Insulin resistance without elevated mammalian target of rapamycin complex 1 activity in muscles of mice fed a high-fat diet

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Reynolds TH IV, Cinquino N, Anthony M, Phelps CB, Berk EZ. Insulin resistance without elevated mammalian target of rapamycin complex 1 activity in muscles of mice fed a high-fat diet. J Appl Physiol 107: 1479–1485, 2009. First published September 3, 2009; doi:10.1152/japplphysiol.00574.2009.—The mammalian target of rapamycin complex 1 (mTORC1) appears to mediate the development of insulin resistance in cultured cells. We studied in vivo insulin action and mTORC1 signaling in skeletal muscles of mice fed a normal chow (control [CON]) diet or a high-fat diet (HFD) for 16 wk. We assessed in vivo insulin action by measuring glucose tolerance (GT), insulin tolerance (IT), and insulin-assisted GT (IAGT). Although GT was not altered, the HFD significantly reduced IT and IAGT. Acute treatment with rapamycin, a highly specific inhibitor of mTORC1, did not improve GT, IT, or IAGT in mice fed the CON diet or the HFD. Phosphorylation of S6 kinase (S6K) on Thr389, a surrogate measure of mTORC1 kinase activity, was assessed in skeletal muscles of mice 15 min after an intraperitoneal injection of insulin or saline. In the basal state and after insulin stimulation, phosphorylation of S6K on Thr389 was similar in muscles of mice fed the HFD and mice fed the CON diet, indicating that mTORC1 activity is not elevated. Furthermore, phosphorylation of insulin receptor substrate 1 on Ser636, a site phosphorylated by mTORC1, was similar in muscles of mice fed the HFD and mice fed the CON diet. Taken together, these findings indicate that in vivo insulin resistance can occur without an increase in mTORC1 activity in skeletal muscle and that inhibition of mTORC1 with rapamycin does not improve insulin action.

signal transduction; skeletal muscle

Type 2 diabetes mellitus is a major public health problem affecting ~18 million Americans and resulting in annual health care costs of approximately $120 million (14). Common to all type 2 diabetic patients is insulin resistance, a pathophysiology that precedes the onset of type 2 diabetes. Since skeletal muscle is the primary site for postprandial glucose disposal, it is thought that defects in insulin signal transduction in skeletal muscle contribute significantly to the development of insulin resistance and type 2 diabetes. In recent years, studies of cultured cells have shown that activation of the mammalian target of rapamycin (mTOR) signaling pathway mediates the development of insulin resistance (2, 22, 24–26).

mTOR is an insulin-responsive and amino acid-sensitive Ser/Thr kinase that promotes cellular growth by enhancing mRNA translation initiation and protein synthesis (3, 29). Insulin activates mTOR in a PKB-dependent manner, and amino acids, particularly the branched-chain amino acid leucine, activate mTOR independent of PKB. Recently, mTOR has been shown to exist in at least two complexes, mTOR complex 1 (mTORC1) and mTOR complex 2. mTORC1 contains raptor, and its activity is inhibited by rapamycin (12), whereas mTOR complex 2 contains rictor and is the rapamycin-insensitive complex responsible for phosphorylating PKB on Ser473 (21). In cultured cells, mTORC1 has been shown to contribute to insulin resistance through a negative-feedback mechanism by promoting Ser phosphorylation and subsequent degradation of insulin receptor substrate 1 (IRS1) (4, 22, 26, 30). Evidence indicating that mTORC1 mediates the development of insulin resistance through feedback inhibition of the phosphatidylinositol 3-kinase (PI3K)/PKB pathway is strengthened by the ability of rapamycin to prevent Ser phosphorylation of IRS1 (2, 4, 26, 30) as well as enhance PKB activity (24). Whether mTORC1 plays a role in the development of in vivo insulin resistance is controversial (8, 11, 15, 24, 27). A recent study reveals that acute rapamycin treatment can reverse insulin resistance caused by a high-fat diet that is supplemented with branched-chain amino acids (15). Alternatively, other investigators demonstrate that short-term fat feeding (4 wk) without amino acid supplementation activates mTORC1 signaling in skeletal muscle (11).

