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## Lipid-Induced Insulin Resistance Is Associated With an Impaired Skeletal Muscle Protein Synthetic Response to Amino Acid Ingestion in Healthy Young Men

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**The ability to maintain skeletal muscle mass appears to be impaired in insulin-resistant conditions, such as type 2 diabetes, that are characterized by muscle lipid accumulation. The current study investigated the effect of acutely increasing lipid availability on muscle protein synthesis. Seven healthy young male volunteers underwent a 7-h intravenous infusion of L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine on two randomized occasions combined with 0.9% saline or 10% Intralipid at 100 mL/h. After a 4-h “basal” period, a 21-g bolus of amino acids was administered and a 3-h hyperinsulinemic-euglycemic clamp was commenced (“fed” period). Muscle biopsy specimens were obtained from the vastus lateralis at 1.5, 4, and 7 h. Lipid infusion reduced fed whole-body glucose disposal by 20%. Furthermore, whereas the mixed muscle fractional synthetic rate increased from the basal to the fed period during saline infusion by 2.2-fold, no change occurred during lipid infusion, despite similar circulating insulin and leucine concentrations. This “anabolic resistance” to insulin and amino acids with lipid infusion was associated with a complete suppression of muscle 4E-BP1 phosphorylation. We propose that increased muscle lipid availability may contribute to anabolic resistance in insulin-resistant conditions by impairing translation initiation.**

It has been proposed that the inability of skeletal muscle to adequately synthesize new protein in response to

anabolic stimuli, such as amino acids (termed “anabolic resistance”), is a key contributory factor to the muscle mass loss observed in a variety of conditions such as aging, type 2 diabetes, disuse, and critical illness (1,2). A common feature of all of these conditions is the inability of skeletal muscle glucose metabolism to respond adequately to insulin signaling (insulin resistance), which is thought to be a consequence of the intracellular accumulation of lipid within skeletal muscle (3). Because insulin signaling is also integral to skeletal muscle amino acid delivery and metabolism, in particular playing a permissive role in regulating muscle protein synthesis via activation of the mammalian target of rapamycin complex 1 (mTOR) pathway (4,5), it is possible that lipid-induced insulin resistance may contribute toward skeletal muscle anabolic resistance. Indeed, skeletal muscle protein synthesis in response to insulin and amino acids appears to be negatively related to whole-body fat mass and insulin sensitivity in humans (6). Thus, it is important to elucidate the effect of excess lipid per se on insulin- and amino acid-stimulated skeletal muscle protein synthesis and the associated signaling pathways in vivo in humans, particularly if strategies to treat anabolic resistance are to focus on reducing skeletal muscle lipid accumulation and insulin resistance.

The intravenous infusion of a lipid emulsion with heparin is routinely used to elevate free fatty acid availability and allows researchers to investigate the

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acute effects of lipid-induced insulin resistance on insulin signaling (7) and subsequent impairments in glucose uptake, storage, and oxidation (8,9). The current study investigated the effect of acutely elevating fatty acid availability to a concentration known to induce insulin resistance of glucose metabolism on the muscle protein synthetic response and associated signaling to amino acid ingestion in the presence of a controlled, steady-state circulating insulin concentration in humans.

