Title: Postprandial muscle protein synthetic responses after high quality pork consumption in lean, overweight, and obese adults – **NPB #14-205**

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Industry Summary:

Obesity is associated with numerous chronic diseases. Many obese individuals, however, do not display overt disease. This is due to obese people having altered metabolism that does not meet the threshold needed for a clinical diagnosis. Muscle protein turnover appears to be reduced in obesity, where proteins that are damaged and worn out are not replaced as efficiently. However, muscle protein metabolism in obese people has only been measured in situations that do not reflect real-life (i.e. highly controlled laboratory settings with the amino acids being delivered intravenously). The consumption of dietary protein is a well described strategy to stimulate muscle protein synthesis rates in young healthy weight adults. The most effective protein sources to consume to maximize the muscle protein synthetic response generally have a high degree of digestibility leading a high proportion of the dietary protein derived amino acids becoming available into circulation. Animal based protein sources, such as pork, are highly digestible and thus provide a strong stimulatory signal to muscle after their ingestion. Importantly, pork is also rich in the amino acid leucine, which is an important nutrient signaling molecule to muscle.

      Thus, the main objectives of this study were 1) determine the impact of consuming high quality protein derived from pork on the synthesis rate of new muscle proteins, and 2) measure the activation of anabolic signals in the muscle after consuming pork in overweight and obese young adults. We also sought to describe the systemic responses in blood glucose and plasma insulin and amino acids to pork ingestion. This was accomplished by measuring muscle protein synthesis before and after eating 6 oz. of lean pork in overweight and obese young adults and comparing their responses to those of healthy-weight controls.

Our findings were:

1) Systemic factors were improved by eating pork in all groups. Specifically, pork ingestion resulted in a large rise in blood amino acids without causing an increase in blood sugar.

2) Anabolic signals in the muscle of overweight and obese adults are hyperactive (~3 times greater) at baseline when compared to the healthy-weight controls. Thus, the ability of blood amino acids to signal to skeletal muscle seems to be diminished with greater fat mass. However, pork ingestion strongly activated anabolic signaling molecules in muscle in healthy weight adults.

3) Muscle protein synthesis in overweight and obese adults is less responsive to pork ingestion than healthy-weight controls. This finding is in agreement with previous reports that used constant amino acid infusions, suggesting that overweight and obese people have intrinsic differences in anabolic signaling that is not overcome by increasing circulating amino acids or insulin by ingesting pork. However, pork ingestion strongly stimulates muscle protein synthesis rates in healthy weight adults and to a degree that is commonly observed after the ingestion of other high quality proteins such as beef, eggs, or milk.

Overall, pork ingestion may have beneficial effects on glucose tolerance in obese people, but intrinsic defects in the muscle of overweight and obese people limit their skeletal muscle anabolic response to food ingestion.

Keywords: Muscle health, high quality protein, obesity, muscle recovery, muscle mass

Scientific Abstract:

Background: Excess body fat leads to diminished muscle protein synthesis rates in response to a hyperinsulinemic hyperaminoacidemic clamp. To our knowledge, no studies have compared the postprandial muscle protein synthetic response after the ingestion of a single meal containing a protein dense food source across a range of body mass indices and fat masses. ***Objective:*** We aimed to compare the myofibrillar protein synthetic (MPS) response and underlying nutrient sensing mechanisms after the ingestion of lean pork loin between obese, overweight, and healthy-weight adults. ***Design:*** 10 healthy-weight (HW; Age 24±1 y, BMI 22.7±0.4 kg/m^2, HOMA-IR 1.4±0.2), 10 overweight (OW; Age 26±2 y, BMI 27.1±0.5 kg/m^2, HOMA-IR 1.25±0.11), and 10 obese males and females (OB; Age 27±3 y, BMI 35.9±1.3 kg/m^2, HOMA-IR 5.8±0.8) received primed continuous L-[*ring*-^{13}C$_6$]phenylalanine infusions. Blood and muscle biopsy samples were collected before and after ingestion of 170 g of pork (36 g protein and 5 g fat) to assess skeletal muscle anabolic signaling, amino acid transporters (LAT1, CD98, SNAT2), and MPS. ***Results:*** At baseline, OW and OB showed greater relative amounts of mTORC1 protein compared to the HW group. However, pork ingestion only increased phosphorylation of mTORC1 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 MPS were not different between groups (*P*=0.43). However, postprandial MPS (0-300 min) was greater in the HW group (1.6-fold; *P*=0.005) after pork ingestion, compared with the OW and OB groups. ***Conclusions:*** There is diminished responsiveness of postprandial MPS to the ingestion of a protein dense food in overweight and obese adults as compared to healthy-weight controls. These data indicate that impaired postprandial MPS may be an early defect with increasing fat mass and may be dependent on altered anabolic signals leading to poor sensitivity to protein ingestion.