The purpose of the present study was to determine whether mTORC1 signaling in skeletal muscle is associated with in vivo insulin resistance in mice fed a high-fat diet compared with mice fed a normal chow diet. Our hypothesis is that basal phosphorylation of S6 kinase (S6K) on Thr389 and IRS1 on Ser636 will be elevated in muscles of mice fed a high-fat diet for 16 wk. We also hypothesized that acute rapamycin treatment would improve the insulin resistance of mice fed a high-fat diet.

METHODS

Ethical Information

All animal care and surgery were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (DHEW Publication No. 85-23). All experimental protocols were approved by the University Committee for the Use and Care of Animals.

Glucose and Insulin Tolerance Testing

For assessment of the effect of a high-fat diet on in vivo insulin sensitivity, mice were subjected to a glucose tolerance (GT) test, an insulin tolerance (IT) test, and an insulin-assisted GT (IAGT) test. The GT test was conducted after a 16-h fast, whereas the IT and IAGT tests were conducted after a 6-h fast. GT was assessed by injection of mice with glucose (1 g/kg body wt) and measurement of glucose in blood collected via the tail vein at 0, 15, 30, 45, 60, and 90 min after the glucose injection. IT was assessed by injection of mice with insulin (0.25 U/kg body wt) and measurement of glucose in blood collected via the tail vein at 0, 15, 30, 45, and 60 min after the insulin injection. IAGT was assessed by injection of mice with insulin (0.25 U/kg body wt) 5 min after glucose injection (1 g/kg body wt) and measurement of glucose in blood collected via the tail vein at 0, 15, 30, 45, and 60 min after the insulin injection.
measurement of glucose in blood collected via the tail vein at 0, 15, 30, 45, and 60 min after the glucose injection. For assessment of the role of mTORC1 activity on in vivo insulin sensitivity, mice fed a high-fat diet were injected intraperitoneally with rapamycin (0.005 g/kg body wt) or an equivalent volume of saline 2 h before the GT, IT, and IAGT tests. Figure 1 demonstrates that this dose of rapamycin abolishes mTORC1 activity in skeletal muscle, as reported previously (13). We chose acute rapamycin treatment, because prolonged treatment can inhibit Akt/PKB activity (20) and cause insulin resistance (6, 23).

Surgical Procedures

Male C57/B6 mice (n = 42; Taconic Farms, Germantown, NY) were caged individually, maintained on a 12:12-h light-dark cycle, fed a normal chow [control (CON) diet, were caged individually, maintained on a 12:12-h light-dark cycle, fed a normal chow [control (CON) diet, or a high-fat diet (HFD, n = 22), and allowed ad libitum access to water for 16 wk. The calories from fat were 59.2% and 10.6% for the HFD and CON diet, respectively (Test Diets, Richmond, IN). Skeletal muscle was harvested from mice anesthetized by injection of 1:1:1 promace-ketamine hydrochloride-xyalzine (0.015 ml/10 g body wt ip). At 10 min before administration of the anesthetics, mice were injected with insulin (0.25 U/kg body wt) or an equivalent volume of saline (0.9% NaCl). At 15 min after insulin or saline injection, gastrocnemius muscle was rapidly dissected, frozen in liquid nitrogen, and stored at −80°C until analysis.

