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**ER Stress Induces Anabolic Resistance in Muscle Cells through PKB-Induced Blockade of mTORC1**

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**Abstract**

**Background:** Anabolic resistance is the inability to increase protein synthesis in response to an increase in amino acids following a meal. One potential mediator of anabolic resistance is endoplasmic reticulum (ER) stress. The purpose of the present study was to test whether ER stress impairs the response to growth factors and leucine in muscle cells.

**Methods:** Muscle cells were incubated overnight with tunicamycin or thapsigargin to induce ER stress and the activation of the unfolded protein response, mTORC1 activity at baseline and following insulin and amino acids, as well as amino acid transport were determined.

**Results:** ER stress decreased basal phosphorylation of PKB and S6K1 in a dose-dependent manner. In spite of the decrease in basal PKB phosphorylation, insulin (10–50 nM) could still activate both PKB and S6K1. The leucine (2.5–5 mM)-induced phosphorylation of S6K1 on the other hand was repressed by low concentrations of both tunicamycin and thapsigargin. To determine the mechanism underlying this anabolic resistance, several inhibitors of mTORC1 activation were measured. Tunicamycin and thapsigargin did not change the phosphorylation or content of either AMPK or JNK, both increased TRB3 mRNA expression and thapsigargin increased REDD1 mRNA. Tunicamycin and thapsigargin both decreased the basal phosphorylation state of PRAS40. Neither tunicamycin nor thapsigargin prevented phosphorylation of PRAS40 by insulin. However, since PKB is not activated by amino acids, PRAS40 phosphorylation remained low following the addition of leucine. Blocking PKB using a specific inhibitor had the same effect on both PRAS40 and leucine-induced phosphorylation of S6K1.

**Conclusion:** ER stress induces anabolic resistance in muscle cells through a PKB/PRAS40-induced blockade of mTORC1.

**Introduction**

Amino acids activate mTORC1 (mammalian target of rapamycin complex 1) by promoting the exchange of GDP for GTP in the RagA/B GTPase proteins [1,2]. When RagA/B is bound to GTP, mTORC1 is recruited to the lysosome through its association with the Ragulator scaffolding complex [3]. At the lysosome, mTORC1 interacts with Rheb (ras homologous enriched in brain) and is activated [3]. The requirement of PI-3 (phosphatidylinositol-3) kinase and PKB (protein kinase B/akt) for the activation of mTORC1 by amino acids is controversial [4–7] and it has been suggested that this process could be independent of PKB [8]. However, in some pathological states and during aging, the response to amino acids can be altered. The inability to increase protein synthesis in response to an increase in amino acids following a meal, irrespective of the availability of insulin, insulin-like growth factor 1, and growth hormone has been called anabolic resistance [9]. In skeletal muscle, this anabolic resistance is thought to contribute to the loss of muscle mass in aging [10]; immobilization [11]; and high-fat feeding/obesity [12]. In spite of the fact that PKB is not necessarily required for the amino acid-induced increase in protein synthesis and mTORC1 activation, anabolic resistance is characterized primarily by decreased phosphorylation of PKB.

mTORC1 is the central molecular player in anabolic resistance. mTORC1 is composed of mTOR (a Ser/Thr protein kinase), raptor (regulatory associated protein of mTOR) and mLST8/GβL (G protein beta subunit-like protein) and is sensitive to the macrolide rapamycin [13,14]. It regulates cell growth and protein synthesis through the phosphorylation of 4EBP1 (initiation factor 4E binding protein) and S6K1 (ribosomal protein S6 kinase). Activation of S6K1 and 4E-BP1 requires sequential phosphorylation events. For S6K1, phosphorylation of Ser/Thr residues in the autoinhibitory domain, such as at Thr421 and Ser424, is required for altering its conformation and making Thr389 and

