The effects of amino acids on glucose metabolism of isolated rat skeletal muscle are independent of insulin and the mTOR/S6K pathway

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Stadlbauer K, Brunmair B, Szöcs Z, Krebs M, Luger A, Fürnissn C. The effects of amino acids on glucose metabolism of isolated rat skeletal muscle are independent of insulin and the mTOR/S6K pathway. Am J Physiol Endocrinol Metab 297: E785–E792, 2009. First published July 21, 2009; doi:10.1152/ajpendo.00061.2009.—Two mechanisms have been proposed for the modulation of skeletal muscle glucose metabolism by amino acids. Whereas studies on humans and cultured cells suggested acute insulin desensitization via mammalian target of rapamycin (mTOR) and its downstream target p70 S6 kinase (S6K), investigations using native specimens of rat muscle hinted at impairment of glucose oxidation by competition for mitochondrial oxidation. To better understand these seemingly contradictory findings, we explored the effects of high concentrations of mixed amino acids on fuel metabolism and S6K activity in freshly isolated specimens of rat skeletal muscle. In this setting, increasing concentrations of amino acids dose-dependently reduced the insulin-stimulated rates of CO2 production from glucose and palmitate (decrease in glucose oxidation induced by addition of 5.5, 11, 22, and 44 mmol/l amino acids: −16 ± 3, −25 ± 7, −44 ± 4, −62 ± 4%; P < 0.02 each). This effect could not be attributed to insulin desensitization, because it was not accompanied by any reduction of insulin-stimulated glucose transport (+12 ± 16, +17 ± 22, +21 ± 33, +13 ± 12%; all nonsignificant [NS]) or glycogen synthesis (+1 ± 6, −5 ± 6, −9 ± 8, +6 ± 5%; NS) and because it persisted without insulin stimulation. Abrogation of S6K activity by the mTOR blocker rapamycin failed to counteract amino acid-induced inhibition of glucose and palmitate oxidation, which therefore was obviously independent of mTOR/S6K signaling (decrease in glucose oxidation by addition of 44 mmol/l amino acids: without rapamycin, −60 ± 4%; with rapamycin, −50 ± 13%; NS). We conclude that amino acids can directly affect muscle glucose metabolism via two mechanisms, mTOR/S6K-mediated insulin desensitization and mitochondrial substrate competition, with the latter predominating in isolated rat muscle.

mammalian target of rapamycin; ribosomal protein p70 S6 kinase; glucose transport; glucose oxidation; fatty acid oxidation; species difference; mitochondrion

EXCESSIVE SUPPLY OF ALIMENTARY PROTEIN or red meat is associated with reduced insulin-stimulated glucose disposal and an increased risk of developing type 2 diabetes, attributes that are typically related to insulin resistance of skeletal muscle (16, 21). Experimental investigation of such observations showed that short-term infusions of mixed amino acids impair insulin-stimulated whole body glucose disposal in humans along with decreased glucose transport/phosphorylation and reduced net glycogen storage in skeletal muscle (9, 15, 25). In the search for the underlying mechanism, studies on cultured cell lines, like L6 cells derived from rat skeletal muscle, revealed that high ambient amino acids activate the kinase mammalian target of rapamycin (mTOR) and its downstream target p70 S6 kinase (S6K) (12, 26, 27). Activation of this nutrient-sensitive signaling pathway then stimulates serine phosphorylation of insulin receptor substrate-1 (IRS-1), which in turn impairs the transduction of the insulin signal and, hence, blunts insulin-stimulated glucose uptake (10, 24, 27). Accordingly, pharmacological blockade of the mTOR/S6K pathway by rapamycin abolishes the amino acid-induced decreases in glucose transport and insulin sensitivity of L6 cells (26), whereas infusion of rapamycin in healthy volunteers promotes insulin-stimulated glucose disposal, hinting at a regulatory role of this signaling cascade in human physiology (14).

