

# Anabolic sensitivity of postprandial muscle protein synthesis to the ingestion of a protein-dense food is reduced in overweight and obese young adults<sup>1,2</sup>

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## ABSTRACT

**Background:** Excess body fat diminishes muscle protein synthesis rates in response to hyperinsulinemic-hyperaminoacidemic clamps. However, muscle protein synthetic responses after the ingestion of a protein-dense food source across a range of body mass indexes (BMIs) have not been compared.

**Objective:** We compared the myofibrillar protein synthetic response and underlying nutrient-sensing mechanisms after the ingestion of lean pork between obese, overweight, and healthy-weight adults.

**Design:** Ten healthy-weight [HW; BMI (in kg/m<sup>2</sup>): 22.7 ± 0.4], 10 overweight (OW; BMI: 27.1 ± 0.5), and 10 obese (OB; BMI: 35.9 ± 1.3) adults received primed continuous L-[ring-<sup>13</sup>C<sub>6</sub>]phenylalanine infusions. Blood and muscle biopsy samples were collected before and after the ingestion of 170 g pork (36 g protein and 3 g fat) to assess skeletal muscle anabolic signaling, amino acid transporters [large neutral and small neutral amino acid transporters (LAT1, SNAT2) and CD98], and myofibrillar protein synthesis.

**Results:** At baseline, OW and OB groups showed greater relative amounts of mammalian target of rapamycin complex 1 (mTORC1) protein than the HW group. Pork ingestion increased mTORC1 phosphorylation only in the HW group ( $P = 0.001$ ). LAT1 and SNAT2 protein content increased during the postprandial period in all groups (time effect,  $P < 0.05$ ). Basal myofibrillar protein synthetic responses were similar between groups ( $P = 0.43$ ). However, myofibrillar protein synthetic responses (0–300 min) were greater in the HW group (1.6-fold;  $P = 0.005$ ) after pork ingestion than in the OW and OB groups.

**Conclusions:** There is a diminished myofibrillar protein synthetic response to the ingestion of protein-dense food in overweight and obese adults compared with healthy-weight controls. These data indicate that impaired postprandial myofibrillar protein synthetic response may be an early defect with increasing fat mass, potentially dependent on altered anabolic signals, that reduces muscle sensitivity to food ingestion. This trial was registered at [clinicaltrials.gov](http://clinicaltrials.gov) as NCT02613767. *Am J Clin Nutr* doi: 10.3945/ajcn.116.130385.

**Keywords:** insulin resistance, leucine, muscle mass, inflammation, amino acid transporters, mTORC1

## INTRODUCTION

An estimated 35% of US adults are obese, with a BMI (in kg/m<sup>2</sup>) >30 (1). Obesity and excess fat mass have been linked to metabolic alterations in various tissues (2). This impaired metabolism is prominent in skeletal muscle despite the greater lean body mass generally observed in obese compared with healthy-weight adults (3). Skeletal muscle metabolism has a prominent role in the regulation of blood glucose and blood lipids and is a primary contributor to basal metabolic rate (4). These metabolic functions may be impaired in obese individuals due to reduced protein turnover, which leads to reduced quality and composition of the muscle proteome (5).

Few studies have assessed the effects of adiposity on skeletal muscle protein metabolism in vivo in humans. The available evidence indicates that increased adiposity, and its associated metabolic perturbations, may lead to altered regulation of muscle protein synthesis rates in response to elevated plasma amino acid availability during insulin clamped conditions (5–7). In other studies in which an impaired postprandial muscle protein synthetic response was observed, the obese participants were older adults with no comparison to healthy-weight controls (8, 9). Thus, it is difficult to distinguish between the independent effects of obesity and aging on the regulation of muscle protein synthesis rates in these studies. For example, aging muscle appears to be “anabolically resistant” to dietary protein-derived amino acids, independent of the potential negative effects of increased adiposity (10).

To date, studies that examined protein metabolism with obesity were performed in a setting in which intravenous amino acid infusions are applied under insulin clamped conditions (5–7) or with small intermittent feeding patterns (8, 9) as opposed to a setting that more closely reflects a normal eating pattern of US

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<sup>2</sup> Supplemental Figure 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

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adults, such as bolus protein ingestion. Moreover, we are unaware of any studies that assessed the postprandial muscle protein synthetic response to the ingestion of a protein-dense food source across a continuum of BMIs and fat percentages in young adults. Therefore, the purpose of the current study was to compare the postprandial myofibrillar protein synthetic response to the ingestion of a single protein-dense meal in young healthy-weight, overweight, and obese men and women. We hypothesized that ingesting 6 ounces of lean pork loin (36 g protein, ~3.3 g leucine, 2.8 g fat), representing a typical portion for an American dinner (11), would provide a strong stimulatory signal to skeletal muscle tissue and, as such, result in a similar stimulation of postprandial muscle protein synthesis rates across all conditions.

## METHODS

### Participants and ethical approval

Ten healthy-weight (HW;<sup>7</sup> BMI: 22.7 ± 0.4), 10 overweight (OW; BMI: 27.1 ± 0.5), and 10 obese (OB; BMI 35.9 ± 1.3) adults volunteered to participate in this study. The participants were counterbalanced for age and sex. Participants were not involved in a regular exercise-training program and were considered sedentary. Participant characteristics are shown in **Table 1**. All of the participants were deemed healthy on the basis of responses to a routine medical screening questionnaire and had no previous history of participating in stable isotope amino acid tracer experiments. Each participant was informed of the purpose of the study, experimental procedures, and all of its potential risks before providing written consent to participate. The study was approved by the University of Illinois Institutional Review Board and conformed to standards for the use of human participants in research as outlined in the sixth Declaration of Helsinki. The trial was registered at clinicaltrials.gov as NCT02613767.