Preparation of Muscle Extracts

The frozen gastrocnemius muscles from all mice were manually ground with a porcelain mortar and pestle chilled in liquid N2. Powdered muscles were homogenized on ice using a motor-driven tissue grinder (Teflon-glass) in Cell Extraction Buffer (Biosource; 10 ml buffer/l g muscle wt) containing 0.1 mM dithiothreitol, 500 nM microcin-LR, 0.1 mM phenylmethylsulfonyl fluoride, and 10 μg/ml each of apro tinin, leupeptin, and pepstatin A. The homogenates were rotated at 4°C for 1 h and then centrifuged at 9,000 g for 30 min at 4°C. The protein concentrations of the supernatants were determined by the bicinchoninic acid method (Pierce). The remaining skeletal muscle extract was utilized for electrophoretic analysis and immunoblotting experiments.

Electrophoretic Analysis and Immunoblotting

Skeletal muscle extracts and molecular weight standards (Bio-Rad, Hercules, CA) were subjected to SDS-PAGE. The proteins were then electrophoretically transferred to Immobilon membranes and immunoblotted with phosphospecific antibodies to S6K, IRS1, and PKB. The phosphorylated Thr389 S6K antibody recognizes S6K when phosphorylated on Thr389, a site phosphorylated by mTOR (17), and, therefore, is an ideal measure of mTORC1 kinase activity (5). The phosphorylated Ser636 IRS1 antibody recognizes IRS1 when phosphorylated on Ser636, a site believed to be phosphorylated by mTORC1 (16). The phosphorylated Thr308 PKB antibody recognizes PKB when phosphorylated on Thr308, a site whose phosphorylation is necessary for PKB activity (1). To account for changes in the total amounts of the kinases, the phosphospecific immunoblots were stripped and reprobed with antibodies that recognize total S6K, IRS1, and PKB (generous gift from Dr. M. J. Birnbaum), respectively. As a loading control, all blots were stripped and reprobed with GAPDH or β-tubulin antibody. After the membranes were washed, the light generated by the alkaline phosphatase-conjugated secondary antibody and Tropix reagent was detected using a UVP Chemi Doc-It imaging system. Relative signal intensities of the phosphorylated Thr389 S6K, phosphorylated Ser636 IRS1, and phosphorylated Thr308 PKB bands were determined using Total Lab 100 software (Nonlinear, San Diego, CA). All antibodies were obtained from Cell Signaling Technology (Beverly, MA) unless otherwise noted.

Statistical Analysis

All data were analyzed using ANOVA. To detect statistical significance for basal and insulin-stimulated PKB and S6K phosphorylation, a 2 × 2 ANOVA (diet × insulin) was utilized. Statistically significant differences for basal IRS1 phosphorylation were assessed by a one-way ANOVA. Statistical differences for GT and IT were detected using a 2 × 2 × 2 ANOVA (condition × diet × time) with repeated measures for time of glucose determination (0, 15, 30, 45, 60, and 90 min). For the IAGT, a 1 × 3 ANOVA was used to detect statistical significance. In the case of a significant F ratio, a priori mean comparisons were conducted using Fisher’s least significant difference post hoc test. Values are means ± SE, and the level of statistical significance was set at P < 0.05.