Introduction: An overview of the researchable question and its importance to producers.
There is an increasing prevalence of obesity in US adults and this is a major health concern. Moreover, there is a growing proportion of the US adult population attempting weight loss for improvements in health and/or self-esteem. A major limitation of common weight loss strategies is the loss of muscle mass that accompanies fat loss. The preservation of muscle mass is important for physical performance, metabolic health, and long-term weight management. Pork, as a lean source of high quality protein, has the ability to effectively augment muscle protein accretion and, as such, support muscle mass maintenance and health. However, research was needed to directly show that lean pork consumption is an effective dietary strategy to enhance muscle protein accretion in lean, overweight, and obese adults.

Our proposed research used sophisticated methodology to assess how eating pork acts as a potent anabolic food source to enhance the muscle building response in healthy adults. As such, this work sought to provide the evidence base to prescribe pork as part of a healthy diet and/or weight loss program for muscle mass maintenance. Identifying lean pork as a potent anabolic food for the promotion of muscle health will increase the competitive advantage of the US pork industry by increasing demand for lean pork products within the consumer market.

Objectives:
Primary objective #1. Determine postabsorptive and postprandial muscle protein synthetic response to the consumption of 6 oz. of pork in healthy weight, overweight, and obese adults.

Primary objective #2. Assess the activation of anabolic signaling molecules involved in the regulation of muscle protein metabolism in the postabsorptive and postprandial-states.

Secondary objectives: Examine plasma amino acid, glucose, and insulin concentrations before and after pork ingestion.

Materials & Methods:
Participants and ethical approval
Ten healthy-weight (HW, BMI: 22.7 ± 0.4 kg·m^{-2}), 10 overweight (OW, BMI: 27.1 ± 0.5 kg·m^{-2}), and 10 obese (OB, BMI: 35.9 ± 1.3 kg·m^{-2}) volunteered to participate in this study. The participants were balanced for age and sex. Participants were not involved in a regular exercise-training program. Participant characteristics are presented in Table 1. All participants were deemed healthy based on responses to a routine medical screening questionnaire and had no prior history of participating in stable isotope amino acid tracer experiments. Each participant was informed of the purpose of the study, experimental procedures, and all its potential risks prior to 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.

Experimental design
A parallel group design was employed for this study. Prior to the infusion trial, participants reported to the laboratory in the morning after a 10 hour 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 of water. In addition, body weight and height were measured as well as body composition by dual-energy X-ray absorptiometry (Hologic QDR 4500A, Bedford, MA, USA). Participants waist-to-hip ratio was also measured using the minimum waist, maximum hip method (8). Participants were instructed to refrain from vigorous physical activity and alcohol for three days prior to each tracer infusion. All participants consumed a standardized meal of the same composition (~30% estimated TDEE: providing 50 energy% (En%) carbohydrate, 25 En% fat, and 25 En% of protein) the evening prior to each tracer infusion trial.

Infusion protocol
On the trial days, the participants reported to the laboratory at ~0700 h 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-*$^{13}C_6$]phenylalanine (2 µmol·kg^{-1}). Subsequently, an intravenous infusion of L-[*ring-*$^{13}C_6$]phenylalanine (infusion rate of 0.05 µmol·kg^{-1}·min^{-1}) was initiated and maintained until the end of the trial. A second Teflon catheter was placed in a contralateral dorsal hand vein and placed in a heated blanket for repeated arterialized blood sampling. In the post-absorptive state, muscle biopsies of the *vastus lateralis* were collected at *t*=-135 and -15 min of infusion. Subsequently, the participants consumed 170 g ground pork. During the meal, participants were given 300 mL of water enriched to 4% with L-[*ring-*$^{13}C_6$] phenylalanine according to the phenylalanine content of pork to minimize disturbances in isotopic equilibrium during the infusion. Additional muscle biopsies were collected at 2 and 5 h after pork ingestion. Arterialized blood samples were drawn every 30 or 60 min during the post-absorptive and postprandial states. The biopsies 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 biopsies were obtained from one leg (randomized) and the postprandial biopsies from the contralateral leg. All biopsy samples were freed from any visible adipose, connective tissue and blood, immediately frozen in liquid nitrogen, and stored at –80˚C until subsequent analysis.

Meal composition

Lean center-cut pork loin was homogenized, ground and individually packaged by the Meat Sciences Laboratory at the University of Illinois and stored at –20˚C until each experimental trial. Prior to the infusion, the pork was thawed overnight at 4˚C and grilled until the inner temperature reached 65˚C.