### Experimental design

A parallel-group design was used for this study. Before the infusion trial, participants reported to the laboratory in the morning after a 10-h fast for the determination of oral-glucose tolerance and resting blood pressure. Blood glucose and plasma insulin concentrations were determined before and after the consumption of 75 g glucose dissolved in 500 mL water. In addition, body weight and height were measured as well as body composition by dual-energy X-ray absorptiometry (Hologic QDR 4500A). Participants' waist-to-hip ratio was also measured by using the minimum waist–maximum hip method (12). Participants were instructed to refrain from vigorous physical activity and alcohol for 3 d before the tracer infusion. All of the participants consumed a standardized meal of the same composition (~30% estimated total daily energy expenditure, providing 50% of energy from carbohydrate, 25% of energy from

**TABLE 1**  
Participant characteristics<sup>1</sup>

Variable	Group		
	HW	OW	OB
Participants, <i>n/n</i> females	10/5	10/5	10/5
Age, y	24 ± 1	26 ± 2	27 ± 3
Height, m	1.73 ± 0.03	1.70 ± 0.02	1.71 ± 0.03
Weight, kg	68.5 ± 3.5	78.6 ± 2.2	106.0 ± 5.0*†
BMI, kg/m <sup>2</sup>	22.7 ± 0.4	27.1 ± 0.5	35.9 ± 1.3
Waist-to-hip ratio	0.79 ± 0.02	0.83 ± 0.02	0.92 ± 0.01*†
Body fat, %	22.2 ± 1.8	29.1 ± 1.4*	35.3 ± 1.8*†
Lean mass, kg	51.3 ± 3.7	53.8 ± 1.3	65.5 ± 3.2*†
Systolic BP, mm Hg	120.4 ± 3.0	125.9 ± 2.2	131.5 ± 3.0*
Diastolic BP, mm Hg	75.2 ± 1.7	79.4 ± 1.8	87.2 ± 1.5*†
C-reactive protein, mg/L	0.82 ± 0.29	0.84 ± 0.18	6.55 ± 1.19*†
Fasting glucose, mg/dL	78.0 ± 2.2	78.3 ± 1.2	82.0 ± 2.1
120-min glucose, mg/dL	78.5 ± 6.1	80.6 ± 4.2	99.2 ± 7.7*†
Fasting insulin, μIU/mL	7.1 ± 0.9	6.5 ± 0.5	25.2 ± 3.8*†
HOMA-IR	1.36 ± 0.17	1.25 ± 0.11	5.82 ± 0.81*†

<sup>1</sup>Values are means ± SEMs. Glucose values were obtained from the oral-glucose-tolerance test. Demographic, body-composition, BP, C-reactive protein, and HOMA-IR data were analyzed by 1-factor ANOVA. Fasting glucose and 120-min glucose data were analyzed by using 2-factor repeated-measures ANOVA. A Tukey's post hoc test was used to locate differences between group means when indicated by significant group effects or group × time interactions. \*Different from HW group ( $P < 0.05$ ); †different from OW group ( $P < 0.05$ ). Glucose: group effect,  $P = 0.59$ ; time effect,  $P < 0.001$ ; group × time,  $P < 0.001$ . BP, blood pressure; HW, healthy-weight; OB, obese; OW, overweight.

fat, and 25% of energy from protein) the evening before each tracer infusion trial.

### Infusion protocol

On the day of the infusion trial, participants reported to the laboratory at ~0700 after an overnight fast. A Teflon catheter was inserted into an antecubital vein for baseline blood sample collection ( $t = -195$  min), after which the plasma phenylalanine pool was primed with a single intravenous dose of L-[ring-<sup>13</sup>C<sub>6</sub>] phenylalanine (2 μmol/kg). Subsequently, an intravenous infusion of L-[ring-<sup>13</sup>C<sub>6</sub>]phenylalanine (0.05 μmol · kg<sup>-1</sup> · min<sup>-1</sup>) was initiated ( $t = -180$  min) and maintained until the end of the trial. A second Teflon catheter was placed in a contralateral dorsal hand vein, which was placed in a heated blanket for repeated arterialized blood sampling. In the postabsorptive state, muscle biopsy sample of the vastus lateralis were collected at  $t = -120$  and 0 min of infusion. Subsequently, the participants consumed 170 g ground pork. Participants were also given 300 mL water enriched to 4% with L-[ring-<sup>13</sup>C<sub>6</sub>]phenylalanine according to the phenylalanine content of pork to minimize disturbances in isotopic equilibrium during the infusion. Additional muscle biopsy samples were collected at 120 and 300 min after pork ingestion. Arterialized blood samples were drawn every 30 or 60 min during the postabsorptive and postprandial states. The biopsy samples were collected from the middle region of the vastus lateralis (15 cm above the patella) through separate incisions with a Bergström needle under local anesthesia. The resting muscle biopsy samples were obtained from one leg and the postprandial biopsy samples from the contralateral leg (randomized). All

<sup>7</sup> Abbreviations used: Akt, protein kinase B; AMPK-α, AMP-dependent protein kinase α; BCAA, branched-chain amino acid; CRP, C-reactive protein; EAA, essential amino acid; LAT1, large neutral amino acid transporter; mTORC1, mammalian target of rapamycin complex 1; OGTT, oral-glucose-tolerance test; p70S6K, 70-kDa S6 protein kinase; SNAT2, small neutral amino acid transporter.

muscle biopsy samples were freed from any visible adipose, connective tissue, and blood; immediately frozen in liquid nitrogen; and stored at  $-80^{\circ}\text{C}$  until subsequent analysis.

### Meal composition

Lean center-cut pork loins were homogenized, ground, and individually packaged and stored at  $-20^{\circ}\text{C}$  until each experimental trial. Before the infusion trial, the pork was thawed overnight at  $4^{\circ}\text{C}$  and grilled until the inner temperature reached  $65^{\circ}\text{C}$ . A proximate analysis of center-cut pork loin was performed as previously described (13). In brief, 4 subsamples of the master block of pork were analyzed for fat and protein content. Samples were dried at  $110^{\circ}\text{C}$  for 24 h and extracted in an azeotropic mixture of warm chloroform and methanol. Protein concentrations were determined by measuring nitrogen content with the use of the combustion method (method 990.03; Association of Official Analytical Chemists International, 2000; TruMac; LECO Corporation). Moisture, protein, and extractable lipid analyses were performed in duplicate. The variability between subsamples was within the acceptable range ( $\text{CV} < 5\%$ ). The 170-g ground pork patty provided 36 g protein and 5 g fat. This amount of pork was selected because others have shown that the ingestion of 170 g lean beef (36 g protein) was required to stimulate the postprandial muscle protein synthetic response in healthy adults (14), and it represents a typical amount of protein consumed with dinner by US adults.

