Abnormal protein turnover and anabolic resistance to exercise in sarcopenic obesity

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ABSTRACT Obesity may impair protein synthesis rates and cause anabolic resistance to growth factors, hormones, and exercise, ultimately affecting skeletal muscle mass and function. To better understand muscle wasting and anabolic resistance with obesity, we assessed protein 24-h fractional synthesis rates (24-h FSRs) in selected hind-limb muscles of sedentary and resistance-exercised lean and obese Zucker rats. Despite atrophied hind-limb muscles (−28% vs. lean rats), 24-h FSRs of mixed proteins were significantly higher in quadriceps (+18%) and red or white gastrocnemius (+22 or +38%, respectively) of obese animals when compared to lean littersmates. Basal synthesis rates of myofibrillar (+8%) and mitochondrial proteins (−1%) in quadriceps were not different between phenotypes, while manufacture of cytosolic proteins (+12%) was moderately elevated in obese cohorts. Western blot analyses revealed a robust activation of p70S6k (178%) and a lower expression of the endogenous mTOR inhibitor DEPTOR (−28%) in obese rats, collectively suggesting that there is an obesity-induced increase in net protein turnover favoring degradation. Lastly, the protein synthetic response to exercise of mixed (−7%), myofibrillar (+6%), and cytosolic (+7%) quadriceps subfractions was blunted compared to the lean phenotype (+34, +40, and +17%, respectively), indicating a muscle- and subfraction-specific desensitization to the anabolic stimulus of exercise in obese animals.—Nilsson, M. I., Dobson, J. P., Greene, N. P., Wiggs, M. P., Shimkus, K. L., Wudeck, E. V., Davis, A. R., Laureano, M. L., Fluckey, J. D. Abnormal protein turnover and anabolic resistance to exercise in sarcopenic obesity. FASEB J. 27, 3905–3916 (2013). www.fasebj.org

Key Words: fractional synthesis rates • DEPTOR • muscle • diabetes

Obesity and sarcopenia act synergistically to limit functional capacity and increase the risk of disability and mortality in older adults (sarcopenic obesity; refs. 1–5), but obese individuals may also exhibit an altered lean mass distribution, characterized by a reduction in lower body lean mass, as early as in middle adulthood (6). Although the molecular mechanisms that underlie obesity-associated dysfunctions in lipid and glucose metabolism have been extensively studied to date, the effects on the regulatory processes of muscle protein metabolism (protein turnover) remain largely unknown. Expanding our understanding of the complex etiology underlying muscle impairment in obesity will aid in identifying potential therapeutic targets that can limit disability, morbidity, and mortality in this rapidly expanding population.

Skeletal muscle mass is governed by a complex interplay between mechanisms that regulate satellite cell function, protein degradation, and protein synthesis (7, 8), with muscle loss mainly occurring because of an imbalance between the two latter processes (degradation>synthesis) during disease, inactivity, and aging (9). Considering the anabolic and antiproteolytic actions of insulin and amino acids on skeletal muscle (10, 11), a reduction of insulin sensitivity and an enhancement in plasma and intramuscular amino acid levels with obesity (12–14), may affect protein degradation, protein synthesis, and ultimately muscle growth. Tracer studies in obese and/or insulin-resistant models, whether in humans or rodents, have yielded inconsistent results with regard to skeletal muscle protein fractional synthesis rates (FSRs), with reports ranging from decreased (15, 16), unchanged (17, 18), to increased FSR compared to their lean counterparts (19–21). Our group recently reported that the obese Zucker rat (fa/fa), which is a widely studied model of obesity and skeletal muscle atrophy, exhibits a mild suppres-
regulators of mTOR activity (DEPTOR). We hypothesized that chronic obesity, and/or related comorbidities, would impair the anabolic response to exercise regardless of fiber composition, but that basal protein synthesis rates would not be suppressed in this animal strain despite its diminished muscle mass and insulin-resistant state. Specifically, we theorized that basal FSR would be higher in obese vs. lean rats due to a chronic activation of translation initiation, and that obese animals would be resistant to the anabolic effect of resistance exercise.

**MATERIALS AND METHODS**

**Animals and experimental protocol**

All procedures were approved by the Institutional Animal Care and Use Committee of Texas A&M University and have been described in detail elsewhere (22). The primary events of obesity are overeating and inactivity, irrespective of origin (genetic vs. diet), making the obese Zucker rat (fa/fo), which carries a missense mutation (Gln269Pro) in the extracellular domain of the Ob leptin receptor, a suitable model for studying the effect of obesity on protein synthesis. Leptin may activate mTOR signaling in regulatory T cells (Tregs; ref. 32), and more research is warranted to determine the effect of leptin receptor dysfunction and leptin resistance on protein synthesis rates in skeletal muscle (33). Nevertheless, despite a pathological point mutation, the long form of the leptin receptor retains some function in the fa/fo strain (34), and, similar to sarcopenic obesity and metabolic syndrome in humans, these animals exhibit skeletal muscle atrophy, hyperphagia, hyperinsulinemia, hyperleptinemia, hypertriglyceridemia, hypercholesterolemia, glucose intolerance, insulin resistance, angiopathy, and neuropathy (35).