Blood analyses

Glucose concentrations were analyzed in whole blood using an automated glucose analyzer (YSI 2300 Stat Plus, Yellow Springs, OH, USA). Plasma insulin concentrations were determined using a commercially available enzyme-linked immunosorbent assays (Alpco diagnostics; Salem, NH USA). Plasma amino acid concentrations and enrichments were determined by GC/MS (Agilent 7890A GC/5975C; MSD, Wilmington, Delaware, USA). Plasma was prepared for amino acid analysis using a mixture of isopropanol:acetonitrile:water (3:3:2, v/v) and centrifuged for 10 min at 4ºC. Subsequently, the supernatant was dried and the amino acids converted into tert-butyldimethylsilyl (*t*-BDMS) derivatives prior to GC/MS analysis. The plasma L-[*ring*-^{13}C$_6$]phenylalanine enrichments were determined using electron impact ionization by ion monitoring at mass/charge (*m/z*) 336 (*m*+0) and 342 (*m*+6) for unlabeled and labeled phenylalanine, respectively. Amino acid concentrations were determined using the AMDIS software package (v. 2.71, NISTTM) and comparing to an internal standard. Amino acid peak areas were subsequently compared to an external standard curve developed using known concentrations (10).

Myofibrillar protein synthesis

Intracellular free amino acids were extracted by hand-homogenizing ~10-15 mg of wet muscle using a Teflon pestle in 0.6 M perchloric acid. Following homogenization, samples were centrifuged and the homogenate collected for intracellular enrichments. Myofibrillar protein-enriched fractions were extracted from ~50 mg of wet muscle with a Dounce glass homogenizer in ice-cold homogenization buffer (10 µL·mg^{-1}) supplemented with a Complete Mini, protease inhibitor and phosphatase cocktail tablets (PhosStop, Roche Applied Science). . Homogenates were transferred to a microcentrifuge tube and centrifuged at 700 ×g for 15 min at 4ºC to pellet a fraction enriched with myofibrillar and cytoskeleton proteins. The resultant supernatant was collected and stored at -80ºC for western blot analyses. The remaining pellet was washed with homogenization buffer and centrifuged at 700 ×g for 10 min at 4ºC. The supernatant was discarded. Myofibrillar proteins were solubilized in 0.3 м NaOH at 50ºC for 30 min with vortex mixing in 10 min intervals. Samples were centrifuged at 700 ×g for 10 min at 4ºC. Myofibrillar proteins were precipitated with 1 м PCA and centrifuging at 2500 ×g for 10 min at 4ºC. The myofibrillar protein pellet was washed twice with 70% ethanol. Myofibrillar protein pellets were hydrolyzed overnight in 6 м HCL at 110ºC. Myofibrillar enrichments were determined using LC/MS/MS.

LC/MS/MS analysis

The free amino acids were purified using cation exchange chromatography (Dowex 50W-X8-200 resin; Acros Organics, Geel, Belgium) and dried under vacuum. Free amino acids were re-suspended in 60% methanol and centrifuged before analysis by 5500 QTRAP LC/MS/MS at Metabolomics Lab of Roy J. Carver Biotechnology Center, University of Illinois at Urbana- Champaign. Subsequently, samples were injected (5 µL) and the LC separation was performed on a Thermo Hypercarb column (4.6 × 100 mm, 5µm) with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetontrile). The flow rate was 0.4 mL/min. Mass spectra were acquired under positive electrospray ionization (ESI) with the ion spray voltage at +5500 V. The source temperature was 450 °C. The curtain gas, ion source gas 1, and ion source gas 2 were 35, 65, and 55 psi, respectively. The L-[ring-13C6]phenylalanine enrichments were determined by multiple reaction monitoring (MRM) at m/z 166.0 → 103.0 and 172.0 → 109.0 for unlabeled and labeled L-[ring-13C6]phenylalanine, respectively. Software Analyst 1.6.2 was used for data acquisition and analysis.

Western blotting

A portion of whole muscle homogenates not used for FSR analyses were used to determine phosphorylation status of proteins involved in anabolic signaling, as well as total protein content of amino acid transporters. After determination of protein content by Bradford Assay (Bio-Rad), equal

amounts of protein were separated by SDS-PAGE, and transferred to polyvinyl difluoride membranes. After blocking, membranes were incubated in the following primary antibodies overnight at 4oC: The phosphorylation status of protein kinase B, phosphor-Akt (Ser473; Cell Signaling), mammalian target of rapamycin complex 1, phosphor-mTORC1 (Ser2448; Cell Signaling), 70 kDa S6 protein kinase, phosphor-p70S6K (Thr389; Cell Signaling), and adenosine monophosphate dependent protein kinase α phosphor-AMPK-α (Thr172; Cell Signaling) were determined by Western blotting with antibodies from Cell Signaling Technologies. Membranes were then incubated with appropriate secondary antibodies, and protein content was detected using West Femto Maximum Sensitivity substrate (SuperSignal, Thermo Scientific, USA) and the ChemiDoc-It2 Imaging System (UVP, USA). After detection of phosphorylated proteins, membranes were stripped with western blot stripping buffer (Restore, Thermo Scientific, USA) and re-incubated with antibodies against total protein. Phosphorylated protein content and amino acid transporters were normalized to α-tubulin. All data were normalized to an internal control run on each blot. Data are expressed as fold change from Healthy-weight basal.