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Thr229 available for phosphorylation, thereby fully activating S6K1 [15]. 4E-BP1 also possesses many different phosphorylation sites [16]. Thr37 and Thr46 phosphorylation serves as a priming motif for subsequent phosphorylation events [17,18]. Activation of mTORC2 has been shown to regulate PKB Ser473 phosphorylation [19], PKB, in turn, can regulate the activity of mTORC1 in three ways: 1) PKB can directly phosphorylate mTORC1 [20]; 2) PKB can phosphorylate and inhibit TSC2 (tuberous sclerosis complex 2); a GTPase activating protein that targets Rheb [21]; and 3) PKB can phosphorylate PRAS40 (prolinerich Akt substrate 40), an allosteric inhibitor of mTORC1 [22,23]. When unphosphorylated, PRAS40 binds to raptor and prevents the association of mTORC1 with its downstream substrates. Phosphorylation of PRAS40 by PKB on Thr246 alters its conformation such that a TOS (TOR signaling motif) motif is unmasked. mTORC1 then phosphorylates PRAS40 on Ser183 and Ser221, resulting in dissociation of PRAS40 and allosteric activation of mTORC1 [22].

One potential mediator of anabolic resistance is endoplasmic reticulum (ER) stress [24–28]. Periods of high lipids, glucose deprivation, or increased synthesis of secretory proteins lead to the accumulation of unfolded or misfolded proteins within the ER lumen [29]. To cope with this ER stress, cells activate the unfolded protein response, a series of events that serve to restore ER function [30]. The unfolded protein response has three main effectors: ATF6 (activating transcription factor 6); IRE1α (inositol-requiring enzyme 1 alpha); and PERK (protein kinase R-like ER protein kinase). In the basal (inactive) state, each of these factors associates with the protein chaperone BiP/GRP78 (binding protein/glucose regulated-protein 78); a member of the Hsp70 (heat shock protein 70) family [31]. Upon accumulation of unfolded/misfolded proteins, ATF6, IRE1α, and PERK are released from BiP/GRP78 and become activated [30]. The best characterized downstream effect of ATF6, IRE1α, and PERK release is the induction of genes, such as XBP1 (X box binding protein 1), CHOP (CCAAT/enhancer binding protein C/EBP) homologous protein) and ATF4 (activating transcription factor 4), which increase the protein-folding capacity of the cell [30]. Concomitant with the increase in protein folding, there is a decrease in protein synthesis that is in part due to the phosphorylation and inhibition of eIF2α [32], and might also be dependent on mTORC1 [28]. When the unfolded protein response fails, the cells undergo apoptosis indicating that the unfolded protein response is essential for normal cellular function [33].

In the present study, we sought to determine whether ER stress could induce anabolic resistance in muscle cells. Consistent with this hypothesis, low levels of ER stress were sufficient to prevent the activation of mTORC1 by leucine, assessed by a decrease in S6K1 phosphorylation on Thr389, whereas at the same level of ER stress insulin could still activate mTORC1 normally. The inability to activate mTORC1 was not due to a lack of leucine transport, but rather to the ER stress-induced decrease in basal PKB phosphorylation resulting in PRAS40 hypophosphorylation and allosteric inhibition of mTORC1.