In the current study, 4-mo-old lean (L) and obese (O) male Zucker rats were matched into sedentary (S) and resistance exercise (E) groups, according to DXA-derived measures of body composition [fat sedentary (FS), n = 6; lean sedentary (LS), n = 8; fat exercised (FE), n = 8; and lean exercised (LE), n = 8]. All animals were housed individually in a climate-controlled environment with a reversed 12:12-h light-dark cycle, having free access to water and a standard commercial rat chow (Harlan Teklad 2016 Global; Harlan Bioproducts, Indianapolis, IN, USA). In ad libitum-fed male obese fa/fo rats, food intake and body weight (rate of gain and mass) are significantly higher vs. lean animals from weaning until 48 wk (11 mo) and 60 wk (14 mo) of age, respectively (36). Accrual of body weight and obesity development appears to be the most rapid until 17.5 wk of age (early stage), followed by two slower phases [18.5–29 wk of age (midstage) and 30–60 wk of age (late stage)], and thereafter, an age-related decline until death [61–115 wk of age; end stage]. Because there is no apparent plateau in body weight gain, there is no static phase of obesity in this animal model.

Rats assigned to the exercise condition were entrained to perform a full extension of hind legs wearing a weighted vest, resembling a traditional jump squat in humans. Our resistance exercise model is a well-documented type of exercise in rats, which has previously been shown to augment bone mass (37), muscle mass (38), and myocellular protein synthesis rates (22, 39, 40) in multiple exercise and training studies. To maximally stimulate skeletal muscle protein synthesis rates, 4 progressive exercise sessions (E1–E4) were conducted over a 9-d period, with each bout separated by 48 h. Because no major differences were noted in exercise adherence or maximal voluntary strength at the onset of the study, exercise intensities were not normalized to muscle mass, and the
number of sets (E1, 5 sets; E2, 5 sets; E3, 5 sets; and E4, 5 sets), repetitions (E1, 50 sets; E2, 68 sets; E3, 92 sets; and E4, 84 sets), and absolute weight lifted (E1, 5500 g; E2, 9340 g; E3, 15,460 g; and E4, 14,620 g) were matched between phenotypes.

For the assessment of 24-h FSR, an intraperitoneal 99.9% "H2O bolus injection (20 µl/g body weight; Cambridge Isotopes, Andover, MA, USA) was administered to the rats 24 h prior to tissue harvest. To ensure adequate "H enrichment of body water and proteins, the animals also had free access to 4% "H2O in their drinking water during the last day. Previous research has indicated that skeletal muscle protein synthesis rates are significantly elevated 12-24 h following the final exercise bout in rats (41). As such, the last squat session was completed 16 h before tissue procurement, and the animals were denied access to food for 4 h prior to being euthanized. Whole blood, quadriceps, gastrocnemius (mixed, red, or white), soleus, and plantaris were collected under anesthesia (ketamine hydrochloride and medetomidine), followed by a lethal dose of pentobarbital sodium. Fat, blood, and connective tissue were removed before the muscles were snap-frozen, pulverized into a homogeneous powered mixture, and stored at −80°C. At the time of euthanasia, the obese animals were 20 wk into their 60-wk dynamic phase of obesity development (i.e., midstage; ref. 36).