**RESEARCH DESIGN AND METHODS**

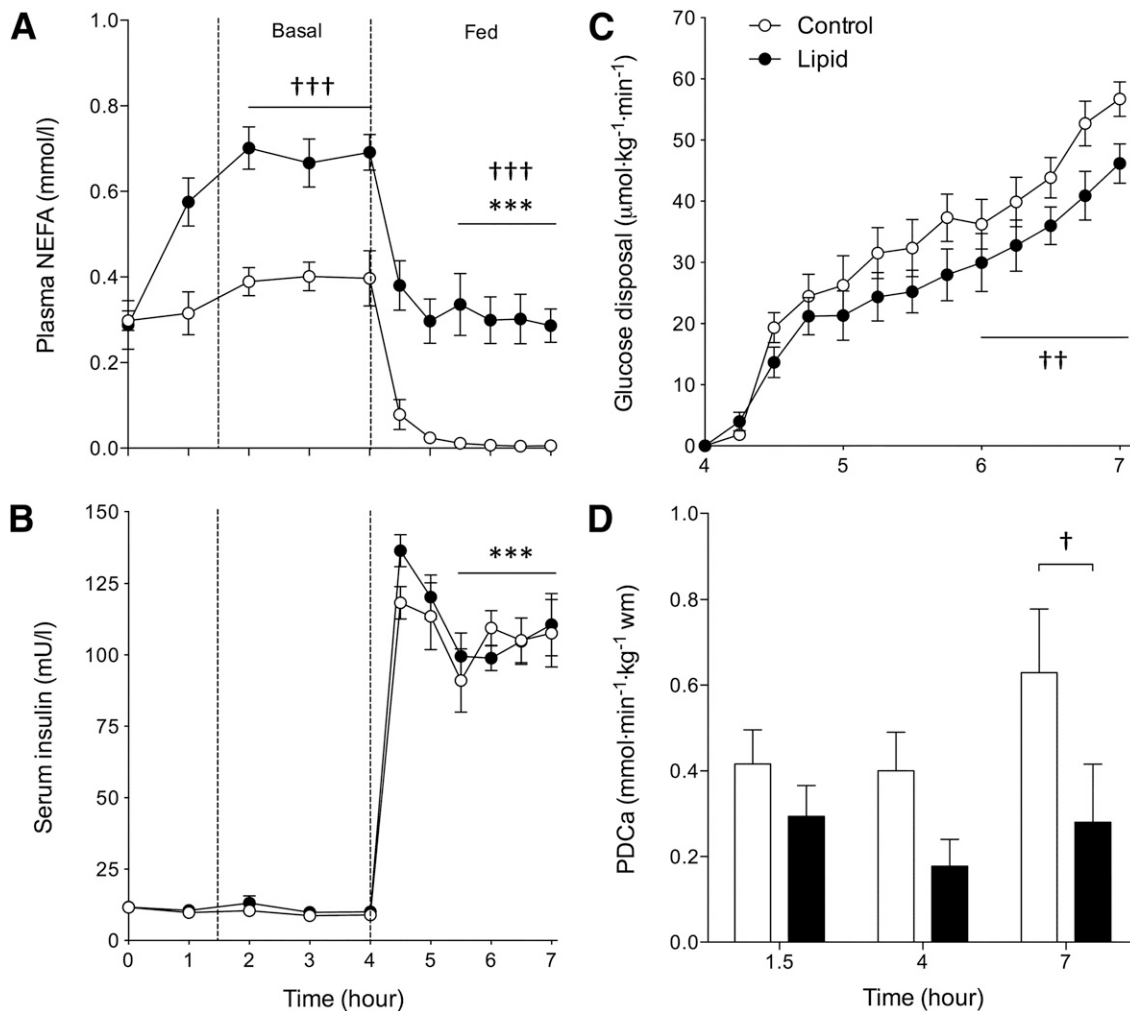
**Subjects**

Seven healthy male participants (age  $23.0 \pm 0.8$  years, body mass  $78.5 \pm 3.8$  kg, BMI  $24.5 \pm 0.9$  kg/m<sup>2</sup>) gave their written informed consent to participate in the current study, which was approved by the University of