Materials and Methods

Cell cultures
C2C12 murine skeletal muscle myoblasts (ATCC, USA) were seeded in 150-mm-diameter culture dishes and grown in Dulbecco’s Modified Eagle Medium (DMEM, Life technologies) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (5000 U/5000 μg/ml). Cells were then plated in 6-well plates until 90% confluent. At this time, the proliferation medium was replaced by a differentiation medium containing 2% horse serum and 1% penicillin/streptomycin (5000 U/5000 μg/ml). After 96 h of differentiation, tunicamycin (MP Biomedicals) or thapsigargin (Tocris Bioscience) was added for 17 h in serum-free DMEM before stimulation of the mTORC1 pathway with insulin (10–50 nM) for 15 min or with leucine (2.5–5 mM) for 30 min. Tunicamycin and thapsigargin were used as they are well-documented chemical inducers of ER stress by blocking N-glycosylation and calcium entry into the ER, respectively. For PKB inhibition experiments, cells were serum-starved for 16 h to maintain the cells in the same conditions as those described for tunicamycin and thapsigargin experiments. PKB inhibitor (100 nM–1 μM), also known as Akt Inhibitor XIII or Akti2 (Calbiochem), was then added 1 h before leucine (2.5–5 mM) stimulation for 30 min. At the end of the incubation period, cells were harvested and cell lysates were immediately frozen at −80°C for subsequent analyses or cells were immediately used for leucine uptake measurements. All experiments were performed at least in duplicate.
quantified by spectrophotometry (260 nm) and its concentration adjusted to 1 μg/μl using RNAse-free water. cDNA was prepared by reverse transcription of 1 μg total RNA using the reverse transcription system (Promega). SYBR Green (Sigma Aldrich) was used for real-time PCR detection using an Eppendorf Light Cycler PCR machine. Real-time PCR primers were designed (Table 1) for mouse CHOP, ATF4, spliced (s) XBP1, unspliced (u) XBP1, TRB3, LAT1, SNAT2, 4F2hc, ASC1, cMyc, MAD1 and GAPDH. Specific primers were designed to recognize the spliced, or active, form of XBP1 (XBP1s) versus the unspliced form (XBP1u). GAPDH was used as the reference gene and the Ct for the amplified product was checked through analysis of the melting curve carried out at the end of amplification.

Leucine uptake measurement

At the end of the 17 h incubation with tunicamycin and thapsigargin, cells were washed with HEPES-buffered saline (HBS: 140 mM NaCl, 20 mM HEPES, 5 mM KCl, 2.5 mM MgSO4 and 1 mM CaCl2, pH 7.4), pre-warmed at 37°C. Cells were incubated for 1 min with 0.25 μM (or 0.5 μM) [35S]leucine diluted in 500 μl HBS containing 10 μM (or 1 mM) leucine. The doses of cold leucine were chosen to be below the Km of the transporter and about 10 times higher (Km = 100 μM) [34,35]. Medium was aspirated before washing cells 2 times with 0.9% (w/v) ice-cold NaCl containing 100 mM leucine. Cells were subsequently lysed in 50 mM NaOH, and radioactivity was quantified using a Beckman LS 6000IC scintillation counter. Specific activity was determined in each well by measuring radioactivity in the incubation medium. Protein concentration in cell lysates was determined using the DC Protein Assay (Bio-Rad). Leucine uptake was reported to protein content and expressed as fold control. The experiment was repeated three times and the results are presented as the mean.

**Results**

**ER stress is induced proportionally to tunicamycin and thapsigargin concentrations**

Increasing doses of tunicamycin (0–5000 ng/ml, Figure 1A) and thapsigargin (0–2000 nM, Figure 1B) increased BiP and IRE1α protein and the phosphorylation of PERK (Thr980) and eIF2α (Ser51) whereas the phosphorylation of PKB (Ser473) and S6K1 (Thr389) decreased. The decrease in phospho-PKB and phospho-S6K1 correlated with the increase in BiP suggesting a common mechanism (Pearson product moment correlation; r = −0.85, P < 0.05). Tunicamycin (Figure 1C and D) and thapsigargin (Figure 1E and F) increased CHOP, XBP1s and TRB3 mRNA more than 30-fold. The activation of TRB3 was of particular interest since it is known to impair PKB activation [36]. ATF4 mRNA was more than doubled by tunicamycin and thapsigargin whereas XBP1 only increased with tunicamycin (Figure 1C–F).

**ER stress decreases leucine-induced phosphorylation of PKB and S6K1**

Tunicamycin (Figure 2A) and thapsigargin (Figure 2B) not only decreased basal phosphorylation of PKB (Ser473) and S6K1 (Thr389) but also repressed leucine-induced phosphorylation of S6K1 (Thr389) at all concentrations from 100 ng/ml for tunicamycin and 200 nM for thapsigargin.