24-h protein synthesis rates

A detailed description of the GC/MS method and the differential centrifugation protocol used for the assessment of 24-h protein synthesis rates in mitochondrial (pooled subsarcolemmal and intermyofibrillar), cytosolic, and myofibrillar subfractions has been given elsewhere (22). Protein concentrations and contents for purposes of separating fractions or Western blot analysis procedures were assessed using a commercially available colorimetric assay (42, 43). In brief, the purity of the skeletal muscle subfractions was verified by Western blot analysis [GAPDH (cytosol), COX-IV (mitochondria), and α-actinin (myofibrils)], which was followed by determination of "H-labeling in body water and proteins. Plasma was reacted with 10 N NaOH and a 5% solution of Na2SO4 and chloroform. Pulverized mixed muscle (soleus, plantaris, red or white gastrocnemius, and quadriceps or isolated subfractions [quadriceps only]) were homogenized in a 10% TCA solution and centrifuged before dissolving the protein-rich pellet in 6 N HCl. An aliquot of the hydrolysate was dried down and derivatized with a 3:2:1 solution of methyl-8, methanol, and acetonitrile to determine "H-labeling of alanine on its methyl-8 derivative. All muscle and plasma samples were measured in duplicate or triplicate with separate preparations using an Agilent 7890A GC (HP-5 ms capillary column) coupled to an Agilent 5975C VL MSD with separate preparations using an Agilent 7890A GC (HP-5 and plasma samples were measured in duplicate or triplicate (44). Briefly, cytosolic proteins were denatured in 4× Laemmlı buffer at 95°C and loaded in equal amounts onto 4–15% polyacrylamide gels. Following 1 h electrophoresis at 20 mA in standard electrode buffer, a wet 16-h 3-step transfer (1 h at 1.3 mA/cm², 14 h at 3.8 mA/cm², and lastly 1 h at 7.5 mA/cm²), or a 40-min semidy transfer (7.5 mA/cm²) was used to transfer proteins onto 0.2-µm nitrocellulose or PVDF membranes soaked in Otter transfer buffer (49.6 mM Tris, 384 mM glycine, 20% methanol, and 0.01% SDS) or absolute methanol.

Membranes were blocked for 1 h in blocking buffer (20 mM Tris base, 5% dry milk, 8% NaCl, 100 mM NF, and 10 mM Na3VO4) and incubated overnight at 4°C under gentle agitation in a heat-sealed plastic bag containing 1-200–1-2500 primary antibody/buffer (see Supplemental Data). Following a serial wash step in 1× TBS (3×5 min), membranes were incubated for 1 h at room temperature with 1:2000 secondary antibody/buffer under continual agitation. After repeating the serial wash step, membranes were incubated for 5 min in ECL (Pierce, Rockford, IL, USA), and bands were developed with a CCD camera, mounted in a FluorChem SP imaging system (Alpha Innotech, San Leandro, CA, USA). Optical density was determined using the AlphaEase FC software (Alpha Innotech), which was automatically set to subtract nonspecific binding from densitometry values. All bands were normalized to GAPDH and expressed as arbitrary units.

Statistical analyses

The effects of phenotype (obesity) and exercise on expression and synthesis of proteins were assessed by 2×2 ANOVA, followed by Student-Newman-Keuls (SNK) post hoc tests when appropriate (SigmaStat 3.5; Systat Software Inc., San Jose, CA, USA). Sedentary conditions (FS vs. LS) were prioritized for this work and analyzed separately by independent t tests in addition to the ANOVA. If homogeneity of data failed, values were transformed (square root) or nonparametric tests were used. Statistical significance was set at P ≤ 0.05 (denoted alphabetically in figures and tables). Exact values of P are included for each of the outcomes. All data are presented as means ± se.

RESULTS

Wet mass, protein content, and protein concentration in skeletal muscle

Previous studies from our laboratory have shown that obese Zucker rats are hyperinsulinemic and display the most essential features of sarcopenic obesity (22). As consistently noted in this animal model, the obese phenotype exhibited significantly higher insulin levels (+537%), body weight (+57%), fat mass (+492%), and bone mineral content (+60%) compared to lean littermates (P<0.001 for all variables; see ref. 22). Obesity was also associated with generalized muscle atrophy (−18% vs. lean, P<0.001), which was further evident when assessing wet mass of hind-leg muscles (Tables 1 and 2) and total protein content of quadriceps in obese rats postmortem (−28 and −31% vs. lean, respectively; Table 1). Cytosolic and myofibrillar subfractions contributed 36 and 62%, respectively, to the absolute loss of skeletal muscle proteins, while mitochondria accounted for the remaining 2%.
Parameter & Group & \( P \)
\hline
\textbf{Quadriceps} & & \\
Wet mass (mg) & 1720 ± 144 & 1725 ± 117 & 2587 ± 203 & 2660 ± 127 & <0.001 & 0.804 & 0.826 \\
Protein content (mg) & & \\
Total & 401 ± 66 & 418 ± 59 & 595 ± 38 & 669 ± 99 & 0.007 & 0.549 & 0.707 \\
Myo & 299 ± 65 & 293 ± 64 & 420 ± 33 & 495 ± 104 & 0.048 & 0.660 & 0.608 \\
Mito & 4.95 ± 0.6 & 6.28 ± 0.5 & 7.94 ± 1 & 8.00 ± 1.3 & 0.021 & 0.472 & 0.512 \\
Cyto & 97 ± 6 & 118 ± 18 & 167 ± 7 & 166 ± 11 & <0.001 & 0.451 & 0.424 \\
Protein concentration (\(\mu g/mg\)) & & \\
Total & 240 ± 34 & 247 ± 34 & 231 ± 10 & 257 ± 40 & 0.643 & 0.985 & 0.789 \\
Myo & 177 ± 34 & 174 ± 36 & 163 ± 9 & 190 ± 42 & 0.982 & 0.734 & 0.675 \\
Mito & 3.06 ± 0.4 & 3.67 ± 0.2 & 3.16 ± 0.5 & 3.14 ± 0.6 & 0.635 & 0.524 & 0.498 \\
Cyto & 60 ± 4 & 69 ± 8 & 65 ± 1 & 63 ± 4 & 0.989 & 0.555 & 0.356 \\
24-h FSR (%/d) & & \\
Mixed & 3.67 ± 0.18 & 3.59 ± 0.12 & 3.06 ± 0.11 & 3.41 ± 0.18 & 0.007 & 0.876 & 0.481 \\
Myo & 2.31 ± 0.23 & 2.46 ± 0.25 & 2.14 ± 0.14 & 2.99 ± 0.42 & 0.527 & 0.081 & 0.215 \\
Mito & 4.01 ± 0.29 & 3.99 ± 0.35 & 4.06 ± 0.34 & 3.94 ± 0.32 & 0.996 & 0.828 & 0.879 \\
Cyto & 8.90 ± 0.47 & 9.55 ± 0.55 & 7.95 ± 0.51 & 9.32 ± 0.55 & 0.221 & 0.042 & 0.455 \\