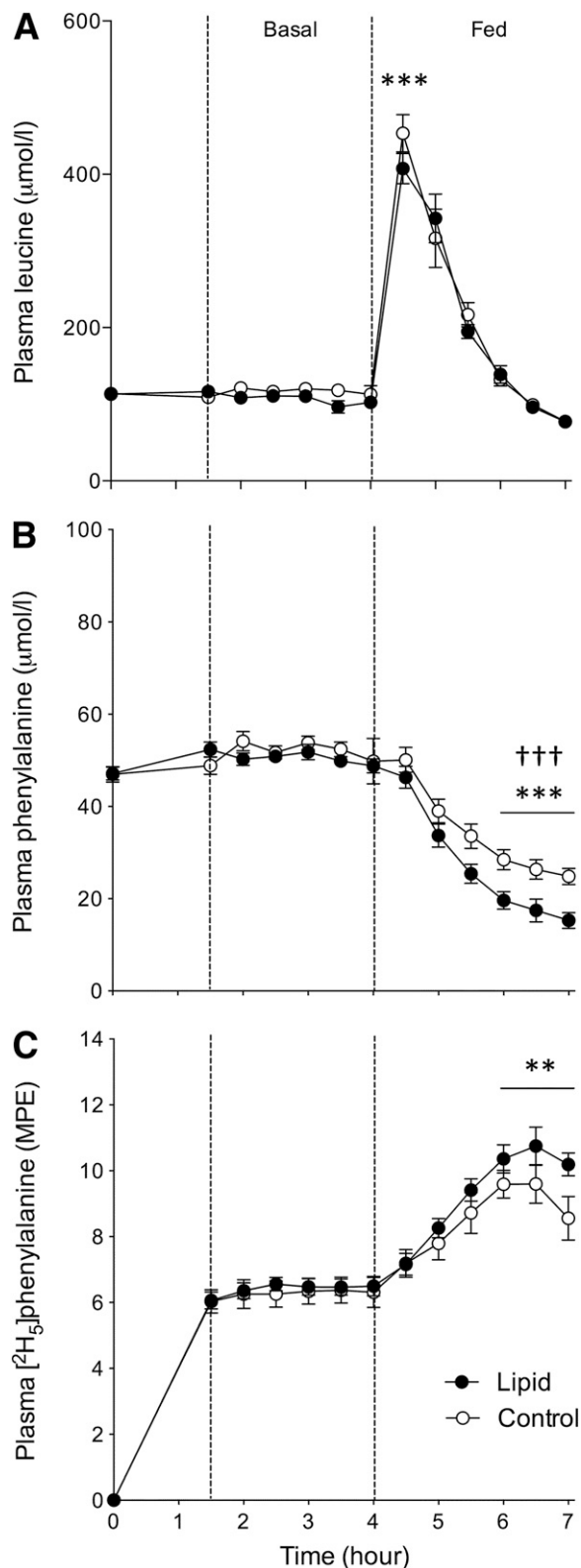
Nottingham Medical School Ethics Committee in accordance with the Declaration of Helsinki.

**Protocol**

Participants reported to the laboratory at 0800 on two randomized occasions, at least 2 weeks apart, after an overnight fast and having abstained from strenuous exercise for the previous 48 h. On each visit, the participants rested semisupine on a bed while a 7-h intravenous infusion of L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine ( $0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ; Cambridge Isotopes Laboratories, Tewksbury, MA) was performed in combination with the infusion of 0.9% saline (“control”) or 10% Intralipid (“lipid”; Fresenius Kabi, Bad Homburg, Germany) at a rate of 100 mL/h. During the lipid infusion, heparin was infused at rate of 600 units/h to elevate plasma nonesterified fatty acid (NEFA) availability. After 4 h, a 21-g bolus of amino acids (except phenylalanine and tyrosine; Tyrosidon, SHS



**Figure 1**—Plasma NEFA concentration (A), plasma insulin concentration (B), whole-body glucose disposal (C), and PDCa (D) before (basal 1.5–4 h) and after (fed 4–7 h) the administration of 21 g amino acids and a 3-h hyperinsulinemic-euglycemic (~100 mU/L) clamp during a 7-h intravenous infusion of saline (control) or 10% Intralipid (lipid) at a rate of 100 mL/h. Values represent means  $\pm$  SEM. †††*P* < 0.001, ††††*P* < 0.01, †*P* < 0.05, lipid significantly different from corresponding control value. \*\*\**P* < 0.001, control and lipid during fed significantly different from corresponding basal steady state.



**Figure 2**—Plasma leucine concentration (A), plasma phenylalanine concentration (B), and plasma L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine enrichment (C) before (basal 1.5–4 h) and after (fed 4–7 h) the administration of 21 g amino acids and a 3-h hyperinsulinemic-euglycemic (~100 mU/L) clamp during a 7-h intravenous infusion of saline (control) or 10% Intralipid (lipid) at a rate of 100 mL/h. Values represent means  $\pm$  SEM. MPE, mole percent excess. †††*P* < 0.001, lipid significantly lower than corresponding control value. \*\*\**P* < 0.001,

International Ltd., Liverpool, U.K.) was administered in a 440-mL solution via a nasogastric tube to avoid issues with palatability. At the same time, a 3-h hyperinsulinemic (Actrapid; Novo Nordisk, Aalborg Øst, Denmark) euglycemic (20% dextrose; Baxter Healthcare, Thetford, U.K.) clamp (10) was commenced at a rate of 50 mU  $\cdot$  m<sup>-2</sup>  $\cdot$  min<sup>-1</sup>. At *t* = 7 h, the Intralipid/saline, L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine, and insulin infusions were stopped, whereas the glucose infusion was continued until the blood glucose concentration was stable.