Calculations
Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the fasting glucose and insulin values from the OGTT (Glucose$_{fast}$ x Insulin$_{fast}$ /22.5 (11)). The fractional synthetic rates (FSR) of myofibrillar protein were calculated using standard precursor-product methods by dividing the increment in tracer enrichment in the myofibrillar protein by the enrichment of the intracellular free precursor pool over time.

Statistics
A parallel group repeated measures design was used for this study. Differences in myofibrillar protein synthesis, muscle anabolic signaling, blood glucose, and plasma insulin were tested by two-factor (treatment × time) repeated measures analysis of variance (ANOVA). Body composition, HOMA-IR, and demographics were analyzed using one-factor ANOVA. When significant effects were observed, Tukey's post-hoc tests were performed to locate these differences. For all analyses, differences will be considered significant at P<0.05. All calculations were performed using IBM SPSS Statistics Version 20. All data are expressed as means ± SEMs.

Results:
Primary objective #1:
Basal myofibrillar protein synthesis rates did not differ between the groups (*P*=0.43) Pork ingestion increased the cumulative myofibrillar protein synthetic response calculated over the entire 300 min postprandial period by 1.6-fold in the HW group (*P*=0.005), but not the OW or OB groups (**Figure 5**). Myofibrillar protein synthesis rates calculated during the early (0-120 min) and late (120-300 min) postprandial period revealed 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 HW, OW, or OB groups *(P*=0.95). However, myofibrillar protein synthetic rates were increased (P<0.001) in the HW group (absolute change from basal: 0.067 ± 0.092 % • hr^{-1}) but not the OW group (0.015 ± 0.011 % • hr^{-1}) or OB group (0.005 ± 0.011 % • hr^{-1}) in the late (120-300 min) postprandial phase. There was a trend for a relationship between total body fat mass and the postprandial muscle protein synthetic response value at 0-300 min (r= -0.35, *P*=0.06) and was significantly correlated with postprandial myofibrillar protein synthesis rates in the late postprandial period (120-300 min; r= -0.45, *P*=0.02)

Primary objective #2:
In the OB and OW groups, the relative concentrations of total mTORC1 protein were significantly greater (*P*<0.001) at baseline compared to the HW group. In contrast, total p70S6k protein was similar in all groups (*P*=0.14; **Figure 4A**). In the postabsorptive-state, mTORC1 phosphorylation was significantly increased in the OB and OW groups compared to the HW group (*P*=0.001; **Figure 4B)**. During the postprandial period, mTORC1 phosphorylation increased at 300 min in the HW group (*P*<0.05) after pork ingestion with no differences in the OB and OW groups. Phosphorylation of p70S6K was increased in the OB group at 300 min after pork ingestion (*P*=0.02; **Figure 4C**), but no

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differences were observed in the OW and HW groups. There were no observed differences in total protein or the phosphorylation-status at any time points for AKT and AMPK (data not shown).

Secondary objectives:
Blood glucose concentrations were higher in the OB group at baseline ($P<0.05$) compared with HW and OW group. The blood glucose values remained stable over the duration of the postprandial period 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±8.0 µIU/mL) compared with the HW (11.9±1.6 µIU/mL) and OW groups (14.3±1.7 µIU/mL; **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 AUC for EAA were similar between all groups ($P=0.11$). Similarly, plasma branched chain amino acid concentrations also increased after pork ingestion with no differences between groups (Time effect: $P<0.001$; **Figure 2B**). Plasma L-[*ring*-^{13}C$_6$]phenylalanine enrichments are shown in **Figure 3**. Plasma L-[*ring*-^{13}C$_6$]phenylalanine enrichments were different between groups (Treatment effect: $P=0.05$). However, plasma L-[*ring*-^{13}C$_6$]phenylalanine enrichments were stable for each respective group throughout the infusion protocol indicating a tracer steady-state was achieved (Time effect: $P=0.20$).

Discussion:
In this study, we compared the nutrient sensing mechanisms and subsequent postprandial myofibrillar protein synthetic response 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 protein (36 g) from pork in the obese and overweight groups as compared to their healthy weight 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 healthy weight group. Interestingly, the overweight group did not demonstrate overt differences in whole body glucose tolerance or systemic inflammation versus the healthy weight group, which may illustrate that skeletal muscle anabolic insensitivity is an early impairment associated with increased adiposity that occurs prior to glucose intolerance (OGTT), chronic low-grade systemic inflammation (e.g., elevated plasma CRP concentrations), and hyperinsulinemia. Moreover, the net exposure of amino acids (total, EAA, BCAA, 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 with excess fat mass. What is noteworthy is that our data demonstrate that consuming a protein quantity that is similar to that provided during an average American meal is incapable of overcoming the poor anabolic sensitivity of skeletal muscle tissue to dietary amino acids with increasing adiposity in young adults.