Many years before the mTOR/S6K pathway was discovered, however, it had been reported that amino acids inhibit the oxidation of glucose and pyruvate by native specimens of hindlimb, diaphragm, and heart muscles from rats. This decrease in carbohydrate oxidation was attributed to intracellular competition of substrates for mitochondrial oxidation rather than to an impairment of insulin sensitivity, because it was not associated with any reduction of insulin-stimulated glucose transport and because it was particularly pronounced in response to leucine, an amino acid readily oxidized to CO2 by muscle cells (4, 5, 23). The present study aimed to reassess this old concept and to bring the seemingly divergent conclusions from former and recent research efforts into context.

METHODS

Rats. Male Sprague-Dawley rats were purchased from the breeding facilities of the Medical University of Vienna (Himberg, Austria). They were kept at an artificial 12:12-h light-dark cycle at constant room temperature and provided with conventional laboratory diet and tap water ad libitum. At an age of 8–10 wk (~180 g body wt), food, but not water, was withdrawn 3–4 h before rats were killed by cervical dislocation for preparation of muscle specimens. All procedures were performed according to local law and to the principles of good laboratory animal care.

Incubation of muscle strips. Immediately after killing, two longitudinal strips of soleus and/or extensor digitorum longus (EDL) muscle per leg were prepared, weighed (~25 mg/strip), and tied under tension on stainless steel clips (6). According to procedures established at our laboratory (2), muscles were immediately put into Erlangen flasks coated with BlueSlick solution (Serva, Heidelberg, Germany) and provided with Cell Culture Medium 199 (M199, pH 7.35, containing 5.5 mmol/l glucose and 11.4 mmol/l mixed amino acids; Sigma-Aldrich, St. Louis, MO; cat. no. M-4530). The medium was supplemented with 5 mmol/l HEPES, 25,000 U/l penicillin G, 25 mg/l streptomycin, 0.2 mg/l ciprofloxacin, 0.3% (wt/vol) fatty acid-free BSA, and 300 μmol/l palmitate (the latter dissolved in ethanol; final concentration of ethanol 0.25% vol/vol). The addition of ethanol did not have any detectable influence in our experimental setting (data available upon request).
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not shown). All incubations were under a continuous atmosphere of 95% O₂-5% CO₂.

Where indicated, an amino acid solution commonly used for parenteral nutrition was added to the medium (Aminoplasmal 10% GB without electrolytes; Braun, Melsungen, Germany). This solution contained the following l-amino acids (in g/l): isoleucine 5.1, leucine 8.9, valine 4.8, lysine 5.6, methionine 3.8, phenylalanine 5.1, tryptophan 1.8, arginine 9.2, histidine 5.2, N-acetyl-cysteine 0.7, proline 8.9, threonine 4.1, glutamate 4.6, serine 2.4, glycine 7.9, alanine 13.7, asparagine 3.2, ornithine 0.3, N-acetyl-tyrosine 1.3, and aspartate 1.3. Aminoplasmal solution at 0.625, 1.25, 2.5, 5, 10, or 20% (vol/vol) was added, equivalent to increases over the prevailing amino acid concentration of 11.4 mmol/l by 5.5, 11, 22, 44, 88, or 176 mmol/l. In some experiments, individual amino acids (Sigma-Aldrich, St. Louis, MO) were tested instead of the mixture, or the mTOR inhibitor rapamycin was added (Cell Signaling, Beverly, MA; dissolved in methanol; final concentration of methanol 0.1% vol/vol).

Within the same experiment, all media contained identical concentrations of methanol and ethanol. Osmotic differences, as would arise from added amino acids, were compensated for by the addition of mannitol.

Where explicitly stated, M199 was replaced by Krebs-Ringer buffer solution (KRBI pH 7.35), with additions resulting with the same concentrations as in M199 of glucose, HEPES, penicillin G, streptomycin, ciprofloxacin, fatty acid-free BSA, palmitate, and ethanol.

Measurements of fuel metabolism. After preincubation for 1 h (if not stated otherwise), isolated muscle specimens were transferred into identical medium additionally supplemented with 0.2 μCi/ml [U-14C]glucose, 0.2 μCi/ml [U-14C]palmitate, 0.3 μCi/ml [14C]leucine, or 0.4 μCi/ml 2-deoxy-D-[2,6-3H]glucose plus 6 μCi/ml [14C]glucose (l-leucine from Moravek Biochemicals, Brea, CA; all others from Amersham, Amersham, UK) and, where not stated otherwise, with 10 mmol/l human insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark). After another hour of incubation, muscles were quickly removed from the flasks, blotted, and frozen in liquid nitrogen.