Values are means ± se. Myo, myofibrillar; Mito, mitochondrial; Cyto, cytosolic. Lean and obese Zucker rats (\(n=30\)) were matched according to lean body mass within each phenotype and grouped into sedentary (LS, \(n=8\)); resistance-exercised (LE, \(n=8\); FS, \(n=6\)) cohorts. Main effects [obesity (OB) and exercise (EX)] and interaction (OB×EX) from 2-way ANOVA are noted for each variable. Group means that are significantly different according to SNK post hoc analyses or \(t\)-tests do not share the same letter (\(P \leq 0.05\)).

days of exercise did not significantly affect muscle weights or protein contents or concentrations in either phenotype (Tables 1 and 2), with the exception of an unexplained lower soleus mass with exercise in lean animals (Table 2).

Cumulative 24-h FSRs

To explore contributing mechanisms to muscle loss and anabolic resistance in sarcopenic obesity, 24-h cumulative synthesis rates of mixed and/or mitochondrial, cytosolic, and myofibrillar proteins in quadriceps, soleus, plantaris, and red and white gastrocnemius of lean and obese Zucker rats were assessed under sedentary conditions or after 4 bouts of progressive resistance exercise (Figs. 1 and 2). As predicted, the obese phenotype exhibited 18, 22, and 38% higher mixed protein synthesis rates in quadriceps, red gastrocnemius, and white gastrocnemius, respectively, compared to its lean counterparts (Figs. 1A and 2C, D). Basal synthesis rates of myofibrillar (+8%) and mitochondrial proteins (−1%) were normal, while manufacture of cytosolic proteins was moderately elevated (+12%) in quadriceps of obese animals (Fig. 1B–D). Subfractions of posterior crural muscle groups either were not

### Table 2. Wet mass and cumulative 24-h FSRs of mixed proteins in posterior crural muscle groups of lean and obese Zucker rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>( P )</th>
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<tbody>
<tr>
<td><strong>Soleus</strong></td>
<td>&amp;</td>
<td><strong>F</strong></td>
</tr>
<tr>
<td>Wet mass (mg)</td>
<td>183 ± 9 &amp; 174 ± 9 &amp; 220 ± 8 &amp; 187 ± 5 &amp; 0.004 &amp; 0.013 &amp; 0.145</td>
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<tr>
<td>24-h FSR, mixed (%/d)</td>
<td>6.38 ± 0.16 &amp; 6.23 ± 0.23 &amp; 6.62 ± 0.14 &amp; 7.00 ± 0.34 &amp; 0.044 &amp; 0.635 &amp; 0.281</td>
<td></td>
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<tr>
<td><strong>Plantaris</strong></td>
<td>&amp;</td>
<td><strong>F</strong></td>
</tr>
<tr>
<td>Wet mass (mg)</td>
<td>275 ± 18 &amp; 280 ± 11 &amp; 387 ± 12 &amp; 364 ± 17 &amp; &lt;0.001 &amp; 0.547 &amp; 0.359</td>
<td></td>
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<tr>
<td>24-h FSR, mixed (%/d)</td>
<td>4.72 ± 0.39 &amp; 4.42 ± 0.25 &amp; 4.97 ± 0.52 &amp; 4.75 ± 0.54 &amp; 0.505 &amp; 0.573 &amp; 0.916</td>
<td></td>
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<tr>
<td><strong>Gastrocnemius</strong></td>
<td>&amp;</td>
<td><strong>F</strong></td>
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<tr>
<td>Wet mass (mg)</td>
<td>1436 ± 80 &amp; 1484 ± 59 &amp; 1840 ± 60 &amp; 1807 ± 59 &amp; &lt;0.001 &amp; 0.895 &amp; 0.480</td>
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<tr>
<td>24-h FSR, mixed (%/d)</td>
<td>3.61 ± 0.16 &amp; 3.27 ± 0.21 &amp; 2.97 ± 0.16 &amp; 3.01 ± 0.08 &amp; 0.008 &amp; 0.346 &amp; 0.235</td>
<td></td>
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</table>