#### Sample Collection and Analysis

Arterialized venous blood was obtained from a heated hand vein (11) at *t* = 0, 1.5 h, and every 30 min thereafter. Plasma treated with tetrahydrolipostatin (30  $\mu$ g/mL plasma) was analyzed for NEFA (NEFA C kit; WAKO Chemicals, Düsseldorf, Germany) on an automated analyzer (ABX Pentra 400; Horiba Medical Ltd., Montpellier, France). Plasma separated from EGTA-treated blood was analyzed for insulin concentration by ELISA (DRG Diagnostics, Marburg, Germany) and, after deproteinization on ice with dry 5-sulfosalicylic acid, phenylalanine, and leucine concentration, and L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine enrichment by gas chromatography–mass spectrometry (Agilent 7890A GC/5975C; MSD, Little Falls, NY), as previously described (12,13).

Muscle samples were obtained from the vastus lateralis at *t* = 1.5, 4, and 7 h using the Bergström needle biopsy technique and immediately frozen in liquid nitrogen. Freeze-dried muscle separated free of visible blood, fat, and connective tissue was analyzed for intracellular tissue free and protein-bound L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine enrichments (12,13), as well as acetylcarnitine and long-chain acyl-CoA (*t* = 1.5 and 7 h only), as previously described (9). The remaining “wet” muscle was used to measure the total muscle protein content (*t* = 4 and 7 h only) of phosphorylated Akt (serine<sup>473</sup>), mTOR (serine<sup>2448</sup>), and 4E-BP1 (threonine<sup>37/46</sup>) by Western blot analysis using commercial antibodies (Cell Signaling, Danvers, MA) normalized to  $\alpha$ -actin (Sigma-Aldrich Company Ltd., Dorset, U.K.) to control for loading, as well as pyruvate dehydrogenase complex activation (PDCa) status, both as described previously (9).

#### Calculations

The mixed muscle protein fractional synthesis rate (FSR) was calculated by dividing the increment in enrichment in the product (i.e., protein-bound L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine) by the enrichment of the precursor (i.e., plasma free L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine) (13). To adjust for nonsteady-state plasma tracer enrichments during the fed period, precursor enrichments were calculated as the integral of the plasma L-[ring-<sup>2</sup>H<sub>5</sub>]phenylalanine enrichment (14,15).

\*\**P* < 0.01, control and lipid during fed significantly different from corresponding basal values.

### Statistics

A two-way ANOVA (time and treatment factors) was performed using GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA) to detect differences within and between groups for all measures described. When a significant effect was observed, a Student *t* test with Bonferroni correction was performed to locate differences. Statistical significance was declared at  $P < 0.05$ . All of the values presented in the text, tables, and figures represent the means  $\pm$  SEM.

## RESULTS

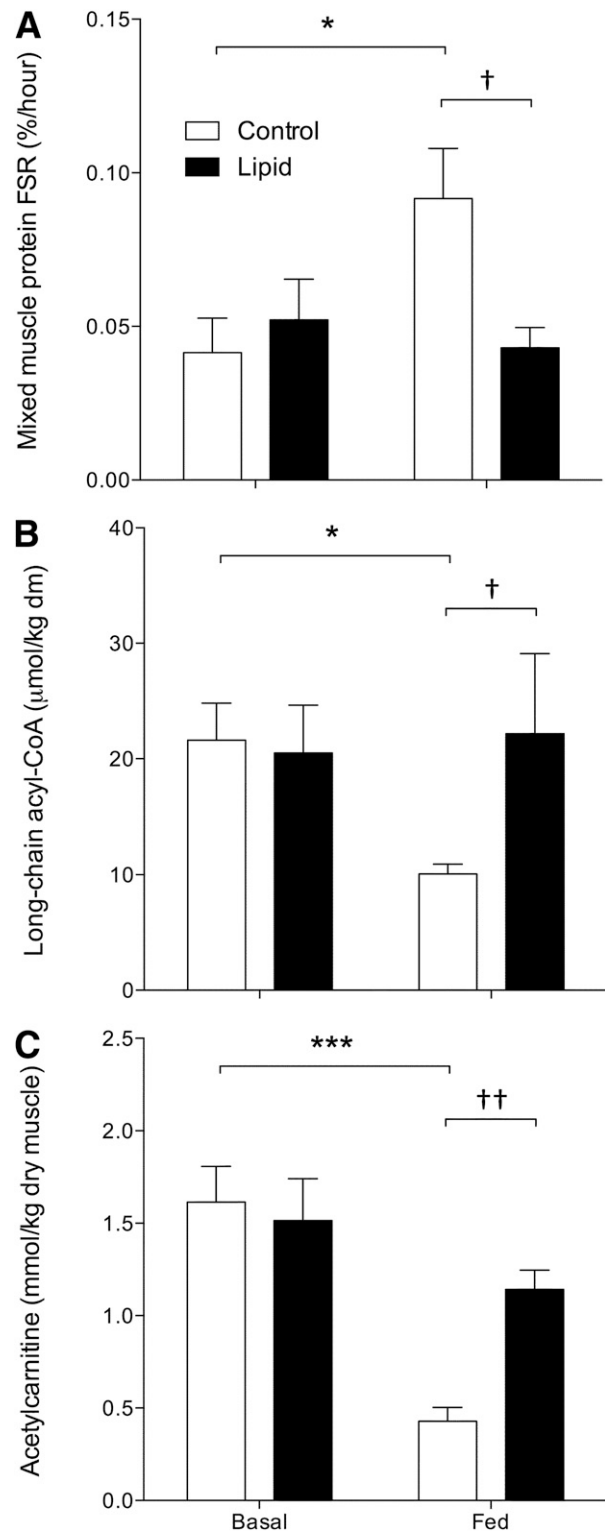
### Insulin Resistance of Glucose Metabolism