Analytic procedures for the determination of metabolic parameters were as described before (7, 8). In brief, rates of CO₂ production from glucose, palmitate, or leucine (referred to as glucose, palmitate, and leucine oxidation, respectively) were calculated from the conversion of [14C]glucose, [14C]palmitate, or [14C]leucine into [14C]CO₂. To avoid loss of CO₂, flasks were sealed during the last 45 min of the incubation procedure. Immediately after termination of the experiment, muscle strips were removed, and hang-in containers with 200 μl of a CO₂ trapping solution (methanol and phenethylamine, 1:1) were placed into the flasks. Perchloric acid (200 μl, 3 mol/l) was injected into the incubation medium to release all CO₂, and after 1 h at room temperature, 14C content of the trapping solution was measured. For the measurement of glycogen storage, muscle strips incubated with [14C]glucose for 1 h were lyzed in 1 mol/l KOH at 70°C. An aliquot of the lysate was subjected to several cycles of glycogen precipitation with 20°C-cold ethanol, centrifugation, and redissolving of the precipitate in water. 14C content of the final aqueous solution, which contained purified glycogen, was measured and used to calculate the net rate of glucose incorporation into glycogen (referred to as glycogen synthesis). Another aliquot of the same lysate was used to determine glycogen content of the muscle specimens. To this end, glycogen in the muscle lysate was degraded to glucose units with amyloglucosidase (special quality for starch determination; Roche Diagnostics, Indianapolis, IN), and the glucose concentration of the resulting solution was measured with an enzymatic kit (Human, Taunusstein, Germany). Rates of lactate release were calculated from the concentration of lactate accumulated in the incubation medium, using the spectrophotometric lactate dehydrogenase method (l-lactate dehydrogenase; Roche Diagnostics, Indianapolis, IN). For determination of glucose transport, muscle specimens were incubated for 1 h with 2-deoxy-[14H]glucose, which is transported into the muscle cell but not further metabolized, and [14C]glucose, which served as a marker of the extracellular space because it does not enter the muscle cell. The tissue specimen was lyzed in 1 mol/l KOH at 70°C, the lysate was neutralized with acetic acid, and 3H and 14C content was measured. From this, the amount of intracellularly accumulated 3H (2-deoxy-glucose) was calculated (referred to as glucose transport).

Western blotting. For Western blotting along procedures described previously (14), muscles were quickly removed from the flasks, blotted, and frozen in liquid nitrogen at the end of the experiment. Later, frozen muscle specimens were crushed into pieces and lyzed in Weinberg buffer. Aliquots of the homogenates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto nitrocellulose membranes, and treated with rabbit anti-S6 kinase IgG [p70(Ser4) (C-18): sc-230, Santa Cruz Biotechnology, Santa Cruz, CA], rabbit anti-phospho-IRS-1 (specific for Ser307 or Ser469) and rabbit anti-IRS-1 IgG (Upstate, Lake Placid, NY), or with rabbit anti-phospho-Akt (Ser473) and rabbit anti-Akt (Biovision, Mountain View, CA). Blots were exposed to Kodak XAR-Omat films and semiquantitative analysis of visualized bands was performed by densitometry using Gene Profiler 3.56 for Windows (Genomic System; MWG Biotech, Ebersberg, Germany).

To quantitate the phosphorylation status of S6K, we exploited the different electrophoretic mobilities of the hyperphosphorylated and hypophosphorylated forms of the kinase. The ratio of more heavily phosphorylated and thus more slowly migrating forms per total S6K immunity found in the same sample was the readout for S6K activity. The amounts of phosphorylated IRS-1 and Akt were normalized to total IRS-1 and Akt protein as measured by homologous immunoblotting. Results on S6K activities and IRS-1 and Akt phosphorylation are given as percent change vs. an intracellular control.