Values are means ± se. Lean and obese Zucker rats (\(n=30\)) were matched according to lean body mass within each phenotype and grouped into sedentary (LS, \(n=8\)); resistance-exercised (LE, \(n=8\); FS, \(n=6\)) cohorts.soleus, plantaris, and gastrocnemius were obtained at end point, and wet mass was calculated as an average of contralateral legs. Main effects [obesity (OB) and exercise (EX)] and interaction (OB×EX) from 2-way ANOVA are noted for each variable. Group means that are significantly different according to SNK post hoc analyses or \(t\)-tests do not share the same letter (\(P \leq 0.05\)).
assessed or were reported elsewhere (22). Because there was no statistical difference between sedentary groups in mixed FSRs of soleus and plantaris (Fig. 2A, B), the main effect of phenotype (on soleus FSR) was partly attributed to a differential exercise response between lean and obese rats (Table 2). The anabolic response to exercise of mixed (11002%, white gastrocnemius), myofibrillar (11001%, quadriceps), and cytosolic (11001%7%, quadriceps) subfractions was blunted compared to the lean phenotype (11001%34, 11001%40, and 11001%17%, respectively), suggesting that obesity is associated with a desensitization to anabolic stimuli (Figs. 1, B–D and 2C).

**Akt-mTOR signaling**

*Signaling by phenotype*

Next, we examined the effects of genetic obesity on expression and phosphorylation of typical regulators of protein synthesis (Figs. 3 and 4) in mixed gastrocnemius muscle. We selected this generally well-characterized muscle on the basis of previous work and the present study showing elevated basal FSRs in obese vs. lean animals (21, 22, 45), whether assessed in specific protein fractions or specific regions of the muscle (red or white; Fig. 2C, D). mTOR levels were moderately lower in obese vs. lean rats, although this did not reach statistical significance (Fig. 3A; P=0.066). Consistent with these findings, the expression of mTOR-associated binding proteins Raptor (positive regulator; Fig. 3B) and DEPTOR (negative regulator; Fig. 3C) were suppressed in obese animals [-35% (P=0.029) and -28% (P=0.025), respectively]. The phenotype difference in DEPTOR expression, but not Raptor, remained after normalizing to total mTOR content (Fig. 3C, inset) and is perhaps indicative of a net dysregulation of mTOR activity, potentially enhancing translation initiation in the obese phenotype. However, we should note that the present findings in skeletal muscle are markedly different from those of a previous study conducted in white adipose tissue of obese rodents and humans (46), where DEPTOR was elevated. This discrepancy between studies may not be surprising given the differing strategies of these metabolically divergent tissues with obesity.

As indicators of mTOR activation, we assessed two primary downstream substrates (p70S6k and eIF4EBP1). Although there were no phenotypic differences for total p70S6k (Fig. 4A), we noted a robust phosphorylation of p70S6k (Fig. 4B) in the fa/fa phenotype, which yielded a significant phospho/total ratio (p70S6kthr389/
p70S6k ratio, +178%, \( P = 0.019 \); Fig. 4C). p70S6k is the main enzyme responsible for phosphorylating the S6 protein in the 40S ribosomal subunit. Likewise, total eIF4EBP1 protein, the major binding protein that negatively regulates eIF4E activity, was not different between lean and obese rats (Fig. 4D), but unlike p70S6k, the phosphorylation of this important binding protein (eIF4EBP1\( \text{Thr}^{37/46} \)) was similar between lean and obese animals (Fig. 4E), at least independent of exercise (\( P > 0.05 \)). Analyses of the phospho/total ratio (Fig. 4F) for lean animals demonstrated that they were not different from the obese groups (\( P > 0.05 \)).