RESULTS

In Vivo Insulin Action

Diet effects. To demonstrate that the HFD produced whole body insulin resistance, we conducted GT, IT, and IAGT tests. GT was not altered by the HFD, inasmuch as blood glucose levels after an intraperitoneal injection of glucose were similar and the GT area under the curve (AUC) in mice fed the HFD was not significantly different (Table 1) from that in mice fed the CON diet. However, after the injection of glucose, fasting basal glucose levels were significantly higher in mice fed the HFD (Table 1). Since insulin resistance can be present without changes in GT, we conducted IT tests by injecting mice with 0.25 U/kg of insulin. After the injection of insulin, blood glucose values were significantly higher and the IT AUC was elevated in mice fed the HFD compared with mice fed the CON diet (Table 2). To further establish the presence of whole body insulin resistance in the mice fed the HFD, we conducted IAGT tests by injecting mice with insulin (0.25 U/kg) 5 min after an injection of glucose (1 g/kg). The IAGT test measures in vivo insulin action during hyperglycemia, rather than during hypoglycemia, as is the case with IT testing. After the injection of glucose and insulin, blood glucose values were significantly higher (Fig. 2A) and the IAGT AUC was elevated in mice fed the HFD compared with mice fed the CON diet (Fig. 2B). Taken together, these results clearly demonstrate whole body insulin resistance in mice fed the HFD. In addition being insulin resistant, mice fed the HFD weighed significantly more than mice fed the CON diet (46.1 ± 0.52 vs. 32.9 ± 0.59, P = 0.0001).
Rapamycin effects. There is little direct evidence to support a role for mTORC1 in the development of obesity-related insulin resistance in intact animals or humans. Therefore, we examined the effects of acute inhibition of mTORC1 with rapamycin 2 h before assessing GT, IT, and IAGT. As shown previously (13) and in Fig. 1, rapamycin abolishes insulin’s ability to promote the phosphorylation of S6K on Thr\textsuperscript{389}. Rapamycin had no effect on GT and IT in mice fed the HFD or the CON diet (Tables 1 and 2, respectively). However, basal glucose values were significantly lower in HFD-fed mice that were treated with rapamycin after a 6-h fast (Table 2), indicating that mTORC1 may control hepatic glucose production. Although the IT AUC of rapamycin-treated HFD-fed mice is not significantly different from that of vehicle-treated mice fed the CON diet, this effect is primarily due to the effect of rapamycin on basal glucose values. When AUC is calculated from 15 to 60 min, the IT AUC is elevated in the HFD mice treated with rapamycin compared with the mice fed the CON diet and treated with vehicle (P = 0.0560). Furthermore, as shown in Fig. 2, rapamycin had no effect on IAGT in mice fed the HFD, providing further evidence that inhibition of mTORC1 activity does not improve insulin action. These results clearly indicate that obesity-related in vivo insulin resistance is not improved by acute rapamycin treatment.

S6K Phosphorylation on Thr\textsuperscript{389}

Phosphorylation on Thr\textsuperscript{389} of S6K is mediated by mTORC1 and serves as an ideal measure of mTORC1 activity (5, 17). Therefore, we have used the phosphorylation of S6K on Thr\textsuperscript{389} as a surrogate measure of mTORC1 kinase activity by using the phosphospecific antibody pThr\textsuperscript{389} S6K. Figure 3A shows a representative immunoblot prepared with the pThr\textsuperscript{389} S6K phosphospecific antibody. As shown in Fig. 3B, basal phosphorylation of S6K on Thr\textsuperscript{389} is similar in skeletal muscle extracts prepared from mice fed the HFD and mice fed the CON diet. pThr\textsuperscript{389} S6K immunoreactivity was significantly higher after insulin stimulation than in the basal state in mice fed the CON diet but not in mice fed the HFD, indicating skeletal muscle insulin resistance (Fig. 3B). An immunoblot prepared with an antibody that recognizes all S6K, regardless of phosphorylation, demonstrates that changes in pThr\textsuperscript{389} S6K immunoreactivity are not due to differences in expression of the kinase (Fig. 3A).

Phosphorylation of IRS1 on Ser\textsuperscript{636}

Because mTORC1 has been shown to promote insulin resistance in cultured cells by promoting serine phosphorylation and degradation of IRS1, we assessed the phosphorylation of IRS1 on Ser\textsuperscript{636}, a site thought to be phosphorylated by mTORC1. As shown in Fig. 4, Ser\textsuperscript{636} immunoreactivity is similar in muscles from mice fed the HFD and mice fed the CON diet. Furthermore, there do not appear to be any significant changes in the total abundance of IRS1 in mice made obese and insulin resistant by the HFD (Fig. 4). Despite the presence of in vivo insulin resistance, Ser\textsuperscript{636} phosphorylation and total IRS1 levels are similar in muscle of mice fed the HFD and mice fed the CON diet.