Statistics. According to the exploratory character of the study, statistics were used in a descriptive sense. Results are given as means ± SE. Differences were analyzed by paired or unpaired two-tailed Student’s t-test as appropriate, with P < 0.05 considered as significant. Four muscle strips were available per rat, allowing paired examination of four different experimental conditions. To minimize the influence of interindividual and interassay variance, comparison of more than four conditions is therefore shown as percent change vs. the intracellular control.

RESULTS

Amino acids insulin-independently inhibit glucose and fatty acid oxidation. The effect of insulin on glucose metabolism is shown in Table 1. The experiment was performed under hyperosmotic conditions as used in other experiments to match for the osmotic effects of amino acids. Hyperosmolarity is known to stimulate glucose transport (3), which explains the relatively modest relative effect of insulin on this parameter.

Under insulin-stimulated conditions (Fig. 1), addition of 0.625% Aminoplasmal to the incubation medium caused a modest, but significant, inhibition of insulin-stimulated glucose

| Table 1. Effect of insulin on glucose metabolism of isolated rat soleus muscle |
|------------------|-----------|-----------|
| Glucose transport, cpn·mg⁻¹·h⁻¹ | 452±24 | 518±26* |
| Glucose oxidation, μmol·g⁻¹·h⁻¹ | 1.70±0.16 | 1.82±0.17 |
| Lactate release, μmol·g⁻¹·h⁻¹ | 13.9±0.9 | 14.8±0.8 |
| Glycogen synthesis, μmol·g⁻¹·h⁻¹ | 1.87±0.15 | 4.38±0.38† |
| Glycogen content, μmol/g | 13.8±0.6 | 16.1±1.0* |

Data are given as means ± SE. Muscle strips were incubated for 2 h, and metabolic rates were measured without or with insulin stimulation (10 mmol/l) during the 2nd hour. Glycogen content was determined at the end of the experiment. *P < 0.05, †P < 0.001.
oxidation in isolated rat soleus muscle (2.85 \pm 0.20 vs. 2.39 \pm 0.18 \text{ mmol g}^{-1} \text{ h}^{-1}, P = 0.002; \text{Fig. 1A}). Stepwise doubling of the added amount of amino acids further increased this effect. The very smooth shape of the dose-response curve indicates that the same mechanism was, at least predominantly, responsible for this effect over the whole range of amino acid concentrations (Fig. 1A).

Marked inhibition of glucose oxidation was not accompanied by any reduction of insulin-stimulated glucose transport, except for the extremely high amino acid concentration resulting from the addition of 20\% Aminoplasmal (Fig. 1B). Likewise, other readouts of glucose metabolism were not reduced by additions of 0.625 to 10\% Aminoplasmal. On some occasions, amino acids even significantly increased the rate of lactate release and muscle glycogen content (Figs. 1, C–E). At variance with this, the amino acid-induced inhibition of insulin-stimulated glucose oxidation was accompanied by a parallel decrease in the rate of palmitate oxidation (Fig. 1F). Prolonged exposure to amino acids over 5 h instead of 2 h further enhanced inhibitory action on insulin-stimulated glucose oxidation with 0.625\% Aminoplasmal causing a reduction by \(-38 \pm 3\% (\text{vs. } -16 \pm 3\% \text{ after } 2 \text{ h}, P < 0.001)\).

In parallel experiments without insulin stimulation, amino acids had almost identical effects as under stimulation with insulin. Although basal rates of glucose and palmitate oxidation were distinctly and dose-dependently inhibited (compare Fig. 1, A and F, with Fig. 2, A and F), other parameters of cellular glucose metabolism were hardly affected, except for modest incremental trends seen in the rates of glycogen storage and lactate release (Fig. 2, B–E). The marked effects of increased ambient amino acid concentrations observed in our experimental setting can therefore not be attributed to modulation of insulin sensitivity.

Effects of amino acids do not depend on the employed incubation medium. Since we intended to relate our findings to those obtained by others, who used L6 cells and amino acid-free KRB (26), parallel experiments were performed with KRB instead of culture medium M199. Except that the lowest addition of amino acids was not effective, inhibitory action on insulin-stimulated glucose oxidation in KRB was as observed in M199. Also in line with our findings from M199, the associated rates of lactate release and glycogen storage were increased rather than decreased (Table 2).