The benefit to the pork producers is that we show that eating high quality protein from pork helps the body in building and maintaining muscles in healthy weight adults. Thus, our data supports the regular incorporation of pork based products into a healthy eating pattern to support the muscle building response. Future work needs to identify more effective strategies to make better use of high quality protein in people with obesity.

Revised 2/10

Appendix A
Subject Characteristics

Variable	Healthy-weight	Overweight	Obese
Sex (females)	10 (5)	10 (5)	10 (5)
Age (y)	24 ± 1	26 ± 2	27 ± 3
Ht (m)	1.73 ± 0.03	1.70 ± 0.02	1.71 ± 0.03
Wt (kg)	68.5 ± 3.5	78.6 ± 2.2	106.0 ± 5.0*†
BMI (kg/m^2)	22.7 ± 0.4	27.1 ± 0.5*	35.9 ± 1.3*†
Waist:Hip	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 (mmHg)	120.4 ± 3.0	125.9 ± 2.2	131.5 ± 3.0*
Diastolic BP (mmHg)	75.2 ± 1.7	79.4 ± 1.8	87.2 ± 1.5*†
Fasting glucose (mg/dL)	78.0 ± 2.2	78.3 ± 1.2	82.0 ± 2.1
2-hour glucose (mg/dL)	78.5 ± 6.1	80.6 ± 4.2	99.2 ± 7.7*†
HOMA-IR	1.36 ± 0.17	1.25 ± 0.11	5.82 ± 0.81*†

Table 1. Data are mean ± SEM. * Indicates a difference from the Healthy-weight group, † Indicates a difference from the Overweight group ($P<0.05$). Data were analyzed with one factor ANOVA. A Tukey's post hoc test was used to locate differences between means for all significant interactions. Glucose: Group effect $P=0.59$, Time effect $P<0.001$, Group × Time $P<0.001$.
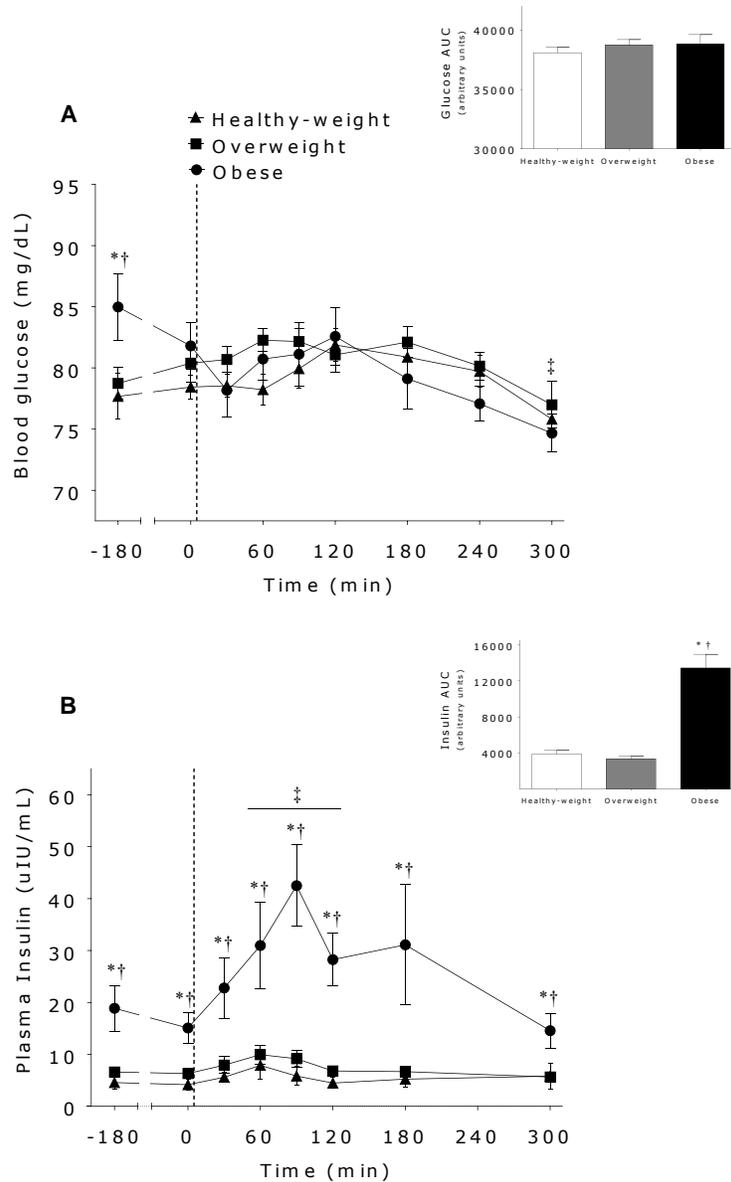
Appendix B
Blood Glucose and Plasma Insulin