### Blood analyses

Glucose concentrations were analyzed in whole blood by using an automated glucose analyzer (YSI 2300 Stat Plus; Yellow Springs Instruments). Plasma insulin and C-reactive protein (CRP) concentrations were determined by using a commercially available high-sensitivity ELISA (Alpco Diagnostics). Plasma amino acid concentrations and enrichments were determined by gas chromatography–mass spectrometry analysis (7890A GC/5975C MSD; Agilent). Plasma samples were prepared for amino acid analysis by using a mixture of isopropanol, acetonitrile, and water (3:3:2, vol:vol) and centrifuged at  $12,000 \times g$  for 10 min at  $4^{\circ}\text{C}$ . Subsequently, the supernatant was dried and the amino acids converted into tert-butyldimethylsilyl derivatives before gas chromatography–mass spectrometry analysis. The plasma L-[ring- $^{13}\text{C}_6$ ]phenylalanine enrichments were determined by using electron impact ionization by ion monitoring at an  $m/z$  of 336 ( $m + 0$ ) and 342 ( $m + 6$ ) for unlabeled and labeled phenylalanine, respectively, with the use of an internal standard (DL-p-chlorophenylalanine;  $10 \mu\text{g}/\text{mL}$ ). Amino acid concentrations were quantified by using the AMDIS software package (version 2.71; National Institute of Standards and Technology) and standards with known concentrations.

### Myofibrillar protein synthesis

Myofibrillar protein-enriched fractions were extracted from  $\sim 50$  mg wet muscle as described previously (15). Myofibrillar protein pellets were hydrolyzed overnight in 6 M HCl at  $110^{\circ}\text{C}$ . The resultant free amino acids were purified by using cation exchange chromatography (Dowex 50W-X8-200 resin; Acros Organics) and dried under vacuum. Free amino acids were resuspended in 60% methanol and centrifuged at  $18,000 \times g$

before analysis by 5500 QTRAP liquid chromatography–tandem mass spectrometry. The samples were dried and resuspended in  $100 \mu\text{L}$  60% methanol and centrifuged. Subsequently, samples were injected ( $5 \mu\text{L}$ ), and the liquid chromatography separation was performed on a Thermo Hypercarb column ( $4.6 \times 100$  mm,  $5 \mu\text{m}$ ) with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). The flow rate was  $0.4 \text{ mL}/\text{min}$ . Mass spectra were acquired under positive electrospray ionization with the ion spray voltage at  $+5500 \text{ V}$ . The source temperature was  $450^{\circ}\text{C}$ . The curtain gas, ion source gas 1, and ion source gas 2 were 35, 65, and 55 pounds/square inch, respectively. The L-[ring- $^{13}\text{C}_6$ ]phenylalanine enrichments were determined by multiple reaction monitoring at  $m/z$  166.0  $\rightarrow$  103.0 and 172.0  $\rightarrow$  109.0 for unlabeled and labeled L-[ring- $^{13}\text{C}_6$ ]phenylalanine, respectively. Software Analyst 1.6.2 (Agilent) was used for data acquisition and analysis.

### Western blotting

A portion of whole-muscle homogenates isolated during the myofibrillar protein extractions was used for Western blotting analysis. The protein content of the homogenates was determined by Bradford Assay (Bio-Rad) and then equal amounts of protein were separated by SDS-PAGE before being transferred to polyvinyl difluoride membranes. After blocking, membranes were incubated in primary antibodies overnight at  $4^{\circ}\text{C}$  to determine the phosphorylation status and total protein content of protein kinase B (Akt) at Ser473, mammalian target of rapamycin complex 1 (mTORC1) at Ser2448, 70-kDa S6 protein kinase 1 (p70S6K) at Thr389, and AMP-dependent protein kinase  $\alpha$  (AMPK- $\alpha$ ) at Thr172 by using antibodies from Cell Signaling Technology. The antibodies used for the detection of skeletal muscle amino acid transporters were as follows: large neutral amino acid transporter (LAT1; Bioss), small neutral amino acid transporter (SNAT2; Abcam), and CD98 (Abcam). Membranes from the respective proteins were then incubated with appropriate secondary antibodies and protein content was detected by using West Femto Maximum Sensitivity substrate (SuperSignal; Thermo Scientific) and the ChemiDoc-It<sup>2</sup> Imaging System (Ultra-Violet Products). After detection of phosphorylated proteins, membranes were stripped with Western blot stripping buffer (Restore; Thermo Scientific) and reincubated with antibodies against total protein (Cell Signaling Technology). Western blot data were normalized to an internal control ( $\alpha$ -tubulin). Bands were quantified by using ImageJ software (NIH), normalized to a control sample run on each blot to account for interblot variability, then expressed as the fold change from HW group basal values.

### Calculations

HOMA-IR was calculated by using the fasting glucose and insulin values from the oral-glucose-tolerance test (OGTT):  $\text{glucose}_{\text{fast}} \times \text{insulin}_{\text{basal}}/22.5$  (16). The fractional synthetic rates of myofibrillar protein were calculated by using standard precursor-product methods by dividing the increment in tracer enrichment in the myofibrillar protein by the enrichment of the plasma free precursor pool over time.

## Statistical analysis

A parallel-group repeated-measures design was used for this study. A power analysis based on previous research (6, 8, 14) showed that  $n = 9/\text{group}$  was sufficient to detect differences in postprandial muscle protein synthesis between groups when using a 2-sided statistical test ( $P < 0.05$ , 80% power,  $f = 0.7$ ; G\*power version 3.1.9.2; Heinrich Heine University Dusseldorf). Considering a potential drop-out rate of 10% during the protocol, the final number of participants recruited was 10/group. Differences in myofibrillar protein synthesis, muscle anabolic signaling, blood glucose, and plasma insulin were tested by 2-factor (group  $\times$  time) repeated-measures ANOVA. Group comparisons were performed when indicated by a significant interaction. Body composition, HOMA-IR, plasma CRP, demographic characteristics, and net areas under the blood glucose, plasma insulin, and AA curves (AUCs) were analyzed by using 1-factor ANOVA. Group comparisons were performed when indicated by a significant group effect. Tukey's post hoc tests were performed to locate group differences. Pearson's product-moment correlation ( $r$ ) was used to examine the relation between fat mass and postprandial myofibrillar protein synthesis rates. For all analyses, differences were considered significant at  $P < 0.05$ . All of the calculations were performed by using IBM SPSS Statistics version 20. All of the data are expressed as means  $\pm$  SEMs.