Leucine is superior to other amino acids. We next compared the effects of selected individual amino acids (4 mmol/l) on muscle glucose oxidation. Among the amino acids examined, leucine was clearly superior as an inhibitor of glucose oxidation (\(-39 \pm 5\%, P < 0.001; \text{Fig. 3} \)). This leucine-induced inhibition of glucose oxidation was associated with an approximately sixfold increase of the oxidation of leucine itself (0.48 \pm 0.04 vs. 2.89 \pm 0.17 \text{ mmol g}^{-1} \text{ h}^{-1}, P < 0.001). Isoleucine (\(-29 \pm 9\%, P = 0.02\)) and glycine (\(-20 \pm 5\%, P = 0.01\)) also reduced the rate of glucose oxidation, whereas threonine, arginine, and methionine did not have significant effects (Fig. 3).
Amino acids activate S6K. Most measurements of the phosphorylation state of S6K were done in EDL muscle, which has a higher content of S6K than soleus (1). In line with previous reports, amino acids as well as insulin stimulated the activity/phosphorylation of S6K in EDL muscle (S6K in phosphorylated state: basal 29.3 ± 3.1%, 5% Aminoplasmal 50.6 ± 4.1%, P = 0.005 vs. basal; 10 nmol/l insulin 46.5 ± 3.2%, P < 0.001 vs. basal; Aminoplasmal and insulin combined 68.7 ± 2.7%, P = 0.002 vs. insulin alone and P < 0.001 vs. Aminoplasmal alone). Figure 4 depicts dose dependence of amino acids as stimulators of S6K in insulin-stimulated EDL muscle and shows that rapamycin almost nullified S6K phosphorylation, even in the concomitant presence of both insulin and high amino acids (Fig. 4). Specimens of soleus muscle showed similar responses but seemed less sensitive to amino acid-induced S6K activation than EDL (increase in phosphorylated fraction induced by 5% Aminoplasmal: EDL +20.9 ± 3.1% vs. soleus +7.2 ± 3.0%, P = 0.004; Fig. 4). These findings confirm modulation of S6K activity by insulin, amino acids, and rapamycin as has been described in other experimental settings (12, 26, 27).

In insulin-stimulated EDL muscle, amino acid-activated phosphorylation of S6K was not associated with a change in the phosphorylation state of IRS-1 on Ser636, but with a trend toward increased phosphorylation of IRS-1 on Ser307 (corresponding to mouse Ser302) reported by others (10). The modest increase in serine phosphorylation of IRS-1 by amino acids was not associated with significant inhibition of Akt phosphorylation, but rapamycin-induced blockade of S6K resulted in increased Akt phosphorylation (Fig. 5), which fits with the idea that active S6K blunts the insulin signal.

**Inhibition of glucose and fatty acid oxidation is independent of S6K.** To examine whether activation of the mTOR/S6K pathway mediates the amino acid-induced metabolic alterations, we measured the rates of insulin-stimulated fuel metabolism in the combined presence of high amino acids and rapamycin. Despite its ability to almost completely turn off the activity of S6K (Fig. 4), rapamycin failed to counteract amino

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**Table 2. Effects of amino acids on insulin-stimulated fuel metabolism of isolated rat soleus muscle incubated in Krebs-Ringer buffer**

<table>
<thead>
<tr>
<th>%Aminoplasmal</th>
<th>0</th>
<th>0.625</th>
<th>2.5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose oxidation, µmol·g⁻¹·h⁻¹</td>
<td>4.5±0.6</td>
<td>4.5±0.5</td>
<td>3.1±0.2*</td>
<td>1.1±0.1†</td>
</tr>
<tr>
<td>Lactate release, µmol·g⁻¹·h⁻¹</td>
<td>6.8±0.8</td>
<td>9.4±0.7*</td>
<td>9.0±0.5†</td>
<td>8.1±1.3</td>
</tr>
<tr>
<td>Glycogen synthesis, µmol·g⁻¹·h⁻¹</td>
<td>2.36±0.24</td>
<td>2.68±0.47</td>
<td>1.91±0.36</td>
<td>3.68±0.47*</td>
</tr>
<tr>
<td>Glycogen content, µmol/g</td>
<td>7.9±1.0</td>
<td>7.4±0.6</td>
<td>7.7±0.7</td>
<td>9.6±1.7</td>
</tr>
</tbody>
</table>