Steady-state plasma NEFA concentrations were elevated throughout the basal period in lipid compared with control ( $P < 0.001$ ; Fig. 1A). Furthermore, plasma NEFA concentrations were suppressed ( $P < 0.001$ ) by insulin and amino acid administration to a similar degree ( $-0.38 \pm 0.01$  vs.  $-0.39 \pm 0.01$  mmol/L; Fig. 1A), such that they remained greater in lipid throughout the fed period ( $P < 0.001$ ; Fig. 1A). This similar suppression of plasma NEFA concentration between lipid and control was reflected by similar steady-state blood glucose ( $4.51 \pm 0.05$  vs.  $4.52 \pm 0.05$  mmol/L, respectively) and plasma insulin concentrations ( $104 \pm 5$  vs.  $99 \pm 3$  mU/L, respectively; Fig. 1B). However, there was a  $20 \pm 6\%$  lower average glucose disposal during the final hour of the fed period in lipid compared with control ( $P < 0.01$ ; Fig. 1C) and a  $56 \pm 12\%$  lower PDca by the end of the fed period at 7 h ( $P < 0.05$ ; Fig. 1D).

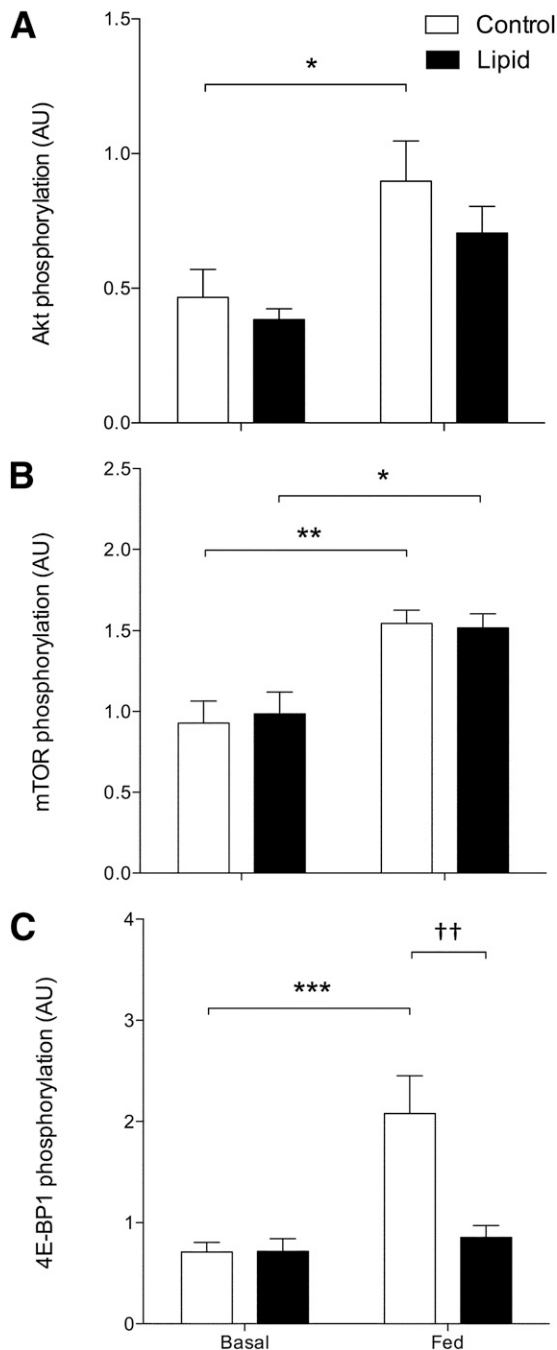
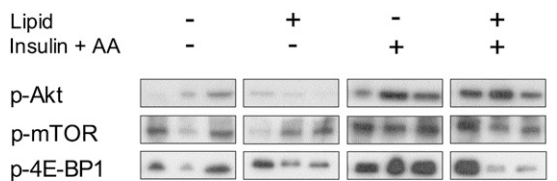
### Amino Acid and Lipid Metabolism