**ER stress decreases insulin-induced phosphorylation of PKB and S6K1**

Tunicamycin (Figure 3A) and thapsigargin (Figure 3B) also repressed insulin-induced phosphorylation of PKB and S6K1, but this repression was not complete. The effects of tunicamycin were stronger than thapsigargin at all concentrations. Insulin-induced phosphorylation of PKB was already partially repressed by 10 ng/ml tunicamycin, considered as a low dose according to previous reports [37], whereas 1000 nM thapsigargin, a large dose [38], was necessary to observe a similar inhibition. Dithiothreitol (1 mM, Figure 3A) and palmitic acid (1 mM, Figure 3B) were used as additional controls to which tunicamycin and thapsigargin could be compared, as they are also known to induce ER stress. It is important to note that the concentration of TG required to reduce PKB/S6K1 phosphorylation following leucine stimulation was much less than after insulin stimulation (200 nM vs. 1000 nM).

Leucine transport is not involved in the impairment of mTORC1 activity by ER stress

We first hypothesized that the greater sensitivity of leucine stimulation to ER stress was due to a decrease in leucine uptake. To test this hypothesis, we analysed the expression of transporters known to be directly or indirectly involved in leucine transport in skeletal muscle: LAT1, SNAT2 and ASC1, as well as adaptors and regulators of these transporters: 4F2hc, cMyc, and MAD. Tunicamycin and thapsigargin increased expression of 4F2hc and cMyc and

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**Table 1. Sequences of primers used for mRNA quantification by real-time RT-PCR.**

|        | Forward | Reverse |
|--------|---------|---------|
| CHOP   | CCT AGC TTG GCT | CTG CTC CTT TCT CTT |
|        | GAC AGA GG | CAT GC |
| ATF4   | GAG CTT CCT GAA | TGG CCA CCT CCA GAT |
|        | CAG CGA AGT G | AGT CAT C |
| XBP1s  | GAG TCC GCA GCA | GTG TCA GAG TCC ATG |
|        | GGT G |
| XBP1u  | AAG AAC ACG CTT | ACT CCC CCT GGC GTC |
|        | GGG AAT GG | CAC |
| TRB3   | TGT GAG AGG ACG | TCG TGA AAT TCC TGC |
|        | AAG CTT GG | CTG |
| LAT1   | GCT GCC TGC ATC | CTG CCA TGG CCA ACA C |
|        | TGT CTC TTA AA | ACA C |
| SNAT2  | TCT TGT CCT CCA | CCC CCA TGA ACC CGA AGA TG |
|        | ATT TGC TC | AGC |
| 4F2hc  | CTT CTA CAT CCA AAG | GAG GAA GAC AGT GCA TGG TAC |
|        | ACC TGT GAA | TGG AAG GC |
| ASC1   | CCA GCC GCA TCC | GGT GT A TC AAG CAG |
|        | AGG TTA | GAG CCG ACC ACC |
| cMyc   | CAC CAG CAG CGA | ATG AGC CCG ACC CCA |
|        | CTC TGA AGA | ACC |
| MAD1   | CAA GCC GCA ACA | AGC CTG TGC ATC CGA |
|        | CCA CTC TGA | GTC C |
| GAPDH  | TGG AAA GCT GTG | TGC TTC ACC ACC |
|        | GGCG TGA T | TCT TGC AT |

CHOP, C/EBP (CCAAT/enhancer binding protein) homologous protein; ATF4, activating transcription factor 4; XBP1s, spliced X Box binding protein 1; XBP1u, unspliced X Box binding protein 1; TRB3, tribbles homolog 3; LAT1, L-type amino acid transporter 1; SNAT2, sodium-coupled neutral amino acid transporter 2; 4F2hc, 4F2hc, 4F2hc, sodium-independent alanine-serine-cysteine transporter 1; MAD1, mitotic arrest-deficient 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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decreased the expression of LAT1 and MAD1 in a dose-dependent manner (Figure 4A–D). Since LAT1, the primary leucine transporter in muscle, expression was decreased, leucine uptake was determined at two different concentrations based on the transport kinetics ($K_m = \sim 100 \mu M$, [34,35]): a non-saturating dose (10 $\mu M$, Figure 4E) and a saturating dose (1 mM, Figure 4F). Contrary to our hypothesis, at both concentrations, leucine uptake was slightly increased by tunicamycin (~40%) and thapsigargin (~60%).