Data are given as means ± SE. Experimental conditions were as in Fig. 1, except that Krebs-Ringer buffer solution was used as the incubation medium. In all other experiments of this study, Cell Culture Medium 199 was used. *P < 0.05, †P < 0.01 vs. control (insulin stimulated with no addition of Aminoplasmal).
acid-induced inhibition of glucose and fatty acid oxidation in rat soleus muscle (Fig. 6, A and B). Insulin-stimulated glycogen storage, which is very sensitive to insulin in our setting (Table 1), did not provide any hint either at amino acid-induced impairment of insulin action or at rapamycin-induced improvement of insulin action (Fig. 6, C and D). Parallel evidence against S6K-dependent modulation of mitochondrial oxidation or insulin-stimulated glycogen storage was obtained with EDL muscle, which expresses S6K more abundantly than soleus muscle (Fig. 7).

DISCUSSION

The present study confirms several observations first reported more than 30 years ago, which include that acute exposure of isolated striated rat muscle to high concentrations of amino acids inhibits carbohydrate oxidation and stimulates lactate release without any effect on the rate of glucose transport (5, 23). The noteworthy point is that this response is strikingly different from what was found much more recently in a cultured cell line derived from rat skeletal muscle. Since our results exclude the use of different incubation media as the cause, differing responses to amino acids obviously relate to the difference in the examined cellular material. In L6 cells, increases in the ambient amino acid concentration, which corresponds to the lower doses used in our study, distinctly impaired insulin-stimulated glucose transport but lacked any such effect in the absence of insulin (26). At variance with such insulin desensitization of L6 cells, the amino acid-induced exposure of isolated striated rat muscle to high concentrations of amino acids inhibits carbohydrate oxidation and stimulates lactate release without any effect on the rate of glucose transport (5, 23). The noteworthy point is that this response is strikingly different from what was found much more recently in a cultured cell line derived from rat skeletal muscle. Since our results exclude the use of different incubation media as the cause, differing responses to amino acids obviously relate to the difference in the examined cellular material. In L6 cells, increases in the ambient amino acid concentration, which corresponds to the lower doses used in our study, distinctly impaired insulin-stimulated glucose transport but lacked any such effect in the absence of insulin (26). At variance with such insulin desensitization of L6 cells, the amino acid-induced
suppression of glucose and palmitate oxidation that we found in native muscle was independent of concomitant insulin stimulation and was accompanied by modest increases in glycogen storage and lactate release. This implicates elevated availability of intracellular glucosyl units for utilization via alternative pathways and, hence, argues against shortage of intracellular carbohydrates as the cause of their impaired oxidation. Rather, our findings support the interpretation that amino acids inhibit glucose oxidation in native rat muscle distal to glucose transport and glycolysis, i.e., on the level of the mitochondrion.

Although the changes in glucose handling thus differed in the two experimental settings, amino acids activated the mTOR/S6K pathway in native rat muscle, as likewise described for L6 cells (26). But whereas blockade of this pathway by rapamycin fully reversed the impairment of insulin-stimulated glucose transport and glycolysis, i.e., on the level of the mitochondrion.

Although our findings do not argue against rapid modulation of S6K and insulin-signaling proteins, they clearly rule out such changes as the cause of metabolic effects observed in native rat muscle. Taken together, high ambient amino acids thus seem to affect carbohydrate metabolism in native rat muscle primarily by competing with glucose for mitochondrial oxidation (Ref. 4 and present study), whereas in L6 cells they cause insulin resistance via mTOR/S6K activation and serine phosphorylation of IRS-1 (24–27). This interpretation is further supported in that leucine, the amino acid most readily oxidized to CO₂, is superior to other amino acids as an inhibitor of glucose oxidation in rat muscle (Ref. 5 and present study), which differs from the hierarchy of individual amino acids as inducers of insulin resistance in L6 cells (26).