Plasma leucine and phenylalanine concentrations (Fig. 2A and B, respectively) were both maintained at fasting concentrations throughout the basal period in lipid and control. Similarly, plasma  $L$ -[ring- $^2$ H $_5$ ]phenylalanine enrichments remained at the same steady-state levels in lipid and control (Fig. 2C). Insulin and amino acid administration resulted in a similar peak in plasma leucine concentration in lipid and control after 30 min of the fed period ( $P < 0.001$ ; Fig. 2A). However, insulin and amino acid administration caused a steady decline in the plasma phenylalanine concentration during control, such that it was  $49 \pm 3\%$  lower during the final hour of the fed period compared with basal ( $P < 0.001$ ; Fig. 2B). Furthermore, the degree of reduction in the plasma phenylalanine concentration in response to insulin and amino acid administration was greater in lipid ( $65 \pm 3\%$ ;  $P < 0.001$ ) than in control, such that the steady-state phenylalanine concentrations were lower ( $P < 0.001$ ; Fig. 2B). However, this did not result in a greater  $L$ -[ring- $^2$ H $_5$ ]phenylalanine enrichment during the final hour of the fed period in lipid compared with control (Fig. 2C).

Basal mixed muscle FSR was the same in lipid compared with control (Fig. 3A). However, mixed muscle FSR increased from the basal to the fed period in control ( $P < 0.05$ ; Fig. 3A) but did not respond to insulin and amino acid administration in lipid, such that it was significantly lower than control ( $P < 0.05$ ; Fig. 3A). This was despite similar intracellular muscle enrichment before and after



**Figure 3**—Skeletal mixed muscle protein FSR (A), long-chain acyl-CoA content (B), and acetylcarnitine content (C) before (basal) and after (fed) the administration of 21 g amino acids and a 3-h hyperinsulinemic-euglycemic ( $\sim 100$  mU/L) clamp during a 7-h intravenous infusion of saline (control) or 10% Intralipid (lipid) at a rate of 100 mL/h. Values represent means  $\pm$  SEM. † $P < 0.05$ , †† $P < 0.01$ , lipid significantly lower than the corresponding control value. \* $P < 0.05$ , \*\*\* $P < 0.001$ , fed significantly greater than corresponding basal values.



**Figure 4**—Representative blots (top panel) and phosphorylation (p) status of skeletal muscle Akt serine<sup>473</sup> (A), mTOR serine<sup>2448</sup> (B), and 4E-BP1 threonine<sup>37/46</sup> (C) before (basal) and after (fed) the administration of 21 g amino acids (AA) and a 3-h hyperinsulinemic-euglycemic (~100 mU/L) clamp during a 7-h intravenous infusion of saline (control) or 10% Intralipid (lipid) at a rate of 100 mL/h. Values represent means ± SEM. AU, arbitrary units. ††*P* < 0.01, lipid significantly lower than corresponding control value. \*\*\**P* < 0.001,

insulin and amino acid administration (4.4 ± 0.3 to 6.0 ± 0.3 vs. 4.6 ± 0.3 to 5.9 ± 0.6 mole percent excess for lipid and control, respectively). Furthermore, this lower rate of protein synthesis in lipid coincided with an inhibition of the insulin- and amino acid-mediated decline in skeletal muscle long-chain acyl-CoA (*P* < 0.05; Fig. 3B) and acetyl-carnitine (*P* < 0.01; Fig. 3C) content at *t* = 7 h in control.