PRAS40 phosphorylation by PKB is required for amino acid-induced mTORC1 activity

After determining that leucine uptake was not limiting, we next established the point at which ER stress interrupted the activation of mTORC1, by assessing changes in the phosphorylation of S6K1 on Thr389. As with the Ser473 site, basal phosphorylation of PKB at Thr308 was decreased following treatment with tunicamycin (1000 ng/ml) and thapsigargin (1000 nM). Tunic-
mycin decreased Thr308 phosphorylation in response to both low and high levels of insulin. The inhibitory action of thapsigargin was less potent, decreasing Thr308 phosphorylation at low but not high levels of insulin. Even though tunicamycin and thapsigargin decreased PKB phosphorylation, the phosphorylation of PRAS40 on Thr246 was still enhanced by insulin, indicating that following insulin stimulation tunicamycin and thapsigargin did not repress mTORC1 via PRAS40. As with Thr389, the phosphorylation of S6K1 on Thr421/Ser424 mirrored that of PKB. When PKB phosphorylation was decreased, S6K1 phosphorylation followed suit (Thr389; Figure 3 and Thr421/Ser424; Figure 5). Neither PDK1, p38, ERK1/2, nor JNK phosphorylation was decreased by either tunicamycin or thapsigargin (data not shown).

Since activation of mTORC1 by amino acids generally occurs independent of PKB, it was not surprising that PKB phosphorylation was not changed by the addition of leucine (Figure 5B). As described above, tunicamycin and thapsigargin decreased basal PKB phosphorylation and this was also true after leucine treatment. The phosphorylation of PRAS40 on Thr246 followed the same pattern, completely absent in the presence of either tunicamycin or thapsigargin. As expected, in untreated cells leucine increased the phosphorylation of S6K1 on Thr421/Ser424 and 4EBP1 on Thr37/46. Tunicamycin and thapsigargin completely prevented both of these leucine-induced changes. As mentioned in the introduction, activation of 4E-BP1 is a two-step event that first requires the phosphorylation of Thr37/46. When 4E-BP1 is less active after

Figure 2. Effect of tunicamycin and thapsigargin on leucine-induced phosphorylation of S6K1 (Thr389). Cells were incubated for 17 h with different doses of tunicamycin (A) or thapsigargin (B) before stimulation with 2.5 mM or 5 mM leucine for 30 min. TN, tunicamycin; TG, thapsigargin; S6K1, ribosomal protein S6 kinase 1; Leu, leucine.
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Figure 3. Effect of tunicamycin and thapsigargin on insulin-induced phosphorylation of PKB (Ser473) and S6K1 (Thr389). Cells were incubated for 17 h with different doses of tunicamycin (A) or thapsigargin (B) before stimulation with 10 nM or 50 nM insulin for 15 min. TN, tunicamycin; TG, thapsigargin; PKB, protein kinase B; S6K1, ribosomal protein S6 kinase 1; Ins, insulin; DTT, dithiotreitol; PA, palmitic acid.
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treatment with tunicamycin or thapsigargin, the phosphorylation state of Thr37 and Thr46 remains high. Under serum-starvation conditions, Thr37 and Thr46 phosphorylation can remain high [16]. Following a second stimulus, such as insulin, 4E-BP1 becomes phosphorylated on Ser65 and Ser70, with a small decrease in phosphorylation of Thr37 and Thr46 being observed [39]. Based on the literature [39], a shift towards less fast migrating bands with insulin can be interpreted as a higher phosphorylation state of 4E-BP1 and a shift towards faster migrating bands with tunicamycin and thapsigargin as a lower phosphorylation state.

