biggest and smallest magnitude observed with
sample size required to detect statistically significant differ-
ences in the rates of MPS in women than in men, although, unlike in the present
study, the difference between men and women in the study by
Henderson et al. (13) remained statistically significant when
using the intracellular phenylalanine labeling as precursor. The
reasons for this discrepancy are not entirely clear but most
likely include both differences in sample size and subject
characteristics. Henderson et al. (13) studied 62 young (30
men; 32 women) and 144 older (87 men; 57 women) adults
during basal postabsorptive conditions and found a statistically
significant main effect for sex with the FSR being greater in
women than in men. However, they did not present the FSR
values obtained using the intracellular phenylalanine labeling
as precursor; therefore the magnitude of the difference using
this analytical approach remains unknown. From the data
presented, we cannot exclude the possibility that this effect was
largely due to differences between older men and women since
we have previously observed a ~30% greater basal FSR in
older women than older men (32).

On the other hand, it is possible that we missed a difference
in the rates of MPS between men and women. Moreover,
in excellent agreement with the results we obtained by using plasma phenylalanine labeling in the calculation of
the FSR, Henderson et al. (13) report similar basal FSR values
as we did in the present study and a ~15% greater basal rate of
MPS in women than in men, although, unlike in the present
study, the difference between men and women in the study by
Henderson et al. (13) remained statistically significant when
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largely due to differences between older men and women since
we have previously observed a ~30% greater basal FSR in
older women than older men (32).

On the other hand, it is possible that we missed a difference
in the rates of MPS between men and women due to a type II
statistical error because the differences in the rates of MPS
between our men and women were small. Overall (main
effect), the average FSR was 13% greater in women than in
men; 32 women) and 144 older (87 men; 57 women) adults
during basal postabsorptive conditions and found a statistically
significant main effect for sex with the FSR being greater in
women than in men. However, they did not present the FSR
values obtained using the intracellular phenylalanine labeling
as precursor; therefore the magnitude of the difference using
this analytical approach remains unknown. From the data
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On the other hand, it is possible that we missed a difference
in the rates of MPS between men and women due to a type II
statistical error because the differences in the rates of MPS
between our men and women were small. Overall (main
effect), the average FSR was 13% greater in women than in
men and the difference in the average anabolic response (i.e.,
the hyperaminoacidemia-hyperinsulinemia-induced increase in
FSR from basal values) was 5%. To obtain an estimate of the
minimum sample size required is as follows: for basal FSR, ≥98
subjects per group assuming the larger of the two SD (i.e.,
that reported for our women) and ≥69 subjects per group assuming
the smaller of the two SD (i.e., that reported for our men); for
anabolic response, >1,220 subjects per group assuming
the larger of the two SD and >605 subjects per group assuming
the smaller of the two SD. Therefore it is conceivable that our
study was underpowered to detect a difference in the absolute
FSR between men and women; however, we consider it un-
likely that the failure to observe a difference in the anabolic
response in men and women was due to a lack of statistical
power—more likely the difference, if it exists, is probably
negligible.

Ideally, the muscle protein FSR is calculated by using the
protein labeling as the numerator and the amino acyl-tRNA
labeling as the denominator in the precursor-product equation.
However, technical difficulties (38, 40) limit this approach, and
the amino acid labeling in muscle fluid [or that of the venous
plasma α-ketosioacropic acid (KIC) for leucine (26)] has been
shown to be a suitable surrogate (3, 20, 38). It has been
demonstrated that the phenylalanine labeling in muscle tissue
fluid is not different from the phenylalanine tRNA labeling,
whereas the plasma phenylalanine labeling is ~40–50%
greater than the phenylalanine tRNA labeling (1, 3). Further-
more, it has been demonstrated that the relationship between
the tissue fluid phenylalanine and the tRNA labeling remains
constant during changing plasma phenylalanine availability

16, 26) but one (13) of the previous studies failed to observe a
difference in the basal rate of MPS between men and women.
Moreover, in excellent agreement with the results we obtained
by using plasma phenylalanine labeling in the calculation of
the FSR, Henderson et al. (13) report similar basal FSR values
as we did in the present study and a ~15% greater basal rate of
MPS in women than in men, although, unlike in the present
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statistical error because the differences in the rates of MPS
between our men and women were small. Overall (main
effect), the average FSR was 13% greater in women than in
men and the difference in the average anabolic response (i.e.,
the hyperaminoacidemia-hyperinsulinemia-induced increase in
FSR from basal values) was 5%. To obtain an estimate of the
minimum sample size required is as follows: for basal FSR, ≥98
subjects per group assuming the larger of the two SD (i.e.,
that reported for our women) and ≥69 subjects per group assuming
the smaller of the two SD (i.e., that reported for our men); for
anabolic response, >1,220 subjects per group assuming
the larger of the two SD and >605 subjects per group assuming
the smaller of the two SD. Therefore it is conceivable that our
study was underpowered to detect a difference in the absolute
FSR between men and women; however, we consider it un-
likely that the failure to observe a difference in the anabolic
response in men and women was due to a lack of statistical
power—more likely the difference, if it exists, is probably
negligible.