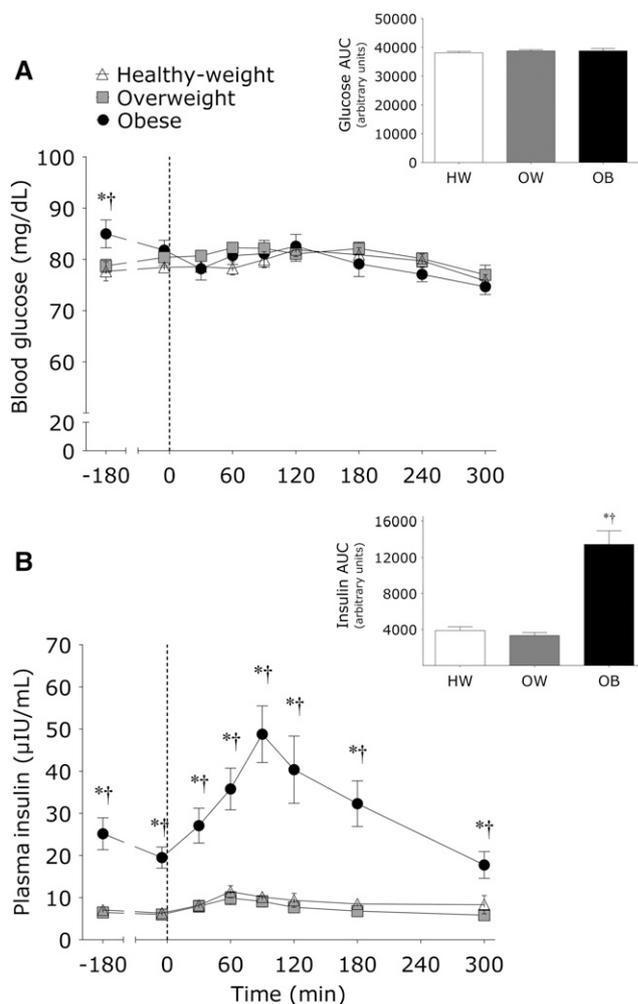
## RESULTS

### Oral-glucose-tolerance testing

During the OGTT, fasting blood glucose was similar across groups ( $P = 0.94$ ). At 120 min post-beverage consumption, blood glucose was greater in the OB group ( $99.2 \pm 7.7$  mg/dL) than in the OW ( $80.6 \pm 4.2$  mg/dL) and HW ( $78.5 \pm 6.1$  mg/dL) groups (all  $P < 0.05$ ). Fasting insulin was greater ( $P < 0.001$ ) in the OB group ( $25.2 \pm 3.8$   $\mu\text{IU/mL}$ ) than in the OW ( $6.5 \pm 0.5$   $\mu\text{IU/mL}$ ) and HW ( $7.1 \pm 0.9$   $\mu\text{IU/mL}$ ) groups. HOMA-IR was greater ( $P < 0.001$ ) in the OB group ( $5.82 \pm 0.81$ ) than in the OW ( $1.25 \pm 0.11$ ) and HW ( $1.36 \pm 0.17$ ) groups (Table 1).

### Blood variables during the infusion trial

Fasting plasma CRP concentrations were elevated ( $-180$  min;  $P < 0.001$ ) in the OB group ( $6.55 \pm 1.19$  mg/L) compared with the OW ( $0.84 \pm 0.18$  mg/L) and HW ( $0.82 \pm 0.29$  mg/L) groups (Table 1). Similarly, blood glucose concentrations were higher in the OB group at  $-180$  min of the infusion trial ( $P < 0.05$ ) than in the OW and HW groups. The blood glucose values remained stable over the duration of the infusion trial in all groups (all  $P > 0.05$ ; **Figure 1A**). Plasma insulin concentrations increased to a greater extent after protein ingestion in the OB group. As such, plasma insulin concentrations reached higher peak values ( $P < 0.001$ ) in the OB group ( $56.7 \pm 8.0$   $\mu\text{IU/mL}$ ) than in the OW ( $14.3 \pm 1.7$   $\mu\text{IU/mL}$ ) and HW ( $11.9 \pm 1.6$   $\mu\text{IU/mL}$ ) groups (Figure 1B). Plasma essential amino acid (EAA) concentrations increased after pork ingestion with no differences between groups (time effect,  $P < 0.001$ ; **Figure 2A**). Moreover, the net AUCs for EAAs were similar between all groups ( $P = 0.11$ ). Similarly, plasma branched-chain amino acid (BCAA) concentrations also increased after pork ingestion, with no differences between groups (time effect,  $P < 0.001$ ; Figure 2B). Plasma L-[ring- $^{13}\text{C}_6$ ]