**Associated Muscle-Signaling Pathways**

The phosphorylation status of Akt, mTOR, and 4E-BP1 were the same after the basal period at *t* = 4 h in lipid compared with control. Insulin and amino acid administration increased the phosphorylation of Akt (*P* < 0.05; Fig. 4A), mTOR (*P* < 0.01; Fig. 4B), and 4E-BP1 (*P* < 0.001; Fig. 4C) by 1.9-, 1.7-, and 2.9-fold, respectively, compared with basal in control. However, insulin and amino acid administration also increased mTOR phosphorylation by 1.5-fold from basal in lipid (*P* < 0.05; Fig. 4B), but the 1.8-fold increase in Akt phosphorylation was not significantly different (*P* = 0.09; Fig. 4A). Furthermore, lipid infusion had no effect on 4E-BP1 phosphorylation, such that it was less than half that of control at the end of the fed period (*P* < 0.01; Fig. 4C).

**DISCUSSION**

The concept that lipid-induced insulin resistance coincides with anabolic resistance is not new. Diet-induced obesity in mice (16) and rats (17) was shown to impair the activation of skeletal muscle protein synthesis in response to feeding, particularly in glycolytic muscle where there was chronic lipid infiltration (17). The whole-body protein anabolic response to combined hyperinsulinemia and hyperaminoacidemia is also blunted in obese women compared with lean individuals (18), and skeletal muscle protein synthesis in response to insulin and amino acids appears to be negatively related to whole-body fat mass in humans (6). However, the contribution of excess lipid and insulin resistance to anabolic resistance cannot be determined from the associations described above because other confounding factors, particularly physical activity status, will also contribute to anabolic resistance (2,19). Thus, the current study clearly demonstrates that excess lipid availability per se within skeletal muscle can induce insulin resistance of skeletal muscle glucose metabolism and also anabolic resistance of amino acid metabolism independently of physical activity levels and diet-induced changes in body composition. This was evidenced by reduced insulin-stimulated peripheral glucose disposal and skeletal muscle PDCa by ~20 and 50%, respectively, and a complete prevention of a 2.2-fold increase in the rate of mixed muscle protein synthesis in response to the ingestion of 21 g amino acids containing

\*\*\**P* < 0.01, \**P* < 0.05, fed significantly greater than corresponding basal values.

2.3 g leucine. Furthermore, this inability of skeletal muscle to increase protein synthesis in response to insulin and amino acid administration appeared to be partly mediated by the repression of translation initiation at the level of 4E-BP1, which is in agreement with previous studies demonstrating that elevating lipid availability in rats and cells decreases muscle protein synthesis (20,21).

This is, to our knowledge, the first study to acutely induce anabolic resistance in humans by lipid administration. However, these findings are in contrast to the study of Katsanos et al. (22), where lipid was intravenously administered at nearly twice the rate and no blunting of the 50% increase in muscle protein synthesis was observed in response to the ingestion of 7 g essential amino acids. The differences are difficult to reconcile but may be due to the hyperinsulinemic-euglycemic clamp in the current study, which likely impaired fat oxidation and caused an accumulation of intracellular lipid metabolites (23). Indeed, this was reflected by greater muscle long-chain acyl-CoA and acetylcarnitine content during lipid infusion under insulin- and amino acid-stimulated conditions, which are sensitive markers of incomplete lipid oxidation and lipid accumulation (9,24). Although a mechanistic link has not been established, these results offer compelling evidence that a common lipid-mediated intracellular signaling defect downstream of the Akt-mTOR signaling pathway may cause both lipid-induced insulin and anabolic resistance and provide insight into how conditions of lipid-induced insulin resistance, such as aging, type 2 diabetes, disuse, and critical illness, may be linked to accelerated muscle mass loss.

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**Duality of Interest.** No potential conflicts of interest relevant to this article were reported.

**Author Contributions.** F.B.S. wrote the manuscript. F.B.S., B.T.W., L.J.C.v.L., and K.T. designed the study. All of the coauthors researched data and reviewed and edited the manuscript. F.B.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Prior Presentation.** Parts of this study were presented as abstracts W217 and LB813 at Experimental Biology 2014, San Diego, CA, 26–30 April 2014.

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