Figure 1. Blood glucose (**A**) and plasma insulin concentrations (**B**) (mg·dL^{-1} and µIU·mL^{-1}, respectively) in the basal state and after pork ingestion (*n*=10 per group). Inset are the area under the glucose and insulin curve. Dashed vertical line refers to pork ingestion. Data were analyzed with a two-factor ANOVA with repeated measures on time. A Tukey's post hoc test was used to locate differences between means for all significant interactions. * Indicates a difference from HW, † Indicates a difference from OW, ‡ Indicates a difference from baseline. Glucose: Group effect *P*=0.59, Time effect *P*<0.001, Group × Time *P*<0.001. Glucose AUC: Group Effect *P*=0.65 Insulin: Group effect *P*<0.001, Time effect *P*<0.001, Group × Time *P*<0.001. Insulin AUC: Group Effect *P*<0.001. Data are Mean ± SEM.

Appendix C

Plasma Amino Acids

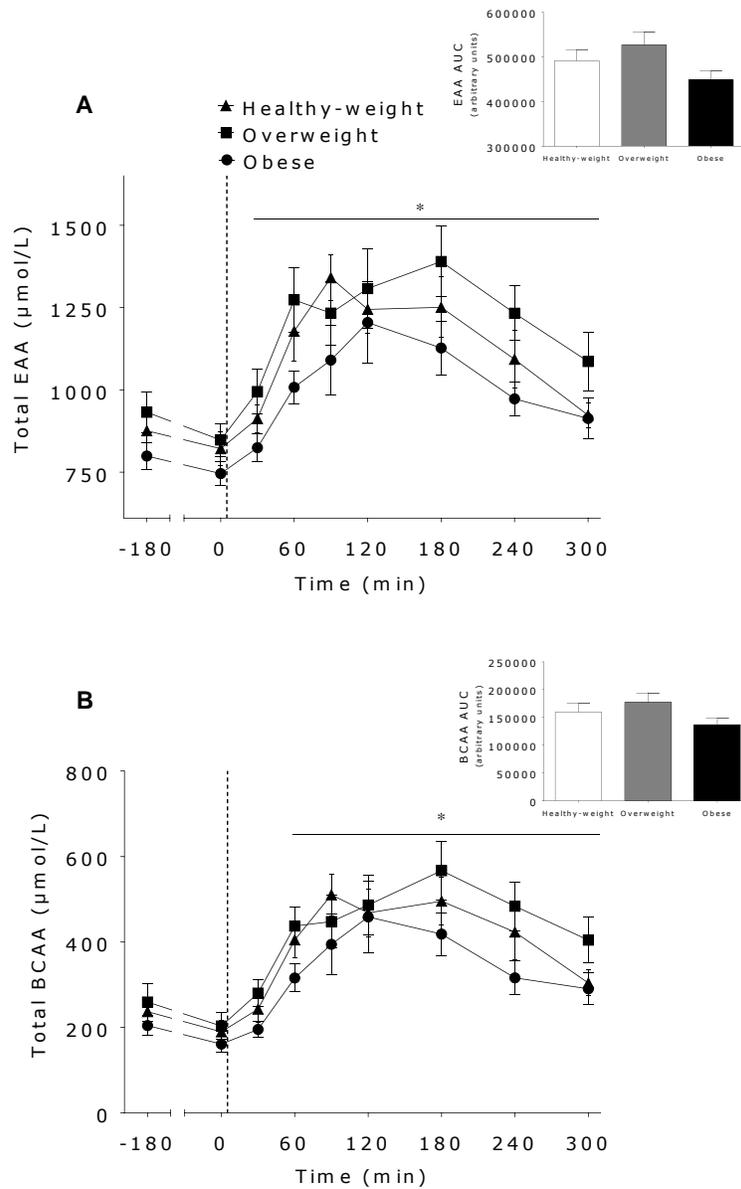**Figure 2.