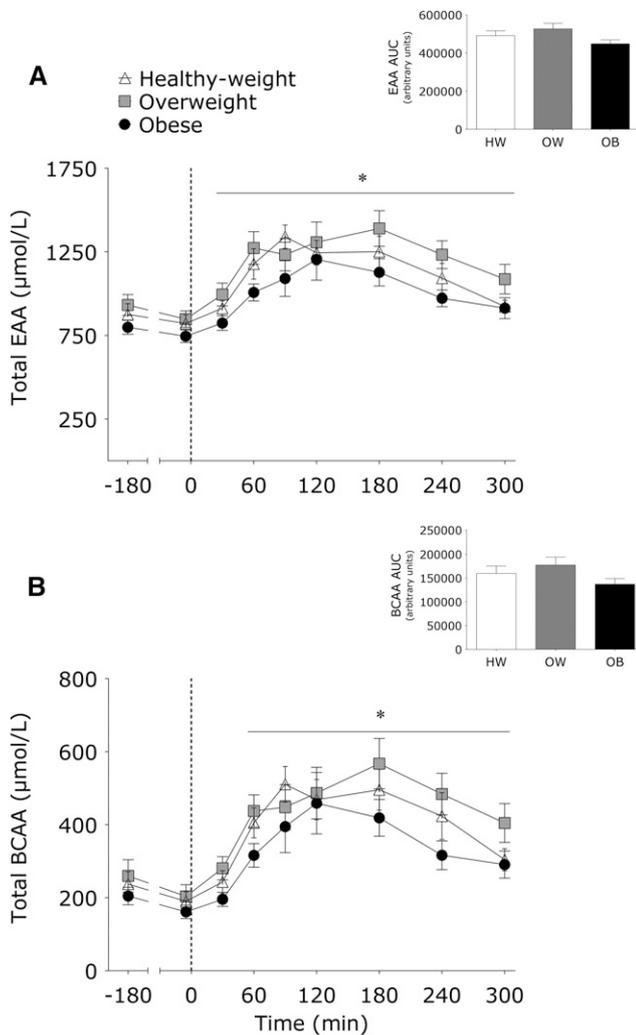


**FIGURE 1** Mean  $\pm$  SEM blood glucose (A) and plasma insulin (B) concentrations in the basal state and after pork ingestion ( $n = 10/\text{group}$ ). Insets show the areas under the glucose and insulin curves. The dashed vertical lines indicate pork ingestion. Blood glucose and plasma insulin data were analyzed by using 2-factor ANOVA with repeated measures on time. The areas under the glucose and insulin curves were analyzed by using 1-factor ANOVA. A Tukey's post hoc test was used to locate differences between means for all significant interactions. \*Different from HW group ( $P < 0.05$ ); †different from OW group ( $P < 0.05$ ). Glucose: group  $\times$  time,  $P = 0.012$ . Glucose AUC: group effect,  $P = 0.65$ . Insulin: time effect,  $P < 0.001$ ; group  $\times$  time,  $P < 0.001$ . Insulin AUC: group effect,  $P < 0.001$ . HW, healthy-weight; OB, obese; OW, overweight.

phenylalanine enrichments are shown in **Figure 3**. Plasma L-[ring- $^{13}\text{C}_6$ ]phenylalanine enrichments were different between groups (group effect,  $P = 0.05$ ). However, plasma L-[ring- $^{13}\text{C}_6$ ]phenylalanine enrichments were stable for each respective group throughout the infusion protocol, indicating a tracer steady state was achieved (time effect,  $P = 0.20$ ).

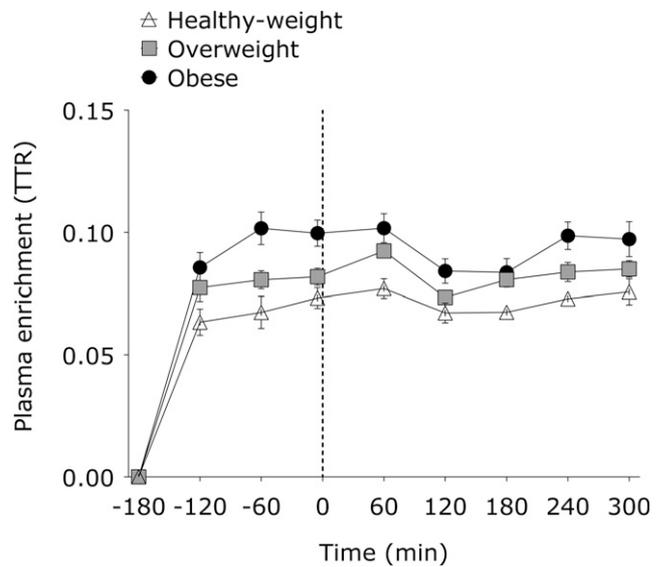
### Anabolic signaling and amino acid transporters

In the OB and OW groups, the relative concentrations of total mTORC1 protein were significantly greater ( $P < 0.001$ ) at baseline than in the HW group. In contrast, total p70S6K protein was similar in all groups ( $P = 0.14$ ; **Figure 4A**). In the post-absorptive state, mTORC1 phosphorylation was significantly higher in the OB and OW groups than in the HW group ( $P = 0.001$ ; Figure 4B). During the postprandial period, mTORC1



**FIGURE 2** Mean  $\pm$  SEM plasma EAA (A) and BCAA (B) concentrations in the basal state and after the consumption of pork ( $n = 10$ /group). Insets show the areas under the EAA and BCAA  $\times$  time curves. The dashed vertical lines indicate pork ingestion. Data were analyzed by using 2-factor ANOVA with repeated measures on time. The areas under the EAA and BCAA curves were analyzed by using 1-factor ANOVA. A Tukey's post hoc test was used to locate differences between means for all significant interactions. \*Different from  $-180$  min ( $P < 0.05$ ). Significant time effects are indicated by a horizontal line above points that differ from  $-180$  min ( $P < 0.05$ ). EAA: time effect,  $P < 0.001$ ; group  $\times$  time,  $P = 0.68$ . EAA AUC: group effect,  $P = 0.11$ . BCAA: time effect,  $P < 0.001$ ; group  $\times$  time,  $P = 0.76$ . BCAA AUC: group effect,  $P = 0.26$ . BCAA, branched-chain amino acids; EAA, essential amino acids; HW, healthy-weight; OB, obese; OW, overweight.

phosphorylation increased at 300 min in the HW group ( $P < 0.05$ ) after pork ingestion, with no changes in the OB and OW groups. Phosphorylation of p70S6K increased in the OB group at 300 min after pork ingestion ( $P = 0.02$ ; Figure 4C), but no changes were observed in the OW and HW groups. There were no observed differences in total protein or in the phosphorylation status at any time points for Akt and AMPK (data not shown). There were no differences in skeletal muscle LAT1, CD98, or SNAT2 protein content between the 3 groups at baseline or after pork ingestion ( $P > 0.05$ ; Figure 5). During the postprandial phase, pork ingestion resulted in increased LAT1 protein content above baseline values (time effect,  $P = 0.003$ ; Figure 5A). Similarly, pork ingestion increased SNAT2 protein content during postprandial period (time effect,  $P < 0.001$ ; Figure 5C). CD98 protein content



**FIGURE 3** Mean  $\pm$  SEM plasma L-[ring- $^{13}\text{C}_6$ ]phenylalanine enrichments (TTR) in the basal state and after pork ingestion ( $n = 10$ /group). The dashed vertical line indicates pork ingestion. Data were analyzed by using 2-factor ANOVA with repeated measures on time. A Tukey's post hoc test was used to locate differences between means for all significant interactions. L-[ring- $^{13}\text{C}_6$ ]phenylalanine: group effect,  $P = 0.05$ ; group  $\times$  time,  $P = 0.19$ . TTR, tracer-to-tracee ratio.

remained unaltered after pork ingestion in all groups ( $P > 0.05$ ; Figure 5B). Representative Western blots for anabolic signaling proteins and skeletal muscle amino acid transporters are shown in Supplemental Figure 1.