We surmised that the low phosphorylation of PKB and PRAS40 resulted in the allosteric inhibition of mTORC1 in response to leucine. To test this hypothesis, PKB activity was blocked chemically [40] and the resulting effect on PRAS40 and leucine uptake was assessed.

**Figure 4. Dose-response curves of tunicamycin and thapsigargin on amino acid transport regulation and leucine uptake.** Effect of low doses of tunicamycin (A) and thapsigargin (C) and high doses of tunicamycin (B) and thapsigargin (D) on the mRNA level of amino acid transporters and amino acid transporters regulators after 17 h incubation. Effect of increasing doses of tunicamycin and thapsigargin on the uptake of 10 μM (E) and 1 mM (F) leucine. TN, tunicamycin; TG, thapsigargin; LAT1, L-type amino acid transporter 1; SNAT2, sodium-coupled neutral amino acid transporter 2; 4F2hc, 4F2 heavy chain; ASC1, sodium-independent alanine-serine-cysteine transporter 1; MAD1, mitotic arrest-deficient 1; leu, leucine.

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stimulation of S6K1 was determined (Figure 5C). Consistent with our hypothesis, in the presence of the PKB inhibitor neither PRAS40 nor S6K1 were phosphorylated, indicating that PKB is permissive for the activation of mTORC1 by amino acids (Figure 5B and [7]).

In summary, whereas ER stress only partially prevents insulin stimulation of PKB and mTORC1, the lack of activation of PKB by amino acids results in a complete block of leucine-stimulated activation of targets downstream of mTORC1 likely through hypophosphorylation of the mTORC1 kinase inhibitor PRAS40.

Discussion

The main finding of the present study is that ER stress induces anabolic resistance in muscle cells through PKB/PRAS40-induced blockade of mTORC1. Anabolic resistance is defined as the inability to increase protein synthesis in response to an increase in amino acids following a meal [9] or other anabolic stimuli [10,41]. The molecular mechanisms behind anabolic resistance are not well understood although decreased expression and phosphorylation of amino acid sensing/signaling proteins, such as mTORC1 and S6K1, seem to be involved [10]. The present study has identified the inactivation of PKB as a likely mechanism underlying ER stress-induced anabolic resistance. High levels of insulin, which continue to activate PKB under ER stress, albeit to a lesser degree, can partially overcome anabolic resistance. However, since amino acids do not normally activate PKB, the competitive inhibitor of mTORC1, PRAS40, remains hypophosphorylated and prevents the phosphorylation of mTORC1 targets such as S6K1 and 4EBP. This phenotype is replicated by the PKB inhibitor Akti suggesting that basal PKB activity is required for amino acid induced activation of mTORC1. We also show that the effect of ER stress on mTORC1 is dose-dependent since the rise in BiP expression occurred proportionally with a decrease in PKB and S6K1 phosphorylation. This relationship suggests that part of the ER stress response is to block PKB and mTORC1 in an effort to decrease protein synthesis.

It should be mentioned that when cells were stimulated with insulin, this was made in presence of basal levels of leucine (0.8 mM) usually found in cell culture medium, whereas when cells were stimulated with extra leucine (2.5 or 5 mM), there was no insulin or serum. As insulin is known to play a permissive role in the action of amino acids on the mTORC1 pathway, it is possible that the effect of additional leucine was relatively less optimal compared to insulin. This could have contributed to the fact that ER stress was more potent in reducing leucine- than insulin-induced stimulation of mTORC1.