PKB Phosphorylation on Thr\textsuperscript{308}

PKB promotes mTORC1 activity by phosphorylating proline-rich Akt substrate (PRAS40) (19, 28) and tuberous sclerosis complex (TSC1/2) (29). Therefore, we assessed PKB phosphorylation on Thr\textsuperscript{308}, a site whose phosphorylation is necessary for PKB kinase activity (1), by immunoblotting with the phosphospecific antibody pThr\textsuperscript{308} PKB. A representative immunoblot prepared with pThr\textsuperscript{308} PKB antibody is shown in Fig. 5A. As shown in Fig. 5B, basal phosphorylation of PKB on Thr\textsuperscript{308} is similar in skeletal muscle extracts prepared from mice fed the HFD and mice fed the CON diet. After insulin stimulation, PKB phosphorylation is significantly lower in muscles of mice fed the HFD than in mice fed the CON diet (Fig. 5B), indicating defects in insulin signaling through the PI3K pathway. In Fig. 5A, a representative immunoblot prepared with an antibody that recognizes all PKBβ, regardless of phosphorylation, demonstrates that changes in pThr\textsuperscript{308} PKB immunoreactivity are significant in muscles from mice fed the HFD and mice fed the CON diet.

Table 1. Effects of rapamycin on glucose tolerance in mice fed CON diet and mice fed HFD

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>90 min</th>
<th>AUC</th>
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<tr>
<td>CON + vehicle</td>
<td>130±9</td>
<td>307±10</td>
<td>289±25</td>
<td>247±38</td>
<td>246±20</td>
<td>216±18</td>
<td>22.37±1.728</td>
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<td>CON + rapamycin</td>
<td>129±17</td>
<td>316±32</td>
<td>248±14</td>
<td>226±9</td>
<td>218±19</td>
<td>194±19</td>
<td>20.61±1.216</td>
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<td>HFD + vehicle</td>
<td>173±19*</td>
<td>355±22</td>
<td>266±15</td>
<td>255±7</td>
<td>238±26</td>
<td>194±19</td>
<td>22.38±2.758</td>
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<tr>
<td>HFD + rapamycin</td>
<td>174±29*</td>
<td>329±29</td>
<td>287±37</td>
<td>262±39</td>
<td>221±37</td>
<td>201±30</td>
<td>22.44±2.872</td>
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Values are means ± SE. *Significantly different from CON + vehicle and CON + rapamycin at respective times, P < 0.05. †Significantly different from basal for HFD + vehicle and CON + vehicle, P < 0.05. ‡Significantly different from CON + vehicle and CON + rapamycin at respective times, P < 0.05.

Table 2. Effects of rapamycin on insulin tolerance in mice fed CON diet and mice fed HFD

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
<th>AUC</th>
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<tr>
<td>CON + vehicle</td>
<td>174±7</td>
<td>95±6</td>
<td>69±2</td>
<td>57±2</td>
<td>47±2</td>
<td>4,85±146</td>
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<tr>
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<td>157±14</td>
<td>91±11</td>
<td>63±6</td>
<td>54±4</td>
<td>46±5</td>
<td>4,63±391</td>
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<tr>
<td>HFD + vehicle</td>
<td>167±9</td>
<td>106±4</td>
<td>88±4*</td>
<td>76±5*</td>
<td>68±5*</td>
<td>5,806±217*</td>
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<tr>
<td>HFD + rapamycin</td>
<td>137±5</td>
<td>100±13</td>
<td>78±9</td>
<td>74±123</td>
<td>67±101</td>
<td>5,293±555</td>
</tr>
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</table>

Values are means ± SE. *Significantly different from CON + vehicle and CON + rapamycin at respective times, P < 0.05. †Significantly different from basal for HFD + vehicle and CON + vehicle, P < 0.05. ‡Significantly different from CON + vehicle and CON + rapamycin at respective times, P < 0.05.
DISCUSSION