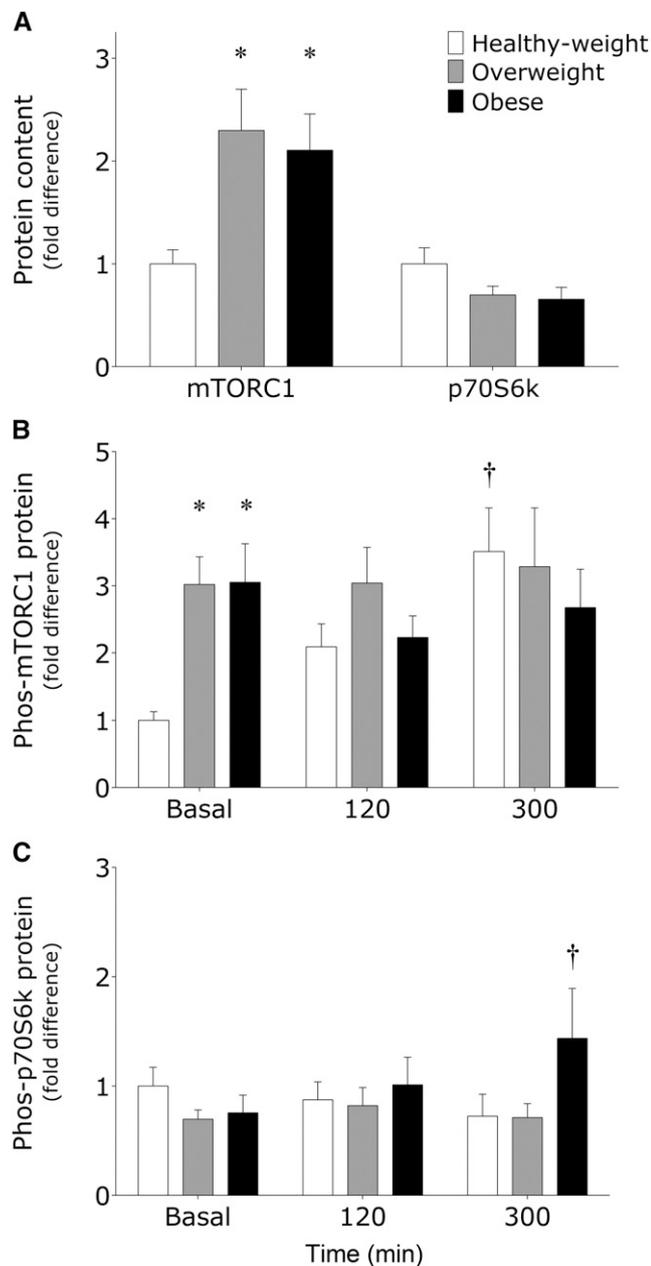
### Myofibrillar protein synthesis

Basal myofibrillar protein synthesis rates did not differ between the groups ( $P = 0.43$ ). Pork ingestion increased cumulative myofibrillar protein synthetic rates calculated over the entire 300-min postprandial period by 1.6-fold above basal values in the HW group ( $P = 0.005$ ), but no increases in postprandial myofibrillar protein synthesis rates were observed in the OW or OB groups (Figure 6).

Myofibrillar protein synthesis rates calculated during the early (0–120 min) and late (120–300 min) postprandial period showed differences in the temporal pattern of change in the stimulation of the postprandial myofibrillar protein synthetic response. During the early postprandial phase (0–120 min), myofibrillar protein synthesis did not differ from basal values in the OB, OW, or HW groups ( $P = 0.95$ ). However, myofibrillar protein synthetic rates increased ( $P < 0.001$ ) in the HW group (absolute change from basal:  $0.067\% \pm 0.092\%/h$ ) but not in the OW group ( $0.015\% \pm 0.011\%/h$ ) or in the OB group ( $0.005\% \pm 0.011\%/h$ ) in the late (120–300 min) postprandial phase. There was a trend for a relation between total body fat mass and the postprandial muscle protein synthetic response value at 0–300 min ( $r = -0.35$ ,  $P = 0.06$ ). Furthermore, total body fat mass was significantly correlated with postprandial myofibrillar protein synthesis rates in the late postprandial period (120–300 min;  $r = -0.45$ ,  $P = 0.02$ ).

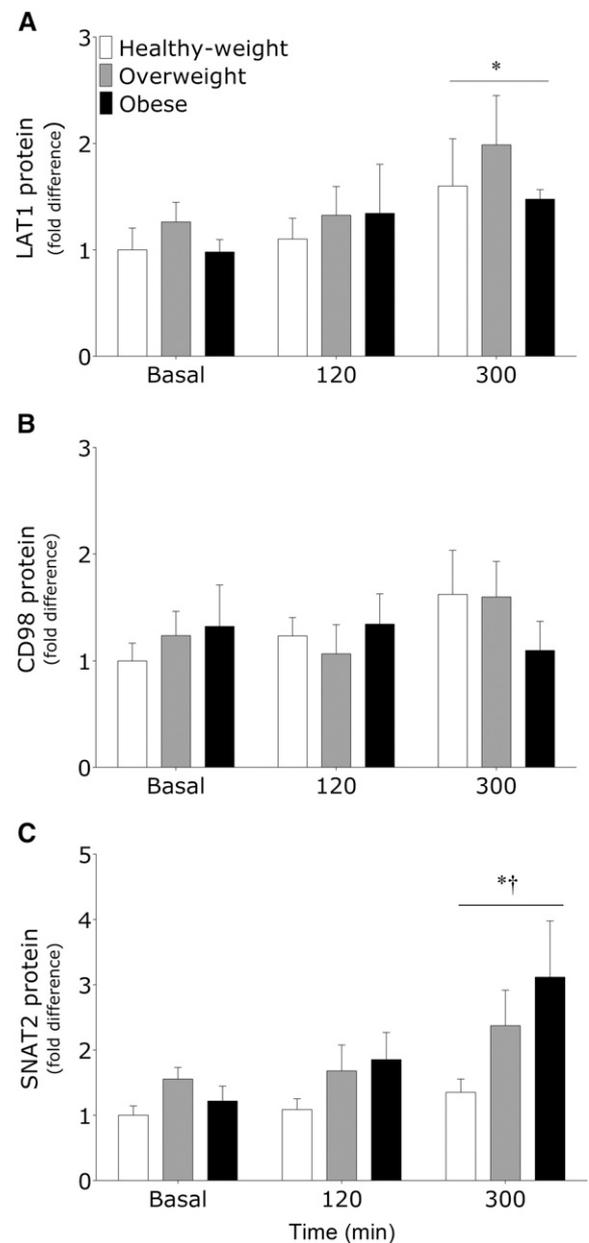
### DISCUSSION

In this study, we compared the nutrient-sensing mechanisms and subsequent postprandial myofibrillar protein synthetic response