Apart from illustrating how much caution must be applied in the extrapolation of findings from a cultured cell line to its native tissue of origin, the existence of two different mechanisms of amino acid-induced modulation of muscle glucose metabolism in vitro raises the question for their respective

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**Fig. 6.** Effects of rapamycin on insulin-stimulated fuel metabolism of isolated rat soleus muscle. Strips of rat soleus muscle were incubated for 2 h without or with addition of mixed amino acids (Aminoplasmal) and with indicated concentrations of rapamycin. Rates of glucose oxidation (A), palmitate oxidation (B), and glycogen synthesis (C) were measured under insulin stimulation (10 nmol/l) during the 2nd hour. Glycogen content (D) was determined at the end of the experiment. Data are given as means ± SE. *P < 0.05, †P < 0.01 vs. insulin stimulated with no addition of Aminoplasmal or rapamycin (open bars).

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**Fig. 7.** Effects of rapamycin on insulin-stimulated fuel metabolism of isolated rat EDL muscle. Strips of rat EDL muscle were incubated for 2 h without or with addition of mixed amino acids (Aminoplasmal) and with indicated concentrations of rapamycin. Rates of glucose oxidation (A) and glycogen synthesis (B) were measured under insulin stimulation (10 nmol/l) during the 2nd hour. Glycogen content (C) was determined at the end of the experiment. Data are given as means ± SE. *P < 0.05, †P < 0.01 vs. insulin stimulated with no addition of Aminoplasmal or rapamycin (open bars).
relevance under physiological circumstances in vivo. Although the prevailing degree of insulin sensitivity is inversely associated with the activity state of the mTOR/S6K pathway in mouse skeletal muscle, rapamycin lacks any acute effect on insulin sensitivity in both normal and genetically obese mice (18, 28). Hence, the inverse association between mTOR/S6K activity and insulin sensitivity can hardly be explained by the rapid signaling mechanism portrayed in L6 muscle cells (26). Such lack of short-term modulation of glucose metabolism via the mTOR/S6K pathway in live rodents is in agreement with our findings from native specimens of rat muscle, which are obviously closer to in vivo physiology than a cultured cell line.

With regard to humans, it cannot be excluded that reductions in glucose oxidation seen with high protein intake or amino acid infusion (9, 17) also relate to an mTOR/S6K-independent mechanism, but apart from this aspect the situation is very different from that in rodents. In humans, infusion of amino acids rapidly improves insulin sensitivity and infusion of rapamycin rapidly improves insulin sensitivity, which strongly suggests that mTOR/S6K-dependent short-term modulation of insulin sensitivity is important not only in L6 cells but also in human physiology (9, 14, 15, 25).

Hence, the comparison of our results from rat muscle to those reported by others for humans hints at a species difference, which is strongly reminiscent of what was found for the glucose-fatty acid cycle. In rat muscle, rapid impairment of glucose utilization by high ambient fatty acids goes along with an increase in intracellular glucose 6-phosphate and, hence, has been attributed to intracellular competition of substrates for mitochondrial oxidation (13, 19). In human muscle, high fatty acids lower intracellular glucose 6-phosphate, indicating inhibitory action on the level of glucose transport/phosphorylation (20). The respective effects of amino acid overload are obviously similar: intracellular substrate competition as the predominant mechanism in rodent muscle (Ref. 5 and present study) vs. inhibition on the level of glucose transport and a decrease in glucose 6-phosphate in human muscle (presumably mediated by mTOR and S6K) (9, 15, 25). More stringent regulation of glucose utilization on the level of transport/phosphorylation in human than in rodent muscle could also explain why muscle glycogen stores are decreased in type 2 diabetic patients but increased in comparable rodent models (11, 22).

In summary, our study helps to unravel seeming discrepancies that have arisen from investigations about the modulation of muscle glucose metabolism by amino acids. The outcome suggests that amino acids can act via two different mechanisms, briefly addressed as mitochondrial substrate competition and mTOR/S6K-mediated insulin desensitization. It seems to depend on the experimental setting employed, on the species examined, and on which of these two mechanisms predominates.

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