**Signaling by exercise**

Generally, total expression and activation (when applicable) of anabolic signaling proteins were not different in exercised vs. unexercised animals at 16 h postexercise, despite an elevation in 24-h mixed FSRs in the lean phenotype. The most notable exceptions were a more robust phosphorylation of eIF4EBP1 (eIF4EBP1\( \text{Thr}^{37/46} \)) vs. eIF4EBP ratio, +30\%, \( P = 0.039 \); Fig. 4E, F) and an increased total expression of rpS6 (+63\%, \( P = 0.024 \)) in exercised vs. unexercised obese rats. Phosphorylation of eIF4EBP1 diminishes its inhibitory effect on mRNA translation (47), but in this case, the elevated phospho/total ratio did not appear to enhance the anabolic response in skeletal muscle. The continued heightened response to exercise was somewhat surprising since we assessed phosphorylation states of these proteins at 16 h postexercise, when others have demonstrated that the exercise-induced hyperphosphorylation of growth signals, such as Akt and mTOR, have returned to normal (48). This delayed exercise response appeared to be more pronounced in obese animals when compared to their lean counterparts (+12 and +5\%, respectively); however, this phenotype-specific finding did not reach statistical significance. Lastly, expression of DEPTOR, an endogenous competitive inhibitor of mTOR, was significantly reduced with resistance exercise in lean (\( P = 0.025 \)) but not obese rats, and total DEPTOR expression after exercise in lean rats was similar to that in obese rats, with or without exercise (Fig. 3).

Consistent with the notion that hyperphosphorylation of enzymes should be normalized by 16 h postexercise, other Western blot analyses (Fig. 5 and Table 3) yielded no differences among groups for eIF2A\( \text{Ser}^{51} \)/total eIF2A, (a key regulator of translation initiation) or eEF2\( \text{Thr}^{56} \)/eEF2 (a key regulator of peptide-chain elongation) ratios. Interpretations regarding the lack of a phosphorylation response should be viewed with extreme caution, particularly because our Western blot analyses were obtained from skeletal muscle at the time of tissue harvest (16 h postexercise), which may or may not be indicative of total FSR measured cumulatively over the previous 24 h. Independent of phosphorylation states of signaling enzymes, other proteins of interest (eIF2\( \alpha \), PRAS40, and REDD1) were also similar among groups (Table 3). Together, the present findings suggest that the augmented FSR is primarily attributed to a dysregulation of the mTOR/p70S6k signaling axis in the obese cohort. Further, the
diminished muscle mass in the obese phenotype is not due to reductions of muscle protein synthesis, indicating that other mechanisms, such as protein degradation, may drive muscle loss during the dynamic phase of obesity development (22, 49).

DISCUSSION

Although obesity and associated comorbidities, such as insulin resistance and hyperaminoacidemia, may affect the ability to make new proteins and thereby impair muscle growth (50), previous research has been inconsistent, with reports ranging from decreased (15, 16) to normal (17, 18) to increased (19–21, 45) FSRs in obese humans and rodents. In the current study, we used a novel tracer method to assess 24-h biosynthesis of proteins in mitochondria, cytosol, and myofibrils in genetically obese rats under free-living conditions, and our results suggest that FSR is significantly elevated in specific subfractions of the muscle cell and that other regulatory processes must contribute to sarcopenic obesity. For the first time, we link DEPTOR expression, an endogenous inhibitor of mTOR and a key player in cancer development and progression, as a potential regulator of the anabolic response to exercise in skeletal muscle.

Elevated 24-h skeletal muscle protein synthesis rates in sarcopenic obesity

A major advantage of our study is the use of a tracer method that allows for assessment of 24-h FSRs in free-living organisms, which incorporates the cumulative effect of all daily and nocturnal activities on skeletal muscle protein synthesis rates. By circumventing the major limitations associated with traditional tracer research (25, 51), we believe that our FSR results, based on long-term enrichment periods with $^2$H$_2$O, are more relevant and generalizable to other species. In this study, we report that 24-h FSRs of mixed proteins are augmented in proximal (quadriceps) and distal (red and white gastrocnemius) hind-leg muscle groups of 4.5-mo-old obese Zucker rats. Consistent with these findings, 24-h FSRs of cytosolic proteins are higher in the aforementioned muscle groups compared to lean rats (current study and ref. 22), which accounts for the observation that synthesis rates of mixed proteins are...
enhanced. We also show that total expression and 24-FSRs of mitochondrial proteins are either normal or elevated with dynamic obesity (current study and ref. 22), indicative of a compensatory mechanism to increase mitochondrial biogenesis and substrate oxidation in the face of nutrient excess (52). Meanwhile, the manufacture of myofibrillar proteins is only mildly suppressed or unaffected (current study and ref. 22), which supports the notion that protein synthesis rates are not impaired during weight gain in obese rats. As theorized by our group (22) and later confirmed by Masgrau et al. (53), the age and stage of disease of the animals will influence the observed degree of dysfunction in protein turnover. Our present results raise the possibility that an up-regulation in protein synthesis compensates for an excess in protein degradation in skeletal muscle of obese Zucker rats, potentially to limit overt muscle loss. A recent report from She et al. (45), demonstrating an overall increase in protein turnover in this animal strain, supports this supposition (although animals were not assessed for 24 h in the free-living state). As such, mechanisms other than protein manufacture, such as protein breakdown and/or satellite cell proliferation (49, 54), must contribute significantly to the atrophic phenotype of obese Zucker rats, and additional time course studies will be necessary to map the progression of events leading to sarcopenic obesity.