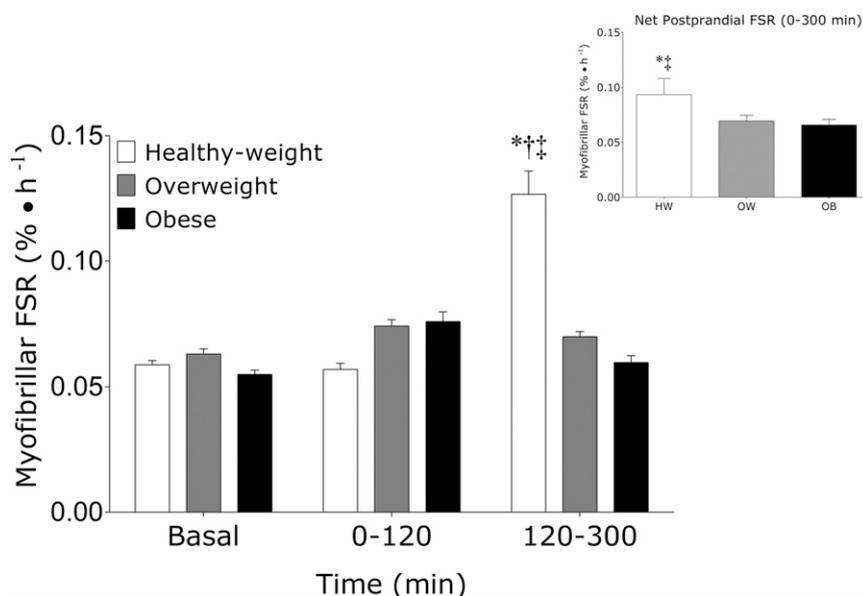
**FIGURE 4** Mean  $\pm$  SEM protein content for mTORC1 and p70S6K in the basal state (A). Phosphorylation of mTORC1 at Ser2448 (B) and p70S6K at Thr389 (C) at the basal state and after the ingestion of pork ( $n = 10$ /group). Data were analyzed by using 2-factor ANOVA with repeated measures on time. A Tukey's post hoc test was used to locate differences between means for all significant interactions. \*Different from healthy-weight group ( $P < 0.05$ ); †different from basal state ( $P < 0.05$ ). mTORC1 protein: group effect,  $P < 0.001$ . p70S6K protein: group effect,  $P = 0.14$ . Phos-mTORC1: group effect,  $P = 0.48$ ; time effect,  $P = 0.11$ ; group  $\times$  time,  $P = 0.04$ . Phos-p70S6K: group effect,  $P = 0.53$ ; time effect,  $P = 0.64$ ; group  $\times$  time,  $P = 0.03$ . Values are expressed as fold changes from healthy-weight basal. mTORC1, mammalian target of rapamycin complex 1; Phos-, phosphorylated; p70S6K, 70-kDa S6 protein kinase.

to the ingestion of a protein-dense food source across a wide range of body fat percentages in humans. We showed that excessive fat mass does not impair the basal myofibrillar protein synthetic response. However, we observed a poor responsiveness of postprandial muscle protein synthesis rates to the ingestion of a meaningful amount of high-quality animal-based protein (36 g



**FIGURE 5** Mean  $\pm$  SEM protein content of large neutral amino acid (LAT1; A) and small neutral amino acid [CD98 (B) and SNAT2 (C)] transporters at the basal state and after the ingestion of pork ( $n = 10$ /group). Data were analyzed by using 2-factor ANOVA with repeated measures on time. A Tukey's post hoc test was used to locate differences between means for all significant interactions. Significant time effects are indicated by a horizontal line above the points that differ. \*Different from basal state in all groups ( $P < 0.05$ ); †different from 120 min in all groups ( $P < 0.05$ ). LAT1: time effect,  $P = 0.003$ ; group  $\times$  time,  $P = 0.64$ . CD98: time effect,  $P = 0.13$ ; group  $\times$  time,  $P = 0.30$ . SNAT2: time effect,  $P < 0.001$ ; group  $\times$  time,  $P = 0.25$ . Values are expressed as fold changes from healthy-weight basal. LAT1, large neutral amino acid transporter; SNAT2, small neutral amino acid transporter.

in the OB and OW groups when compared with their HW counterparts. These differences were driven by the responsiveness of the postprandial myofibrillar protein synthesis rates in the late postprandial phase (2–5 h) in the HW group. Interestingly, the OW group did not show overt differences in whole-body glucose tolerance or systemic inflammation compared with the



**FIGURE 6** Mean  $\pm$  SEM myofibrillar FSR at basal and after (0–120 and 120–300 min) the ingestion of pork ( $n = 10$ /group). The inset shows the cumulative rates of myofibrillar protein synthesis during the postprandial period (0–300 min). Data were analyzed by using 2-factor ANOVA with repeated measures on time. A Tukey's post hoc test was used to locate differences between means for all significant interactions. \*Different from basal state ( $P < 0.05$ ); †different from 0–120 min ( $P < 0.05$ ); ‡different from the OW and OB groups ( $P < 0.05$ ). FSR: group  $\times$  time,  $P = 0.002$ ; inset FSR: group  $\times$  time,  $P = 0.005$ . FSR, fractional synthesis rate; HW, healthy-weight; OB, obese; OW, overweight.