** Plasma essential amino acid (EAA; **A**) and branched chain amino acid concentrations (BCAA; **B**) ($\mu mol \cdot L^{-1}$) in the basal state and after consumption of pork (n=10 per group). Inset are the area under the EAA and BCAA × time curves. Dashed vertical line refers to pork ingestion. Data were analyzed with a two-factor ANOVA with repeated measures on time. A Tukey's post hoc test was used to locate differences between means for all significant interactions. * Indicates a difference from baseline. EAA: Group effect P=0.10, Time effect: P<0.001, Group × Time: P=0.68. EAA AUC: Group Effect P=0.11. BCAA: Group effect P=0.18, Time effect: P<0.001, Group × Time: P=0.76. BCAA AUC: Group Effect P=0.26 Mean ± SEM.
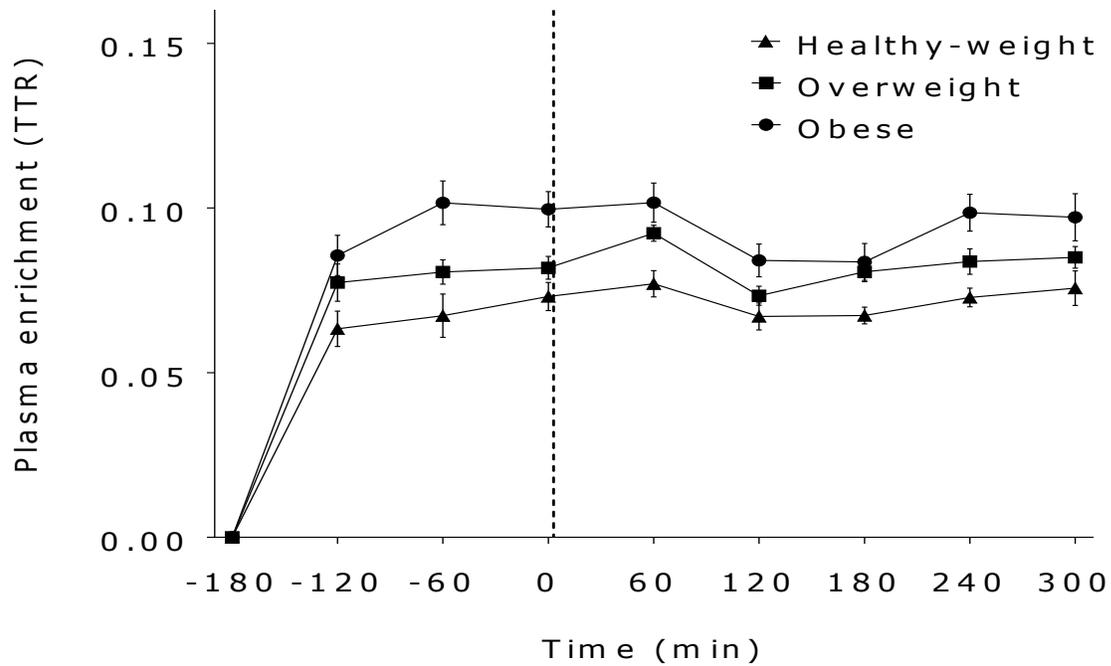
Figure 3. Plasma L-[*ring*-$^{13}C_6$]phenylalanine enrichments (tracer-to-tracee ratio [TTR]) in the basal state and after pork ingestion (*n*=10 per group). Dashed vertical line refers to pork ingestion. Data were analyzed with a two-factor ANOVA with repeated measures on time. L-[*ring*-$^{13}C_6$]phenylalanine: Group effect *P*=0.05, Time effect: *P*=0.20, Group × Time: *P*=0.19. Mean ± SEM.