**Chronic activation of p70S6k with sarcopenic obesity**

Accumulation of intracellular lipid metabolites is one of the first maladies associated with obesity and may antagonize insulin action via activation of serine/threonine kinases and inhibition of the IRS-P13K-Akt-mTOR pathway (55). We found that the obese phenotype exhibits a reduced expression of Akt, mTOR, and Raptor, all of which are important mediators of skeletal muscle growth. Considering that the Akt/mTOR pathway is up-regulated during hypertrophy and down-regulated during muscle atrophy (56), it is not surprising that removal or inactivation of these genes (Akt, mTOR, or Raptor) causes severe muscle loss (57–59). Thus, it would seem that a reduction in Akt/mTOR-Raptor levels would intuitively lead to an attenuation of protein synthesis rates, but our observation that 24-h FSRs of mixed proteins and activation of p70S6k are augmented 1.25- and 2.7-fold, respectively, in obese rats, is inconsistent with that notion and prompted us to investigate alternative regulatory mechanisms of the mTOR-p70S6k signaling axis.

One of the most intriguing findings from this study is that DEPTOR, an endogenous inhibitor of mTORC1 and a novel target for anticancer treatments, (46, 60–64) is significantly suppressed in skeletal muscle of obese Zucker rats. Recent identification of the mTOR-interacting protein DEPTOR and the observation that its expres-
p70S6k and increased protein synthesis, which, in part, may be due to mTORC1 stimulation by nutrients (branched-chain amino acids (BCAAs) and growth factors (insulin/IGF-1) that DEPTOR, which is necessary for subsequent activation of p70S6k activity and enhanced protein synthesis rates in skeletal muscle of obese rats. Considering that p70S6k activation induces insulin resistance (with respect to glucose uptake) through a negative feedback loop on IRS-1 (at Ser307 and Ser636/639) and the deletion of p70S6k protects against diet-induced obesity (65), it is feasible that therapies designed to “restore” DEPTOR protein content may profoundly influence the metabolic syndrome/insulin resistance to glucose uptake with sarcopenic obesity. Whether or not the protection of DEPTOR protein will lead to improved insulin sensitivity with obesity requires further study, but could be problematic if not catastrophic, particularly if the loss of DEPTOR and the resultant anabolic events in the cell are consequent (and compensatory) to uncontrolled protein degradation.

Our results in obese Zucker rats, along with work by others (45), indicate that hyperphagia (36), coupled with excessive protein degradation (49), increases cytoplasmic amino acid availability and facilitates mTOR/p70S6k activation (current study and ref, 12), ultimately augmenting synthesis rates of skeletal muscle proteins in the dynamic phase of obesity (current study and refs. 20–22, 53). Paradoxically, while a BCAA-stimulated activation of the mTOR-p70S6k axis may contribute to limiting overt muscle loss with obesity, it also serves a seemingly opposing purpose by inducing insulin resistance to glucose uptake through a negative feedback loop, which dampens upstream signals that promote insulin-stimulated glucose uptake (see Supplemental Fig. S1 for graphics).

### Anabolic resistance to exercise in sarcopenic obesity

Lifestyle modifications have traditionally been viewed as low-cost, noninvasive, and nonpharmacological alternatives to anorexigenic drugs and bariatric surgery in obesity, but recent evidence suggests that nonsurgical methods may not be cost-effective in morbidly obese populations (66, 67). While exercise undoubtedly will continue to be used in conjunction with other therapies in weight loss management (68), several lines of research indicate that obesity (and/or related comorbidities) desensitizes skeletal muscle to the anabolic effects of contractile activity. An impaired anabolic response following synergistic ablation (28, 29), high-force electrical stimulation (30), and voluntary resistance exercise (22, 31) has been documented in obese rodent models and/or humans previously. The current study expands on those findings by showing a desensitization to resistance exercise in multiple muscle groups (proximal and distal hind limb), fiber types (red and white gastrocnemius), and cellular subfractions (mixed, cytoplasmic, and myofibrillar) of the cell are consequent (and compensatory) to uncontrolled protein degradation.