Ideally, the muscle protein FSR is calculated by using the
protein labeling as the numerator and the amino acyl-tRNA
labeling as the denominator in the precursor-product equation.
However, technical difficulties (38, 40) limit this approach, and
the amino acid labeling in muscle fluid [or that of the venous
plasma α-ketosioacropic acid (KIC) for leucine (26)] has been
shown to be a suitable surrogate (3, 20, 38). It has been
demonstrated that the phenylalanine labeling in muscle tissue
fluid is not different from the phenylalanine tRNA labeling,
whereas the plasma phenylalanine labeling is ~40–50%
greater than the phenylalanine tRNA labeling (1, 3). Further-
more, it has been demonstrated that the relationship between
the tissue fluid phenylalanine and the tRNA labeling remains
constant during changing plasma phenylalanine availability

Fig. 2. Average concentrations (arbitrary units) and repre-
sentative blots (top: protein of interest; bottom: GADPH)
of phosphorylated p70S6kThr389 (A), mTORSer2448 (B),
AktSer473 (C), and AktThr308 (D) during basal postab-
sorptive conditions and during the hyperinsulinemic-
hyperaminoacidemic-euglycemic clamp procedure in
men and women. Values are means ± SE.*ANOVA
revealed a significant main effect for amino acid/insulin
infusion (P’ < 0.05) but no effect of sex and no interac-
tion.
When the phenylalanine labeling is maintained constant, whereas the relationship between plasma phenylalanine and tRNA labeling is not (3). In our view it is therefore valid to use the muscle tissue fluid phenylalanine labeling as the precursor labeling for the calculation of FSR, whereas the use of the plasma phenylalanine labeling may not be valid.

We observed differences between men and women in the labeling of phenylalanine in plasma and muscle free tissue fluid during basal conditions. Since the phenylalanine tracer was infused at the same rate relative to FFM in men and women in our study, this indicates that the rate of appearance of unlabeled phenylalanine into plasma relative to total body FFM is greater in women than men; however, the source of the “extra” phenylalanine is not known and cannot be determined from the data collected in our study. The muscle tissue fluid phenylalanine labeling is determined by the rate of appearance of unlabeled phenylalanine relative to the rate of appearance of labeled phenylalanine into the muscle tissue pool. The difference between men and women in muscle tissue fluid phenylalanine labeling cannot be interpreted conclusively with the data at hand. To our knowledge differences of this kind have either not been observed or not been reported. In any case, although interesting, these findings do not compromise the validity of our MPS measurements.

We were unable to discern substantial differences between the sexes in the capacity for protein synthesis (RNA/protein) or the concentration of a substantial number of the phosphorylated signaling proteins examined in muscle during postabsorptive conditions, except, and in agreement with our previous study in older men and women (32), a trend for decreased eEF2Thr56 and increased Erk 1/Erk 2 MAPKY202/204 in women. These results are novel and have not been reported previously. Furthermore, the anabolic response to hyperinsulinemia-hyperaminoacidemia in the present study, both with regard to signaling events as well as the change in muscle protein FSR (irrespective of the precursor enrichment; i.e., plasma phenylalanine vs. muscle free phenylalanine, used to calculate the FSR), was not different in men and women. We are not aware of any study that compared the muscle protein synthesis response to nutritional stimuli in men and women, except for a recent one we conducted in older adults in whom we found the anabolic response to liquid meal feeding was less in women than in men (32). The differences in experimental design, however, preclude us from drawing firm conclusions regarding potentially different age-associated changes in the anabolic response to nutritional stimuli.

The absence of a difference in the rates of MPS between young and middle-aged men and women or the greater rate of MPS in women than in men reported by us in the present study and others (11, 13, 16, 26) may at first seem counterintuitive, because testosterone is well known to be anabolic and increases the basal rate of MPS and muscle mass in both healthy and hypogonadal young men (2, 6, 12, 35); and there is evidence that ovariectomy increases the rate of MPS, and progesterone and estrogen reintroduction inhibit MPS in ovariectomized rats (34). However, it may be that although the steroid hormones may set the size of peak muscle mass attained during young adulthood, once that has been achieved and hormone levels remain within the normal physiological range they have no further influence on the relative rates of protein turnover. In fact, the difference in lean body mass, even when adjusted for differences in height between the sexes, is evident from infancy and becomes most marked after 15 years of age, due to continued growth in boys (37, 39), probably mediated by the surge in secretion of testosterone (8, 15). Before puberty and after the accelerated growth rate of lean body mass during puberty and early adulthood in boys, however, changes in lean body mass relative to longitudinal growth with time proceed largely in parallel in boys and girls/men and women; once adulthood is attained and sex hormone concentrations are relatively stable there is little change in fat-free/muscle mass in both men and women (9, 17, 18, 39). Furthermore, it has been demonstrated that the stimulatory effect of testosterone on the rate of MPS is lost after chronic administration when a new steady muscle mass is achieved (13, 25).

Although no difference between men and women was observed for the anabolic response of MPS to hyperinsulinemia-hyperaminoacidemia, glucose Rd during the clamp was greater in women than men, indicating greater sensitivity of glucose metabolism to insulin in women. This is consistent with the results of previous studies focused on glucose metabolism (28, 41) and potentially reveals a dissociation between the nutritional regulation of glucose and protein metabolism. Indeed, it
has been demonstrated that muscle glucose uptake is many times more sensitive to the effect of insulin than MPS (14, 19). Since the response of relatively early events in the insulin signaling cascade (i.e., Akt and mTOR phosphorylation) were not different in men and women, divergent signaling events that could explain these findings on glucose metabolism may occur independent of or distal to Akt/mTOR.

In summary, we conclude that there are no major differences in the rate of MPS and its control during basal conditions and during hyperinsulinemia-hyperaminoacidaemia between young adult men and women.

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