Cell culture studies have shown that mTORC1 signaling mediates the development of insulin resistance (2, 22, 26, 30). However, cultured cells are distinct from terminally differentiated insulin-responsive tissues and may not adequately represent the pathogenesis of insulin resistance observed in humans and experimental animals. Because skeletal muscle is the primary tissue for postprandial glucose disposal, we examined the activity of the mTORC1 signaling pathway in skeletal muscle of mice fed a high-fat diet. Our results clearly demonstrate that a diet high in saturated fat produces insulin resistance that is not reversed by acute rapamycin treatment or associated with increased mTORC1 signaling.

Our first line of evidence that mTORC1 signaling is not essential for the presence of in vivo insulin resistance was the observation that acute rapamycin treatment does not improve insulin sensitivity in mice fed the HFD. The inability of rapamycin, a potent and specific mTORC1 inhibitor, to improve insulin action is supported by our immunoblotting experiments that assessed S6K phosphorylation on Thr389, a site phosphorylated by mTORC1. Therefore, our second line of evidence that mTORC1 signaling is not necessary for in vivo insulin resistance is the finding that S6K phosphorylation on Thr389 in skeletal muscle is not elevated in mice fed the HFD compared with mice fed the CON diet for 16 wk. These findings are in contrast to those of Khamzina and colleagues (11), who report increased mTORC1 signaling in muscles of rats fed a high-fat diet for 4 wk. One possible explanation for the differences between the present study and the study of Khamzina et al. is the duration of the high-fat diet intervention. We fed mice the HFD for 16 wk and observed no change in mTORC1 signaling, whereas Khamzina et al. reported an increase in mTORC1 signaling after only 4 wk of a high-fat diet. Perhaps mTORC1 is hyperactive after short-term fat...
feeding (4 wk) but then resolves itself with a long-term HFD (16 wk) and insulin resistance ensues by other mechanisms. However, the long-standing obesity and insulin resistance observed in ob/ob mice are associated with increased basal mTORC1 activity in skeletal muscle (13). Since ob/ob mice typically weigh substantially more than mice fed a high-fat diet, we believe that a threshold of obesity and/or insulin resistance may be needed to persistently activate mTORC1 signaling in skeletal muscle. Another possible explanation for the differences between the present study and the study of Khamzina et al. is the composition of the high-fat diets. Khamzina et al. utilized a lard-corn oil (1:1 mix) diet, whereas the present study used a diet high in hydrogenated coconut oil. Although the sucrose content and percentage of calories derived from fat are similar in these diets, signaling mechanisms by which insulin resistance was induced by the lard-corn oil diet may be different from those by which insulin resistance was induced by the hydrogenated coconut oil diet. However, a high-fat diet consisting of lard-soybean oil produced insulin resistance without activating mTORC1 (15).

Prolonged activation of mTORC1 leads to the serine phosphorylation of IRS1 and insulin resistance in cultured cells (4, 22, 26, 30). Therefore, if mTORC1 activity mediates diet-induced insulin resistance, then increases in Ser phosphorylation of IRS1 would be expected. In this context, our third line of evidence indicating that mTORC1 signaling is not critical for the presence of in vivo insulin resistance was the observation that the HFD does not increase the phosphorylation of IRS1 on Ser636, a site thought to be phosphorylated by mTOR (16). It is possible that a high-fat diet may promote inhibitory serine phosphorylation of IRS1 on sites other than the rapamycin-sensitive Ser636 residue. Herschkovitz et al. (9) demonstrate that IRS1 contains at least seven serine phosphorylation sites and that PKCθ, p38 MAPK, and PKCζ converge on IKKβ, a serine kinase that phosphorylates IRS1. The lack of an effect of rapamycin on insulin sensitivity observed in the present study indicates that kinases other than mTOR may be responsible for promoting IRS1 serine phosphorylation (9). Accordingly, Newgard et al. (15) show an increase in the phosphorylation of IRS1 on Ser636, but not Ser636, in muscles from mice fed a high-fat diet or a high-fat diet supplemented with branched-chain amino acids. This observation indicates that JNK (18) or IKKβ (7), rather than mTOR, disrupts IRS1 function during long-term continual insulin resistance. However, short-term (4 wk) fat feeding appears to result in the phosphorylation of IRS1 on Ser636 in muscle (11), but this response may resolve itself with prolonged (12–16 wk) fat feeding, and inhibitory Ser phosphorylation of IRS1 may persist via other kinases (9). Alternatively, a high-fat diet may induce insulin resistance by increasing ceramide synthesis, a process that appears to impair PKB activity without disrupting IRS1-dependent signaling (10).