HW group, which may show that skeletal muscle anabolic insensitivity is an early impairment associated with increased adiposity that occurs before glucose intolerance (OGTT), chronic low-grade systemic inflammation (e.g., elevated plasma CRP concentrations), and hyperinsulinemia. Moreover, the net exposure of amino acids (total, EAAs, BCAAs, and leucine) during the postprandial period was similar between all groups and further supports that intrinsic defects within skeletal muscle tissue may precede dysregulated metabolism in other tissues. What is noteworthy is that our data show that the consumption of a protein quantity that is similar to that provided during an average American meal (11) is incapable of overcoming the poor anabolic sensitivity of skeletal muscle tissue to dietary amino acids with increasing adiposity in young adults.

Only a handful of previous studies examined the regulation of muscle protein synthesis in obese individuals (5–9). Both basal and postprandial muscle protein synthesis rates have an important role in the maintenance of skeletal muscle mass (e.g., contractile function and metabolic health). Our findings are in agreement with several previous studies that showed similar basal rates of muscle protein synthesis with obesity when compared with their healthy-weight counterparts (5, 7, 17). In a different manner, exogenous amino acid administration has been shown to be both effective (8) and ineffective (5, 9) at stimulating postprandial muscle protein synthesis in obese older adults. Moreover, it has been shown that obese men exhibited a “normal” muscle protein synthetic response during hyperinsulinemic-hyperaminoacidemic clamp (6, 7). Although our data conflict with these findings, the clamped conditions used in these studies were not reflective of the postprandial conditions seen in the present study (i.e., profoundly greater aminoacidemia and insulinemia) (6, 7) and likely created a more anabolic environment for muscle. Here, we show deficits in the responsiveness of postprandial muscle protein synthesis rates to dietary protein-derived

amino acids in both OW and OB groups when applied under conditions more reflective of a typical meal setting. This defect in the postprandial muscle protein synthetic response to food ingestion with increasing adiposity does not negatively affect overall lean mass (Table 1) but is likely contributing to poor metabolic quality of skeletal muscle. For example, impairments in protein turnover and renewal lead to greater protein half-life. These “older” proteins are more susceptible to damage than newly synthesized proteins, which can contribute to poor composition and impaired metabolic function of the muscle proteome (18). Alternatively, the lean body mass in the OW group was not different from that in the HW group despite the elevated fat mass. These findings suggest that reduced postprandial muscle protein synthesis rates in overweight individuals, as evidenced from the blunted myofibrillar protein synthesis to protein ingestion, is a potentially contributing process to the development of metabolic impairments commonly observed with obesity. Murton et al. (5) described similar observations in which a reduced muscle protein synthetic response occurred to amino acid provision in obese older adults, with no impact on muscle strength and muscle fatigue when compared with healthy-weight controls. Moreover, these authors observed a reduced proteolytic response in the obese older adults (5), which is consistent with the notion that protein synthesis and breakdown respond in a coordinated manner (19). Given all this, our data and others (5) support the notion that our data are more reflective of poor skeletal muscle remodeling and are not related to reduced accretion or maintenance of the myofibrillar protein pool with greater adiposity. EAAs are direct activators of muscle anabolic signaling pathways (20), with a pronounced leucinemia being exceptionally important to optimize the postprandial muscle protein synthetic response (21). We observed no differences in plasma EAA or BCAA concentrations or net exposure to amino acids (AUCs) between the 3 groups. These findings

indicate that the defect in postprandial muscle protein synthesis rates in the OW and OB groups is likely not due to reduced plasma amino acid availability because postprandial release of dietary protein-derived amino acids into circulation is a relevant driver of changes in plasma amino acid concentrations (22). Previous research has shown that obesity is associated with elevated postabsorptive plasma BCAA concentrations (23). However, the association between obesity and circulating BCAAs becomes less clear when obese participants are stratified by insulin sensitivity (24). We specifically recruited obese participants without known health concerns, which may explain the discrepancy seen in the present study.

We examined relevant nutrient-sensing mechanisms in muscle in an effort to discern what factor or factors are at the root of the reduced postprandial muscle protein synthetic response with increased adiposity, because it appeared not to be driven by systemic factors. Interestingly, we showed that basal mTORC1 protein concentrations and the phosphorylation of mTORC1 are elevated in both OW and OB groups compared with the HW group. This may represent a compensatory mechanism to maintain a “normal” basal muscle protein synthetic response with increasing adiposity. However, this hyperactivation of mTORC1 in the basal state may diminish the “sensing” capacity of skeletal muscle tissue to protein-derived amino acids during the postprandial period. Our findings are supported by rodent studies, which have shown that mTORC1 is hyperactive with obesity in the fasting state (25–27). We also measured skeletal muscle LAT1, CD98, and SNAT2 protein content for insight into amino acid transport/sensing capacity in muscle. Both LAT1 and SNAT2 protein content increased at 5 h of the postprandial phase in all 3 groups (Figure 5). This suggests that amino acid transport capacity is not impaired with greater adiposity. Overall, these findings suggest that intrinsic anomalies in skeletal muscle anabolic signaling are present and may partly explain the poor sensitivity of postprandial muscle protein synthesis rates to dietary protein-derived amino acids with increasing fat mass.

From a practical perspective, the current findings suggest that simply instructing people with overweight or obesity to eat more protein in a meal to overcome the defect in postprandial muscle protein synthesis would be unproductive. This notion is based on the generous amount of protein (36 g) provided in the present study that mirrors the portion of protein commonly contained in a typical American dinner (11), which was effective in elevating plasma amino acid concentrations and has been shown to be satiating (28). Instead, strategies aimed at improving the amino acid sensitivity of muscle, such as increasing habitual physical activity or incorporation of exercise regimes into the daily lifestyle routine (29), are likely more prudent.

In conclusion, greater adiposity abolishes the increase in postprandial myofibrillar protein synthesis rates seen after the ingestion of a protein-dense meal in apparently “healthy” young overweight and obese adults. In the case of the OW group, this finding was observed independent of many of the typically associated problems that occur with weight gain (e.g., reduced glucose tolerance and/or chronic inflammation). However, key muscle anabolic signaling pathways are clearly disrupted in overweight and obese adults and may underpin the defect in postprandial muscle protein synthesis rates. The focus of future work should aim to increase the anabolic sensitivity of muscle

protein synthesis rates, with exercise before food ingestion representing a logical next step.

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