Having previously established a negative relation between ER stress and the mTORC1 pathway [28], the current work focussed on determining the mechanism underlying this effect. We found that two inhibitors of PKB/mTORC1, TRB3 [36] and REDD1 [25], were transcriptionally increased by ER stress. Even though both of these inhibitors could be increased by ER stress, the effect on REDD1 was minor and only seen with thapsigargin, indicating that REDD1 was not a key player in ER stress-induced anabolic resistance. TRB3 on the other hand was increased over 30-fold by both ER stress agents. TRB3 directly binds to and inhibits the kinase activity of PKB [36]. In our basal conditions, PKB activity, as determined by phosphorylation of PRAS40 on Thr246, was decreased consistent with the published effects of elevated TRB3. Recently, Chen et al showed that both TN and TG could also increase the phosphorylation of rictor at Ser1235 [42]. The phosphorylation of rictor on this residue, like TRB3, decreases mTORC2 activity towards PKB at Ser473, and is completely
consistent with the decrease in PKB and mTORC1 activity we describe here. However, insulin stimulation could partially or even totally reverse the physiologic blockade of PKB, indicating that the effect of TRB3 is reversible. The situation is different with leucine, as amino acids do not activate PKB in muscles. We speculated that if PKB activity was decreased and PKB was permissive in mTORC1 activation by amino acids, that leucine would be unable to activate S6K1 and 4EBP downstream of mTORC1. Consistent with this hypothesis, ER stress prevented S6K1 and 4EBP phosphorylation in response to leucine. The fact that this effect was due to inhibition of PKB was confirmed using a specific inhibitor: Akti [40]. Both ER stress and Akti decreased PRAS40 phosphorylation as well as that of S6K1 and 4EBP. PKB phosphorylates PRAS40 on Thr246 resulting in the unmasking of a TOS motif that allows the phosphorylation of PRAS40 on Ser183 and Ser221 by mTORC1 [22]. Together, these phosphorylation events lead to dissociation from raptor in favor of binding to 14-3-3. The dissociation of PRAS40 allows mTORC1 to bind to its downstream targets such as S6K1 and 4EBP [22]. These data show that a permissive level of PKB activity, likely through phosphorylation of PRAS40, is necessary for leucine to increase the phosphorylation of S6K1 and 4EBP by mTORC1. This is in agreement with recent data from the heart where phosphorylation of PRAS40 is required for leucine to increase S6K1 activity [43]. However, using a knockin mutation where PDK1 phosphorylation of PKB is maintained but S6K activation is prevented, they showed that the phosphorylation of PRAS40 was prevented along with leucine signaling to S6K1 [43]. These data suggest that there may be another PRAS40 kinase responsible for anabolic resistance in response to ER stress.

Another possible mechanism of anabolic resistance is a decrease in amino acid transporter levels and/or amino acid uptake. Several transporters are known to participate directly or indirectly in leucine transport in muscle cells. The preferred transporter for leucine is the tertiary active transport. Tertiary active transport uses the sodium activation [4,46], whereas inhibitors of SNAT2 and 4F2hc reduce growth and their expression has been correlated with mTORC1 SNAT2 and ASC1. LAT1-4F2hc and SNAT2 are involved in muscle amino acid transporter levels and/or amino acid uptake. Several studies that have shown that anabolic resistance is not due to reduced amino acid availability [41].

In C2C12 muscle cells, ER stress seems to impair mTORC1 rather than vice versa [26,27]. We have previously shown that hyperactivation of mTORC1 by insulin for 6 h or 24 h did not trigger the unfolded protein response whereas tunicamycin activated the unfolded protein response before S6K1 phosphorylation decreased, suggesting that in C2C12 muscle cells the induction of ER stress precedes the impairment in mTORC1 activity [20].

The present study suggests that ER stress is a potential mediator of the anabolic resistance observed in the muscle of aged [10], immobilized [11], and obese [12] animals. In vivo, ER stress completely blocks any effect of amino acids on S6K1 and 4EB phosphorylation, whereas the effect on insulin was less dramatic. This is in accordance with the fact that anabolic resistance in vivo is mainly due to decreased sensitivity to amino acids and impaired mTORC1 signaling, rather than an impaired response to insulin [9]. In conclusion, the present study shows that ER stress induces anabolic resistance in muscle cells through a PKB-dependent, PRAS40-induced, blockade of mTORC1.

Author Contributions
Conceived and designed the experiments: LD LB MF KB. Performed the experiments: LD AP KB. Analyzed the data: LD LB MF KB. Contributed reagents/materials/analysis tools: LD LB MF KB. Wrote the paper: LD MF KB.

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