It is well established that insulin and the branched-chain amino acid leucine can independently activate mTORC1 by separate upstream signaling pathways (3, 29). Therefore, the
report of Newgard and et al. (15) that 12–16 wk of a high-fat diet supplemented with branched-chain amino acids, but not a high-fat diet alone, increases mTORC1 activity in skeletal muscle is not surprising. The findings of Newgard et al. reinforce the present results showing that continued, persistent insulin resistance due to 16 wk of a high-fat diet is not associated with enhanced basal mTORC1 signaling in skeletal muscle. However, our results reveal that a high-fat diet produces insulin resistance with respect to mTORC1 activation in skeletal muscle, whereas Newgard et al. show elevated mTORC1 activity in the postprandial state when circulating insulin and amino acids are elevated. In contrast, we injected fasted mice with insulin and observed reduced phosphorylation of S6K on Thr389 in muscles from mice fed the HFD compared with mice fed the CON diet, indicating an impaired ability of insulin to promote mTORC1 activity. Nonetheless, the present study and the study of Newgard et al. demonstrate that rapamycin does not improve glucose tolerance in diet-induced obese mice. Furthermore, we demonstrate that rapamycin does not improve insulin sensitivity in insulin-resistant mice with normal glucose tolerance (Tables 1 and 2, Fig. 2).

One limitation to the present study is the possibility that changes in hepatic glucose production may have played a role in the development of diet-induced insulin resistance. Although the role of hepatic glucose production in diet-induced insulin resistance cannot be ruled out, we demonstrate the presence of skeletal muscle insulin resistance with two lines of evidence. 1) The phosphorylation of PKB on Thr308 is significantly lower in muscles of mice fed the HFD than in muscles of mice fed the CON diet (Fig. 5). 2) Insulin fails to promote the phosphorylation of S6K on Thr389 in muscles of mice fed the HFD, whereas insulin significantly increases the phosphorylation of S6K on Thr389 in muscles of mice fed the CON diet (Fig. 3). Taken together, this evidence clearly shows the presence of diet-induced skeletal muscle insulin resistance, a process that appears to be independent of mTORC1 activity.

In contrast to studies of cultured cells and short-term dietary interventions in mice, the present findings provide evidence indicating that in vivo insulin resistance can occur without increased mTORC1 activity in skeletal muscle. We clearly demonstrate that mice fed the HFD are insulin resistant, as shown by IT and IAGT testing, as well as impaired insulin-stimulated PKB and S6K phosphorylation. Despite the presence of in vivo insulin resistance, basal S6K phosphorylation on Thr389 and IRS1 phosphorylation on Ser436 are unchanged in muscles from mice fed the HFD for 16 wk. Furthermore, when mice fed the HFD are treated with rapamycin, a highly specific inhibitor of mTORC1, insulin resistance is not improved. Taken together, these findings indicate that prolonged diet-induced insulin resistance is not dependent on the activation of mTORC1 in skeletal muscle. Finally, our results highlight the importance of examining terminally differentiated tissues from intact organisms when insulin action is studied.

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REFERENCES