Figure 5. Representative blot images of selected signaling proteins of lean and obese rats, with or without resistance exercise. Analyses of signaling proteins were conducted on FS, FE, LS, and LE rats and are segregated by function. See Table 3 for specific data and the statistical relevance for these images.

- Akt
- eIF2B-ε
- p-eIF2B-εser539
- mTOR Regulative Proteins
  - REDD1
  - PRAS40
- Peptide-Chain Elongation Proteins
  - eEF2
  - p-eEF2thr56
  - eEF2K
- Ribosomal Targets of mTOR
  - rpS6
  - p-rpS6ser240/244
- GAPDH
- Loading Standard

<table>
<thead>
<tr>
<th>FS</th>
<th>FE</th>
<th>LS</th>
<th>LE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akt</td>
<td>eIF2B-ε</td>
<td>p-eIF2B-εser539</td>
<td>mTOR Regulative Proteins</td>
</tr>
<tr>
<td>REDD1</td>
<td>PRAS40</td>
<td>Peptide-Chain Elongation Proteins</td>
<td></td>
</tr>
<tr>
<td>eEF2</td>
<td>p-eEF2thr56</td>
<td>eEF2K</td>
<td></td>
</tr>
<tr>
<td>rpS6</td>
<td>p-rpS6ser240/244</td>
<td>Ribosomal Targets of mTOR</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>Loading Standard</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 3. Western blot analyses of selected signaling proteins of lean and obese rats, with or without resistance exercise

<table>
<thead>
<tr>
<th>Protein</th>
<th>Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FS, n = 6</td>
<td>FE, n = 8</td>
</tr>
<tr>
<td>Akt and downstream peptide-chain-initiation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>substrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akt</td>
<td>279 ± 49</td>
<td>290 ± 33</td>
</tr>
<tr>
<td>eIF2B-ε</td>
<td>252 ± 44</td>
<td>345 ± 40*</td>
</tr>
<tr>
<td>p-eIF2BsεΨ559</td>
<td>532 ± 57</td>
<td>863 ± 114</td>
</tr>
<tr>
<td>mTOR regulative proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>REDD1</td>
<td>81 ± 17</td>
<td>99 ± 13</td>
</tr>
<tr>
<td>PRAS40</td>
<td>436 ± 29</td>
<td>386 ± 13</td>
</tr>
<tr>
<td>Peptide-chain-elongation proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eIF2</td>
<td>109 ± 19</td>
<td>106 ± 16</td>
</tr>
<tr>
<td>p-eIF2hr56</td>
<td>111 ± 22</td>
<td>99 ± 24</td>
</tr>
<tr>
<td>eIF2K</td>
<td>61 ± 13</td>
<td>43 ± 5</td>
</tr>
<tr>
<td>Ribosomal targets of mTOR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rpS6</td>
<td>57 ± 6*</td>
<td>92 ± 10*</td>
</tr>
<tr>
<td>p-rpS6Ψ240/244</td>
<td>15 ± 4</td>
<td>32 ± 11</td>
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<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± se of arbitrary units of density obtained during Western blotting imaging. Western blots of mixed gastrocnemius muscle homogenates in lean and obese Zucker rats. Each gel was run in a balanced fashion (all experimental groups included) with identical quantities of protein per lane, and the most representative blot was chosen for depiction (Fig. 5). GAPDH was probed on all gels to verify similar loading among lanes. Specific signaling factors were grouped on the basis of Akt and its effect on proteins involving peptide-chain initiation, mTOR regulative factors, elongation factors, and ribosomal proteins. Independent of mTOR-related pathways, ERK1/2 expression was also probed. Proteins depicted in the table were not statistically different either within phenotype and/or exercise treatment and represent conserved expression between phenotypes, with or without exercise except when noted. Exact values of P are provided. Main effects [obesity (OB) and exercise (EX)] and interaction (OB×EX) from 2-way ANOVA are noted for each variable. Group means that are significantly different according to SNK post hoc analyses or t tests do not share the same letter (P ≤ 0.05). *P < 0.05 within phenotype.

In summary, we show that sarcopenic obesity is associated with an enhancement of basal protein synthesis rates and confirm that insulin-resistant skeletal muscle is refractory to the anabolic stimulus of resistance exercise. Our study is the first to suggest that changes in DEPTOR expression play an important role in the regulation of protein manufacture after resistance exercise, and the lack of an anabolic response in the obese state may be due to an already diminished expression of this important regulatory protein. These findings may have relevance for the development of new therapeutic strategies to counteract muscle wasting in various medical conditions. Further studies are required to fully elucidate the role of DEPTOR in protein metabolism and the mechanisms underlying impairments in protein turnover and anabolic resistance in sarcopenic obesity.

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