Appendix E
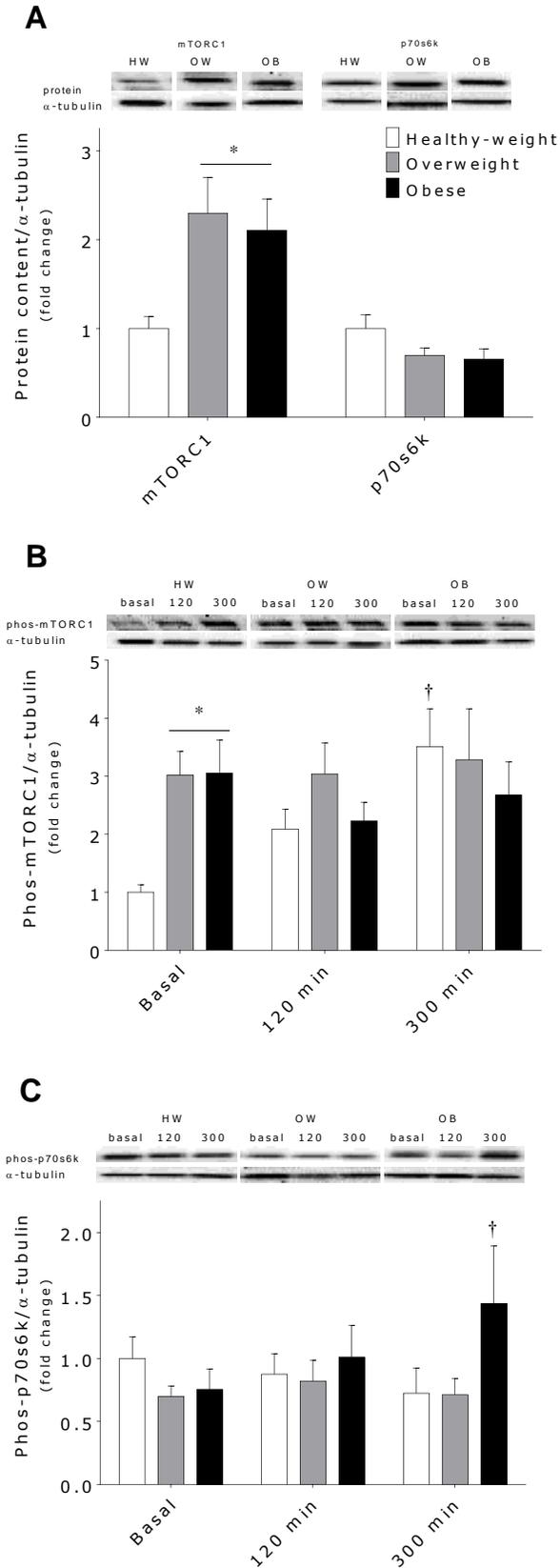Anabolic Signaling

A

Figure 4. Protein content for mammalian target of rapamycin complex 1 (mTORC1) and 70 kDa S6 protein kinase (p70S6K) in the basal-state (**A**). Phosphorylation of mTORC1 at Ser2448 (**B**), and p70S6K at Thr389 (**C**) at basal and after the ingestion of pork (n=10 per group). Data were analyzed with a two-factor ANOVA with repeated measures on time. A Tukey's post hoc test was used to locate differences between means for all significant interactions. * Indicates a difference from the HW. † Indicates a difference from basal. mTORC1 protein: Group effect P=0.26, Time effect: P=0.23, Group × Time: P=0.76. Phos-mTORC1: Group effect P=0.48, Time effect:
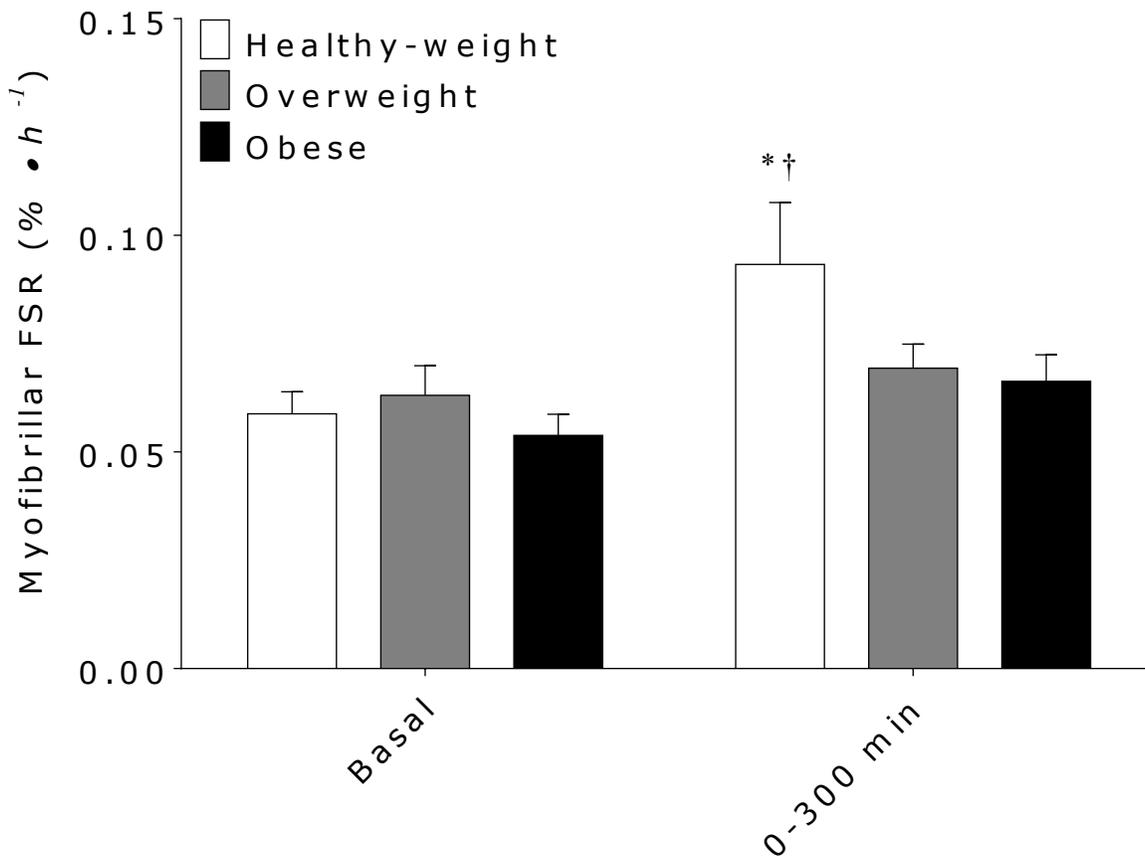
B

C

Figure 5. Myofibrillar fractional synthesis rates (FSR) at basal and after the ingestion of pork. Data were analyzed with a two-factor ANOVA with repeated measures on time. A Tukey's post hoc test was used to locate differences between means for all significant interactions. * Indicates a difference from basal, † Indicates a difference from the OW and OB groups. FSR: Group effect P=0.36, Time effect: P<0.02, Group × Time: P=0.002. Mean ± SEM.