Adipose-Specific Knockout of *raptor* Results in Lean Mice with Enhanced Mitochondrial Respiration

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**Summary**

*raptor* is a specific and essential component of mammalian TOR complex 1 (mTORC1), a key regulator of cell growth and metabolism. To investigate a role of adipose mTORC1 in regulation of adipose and whole-body metabolism, we generated mice with an adipose-specific knockout of *raptor* (*raptor*^ad/C0^). Compared to control littermates, *raptor*^ad/C0^ mice had substantially less adipose tissue, were protected against diet-induced obesity and hypercholesterolemia, and exhibited improved insulin sensitivity. Leanness was in spite of reduced physical activity and unaffected caloric intake, lipolysis, and absorption of lipids from the food. White adipose tissue of *raptor*^ad/C0^ mice displayed enhanced expression of genes encoding mitochondrial uncoupling proteins characteristic of brown fat. Leanness of the *raptor*^ad/C0^ mice was attributed to elevated energy expenditure due to mitochondrial uncoupling. These results suggest that adipose mTORC1 is a regulator of adipose metabolism and, thereby, controls whole-body energy homeostasis.

**Introduction**

The protein kinase target of rapamycin (TOR) is a highly conserved, central controller of cell growth and metabolism (Corradetti and Guan, 2006; Guertin and Sabatini, 2007; Wullschleger et al., 2006). TOR is found in two functionally and structurally distinct multiprotein complexes termed TOR complex 1 (TORC1) and TORC2 (Jacinto et al., 2004; Loewith et al., 2002; Sarbassov et al., 2004). In mammals, mTORC1 consists of mTOR, raptor, PRAS40, and mLST8 and is sensitive to rapamycin (Vander Haar et al., 2007; Hera et al., 2002; Kim et al., 2002, 2003; Loewith et al., 2002). mTORC2 contains mTOR, rictor, mSin1, and mLST8 (Frias et al., 2006; Jacinto et al., 2004, 2006; Sarbassov et al., 2004; Yang et al., 2006) and is not directly inhibited by rapamycin, although long-term rapamycin treatment can inhibit mTORC2 indirectly in certain cell types (Sarbassov et al., 2006). As a central controller of cell growth, TOR plays a key role in development and aging and is implicated in disorders such as cancer, cardiovascular disease, obesity, and diabetes (Guertin and Sabatini, 2007; Wullschleger et al., 2006).

mTORC1 controls many cellular processes that ultimately determine cell growth, including protein synthesis, ribosome biogenesis, nutrient transport, and autophagy. The two best-characterized substrates of mTORC1 are S6 kinase (S6K) and 4E-BP, via which mTORC1 controls protein synthesis (Beretta et al., 1996; Brunn et al., 1997; Burnett et al., 1998; Hay and Sonenberg, 2004). mTORC1 is controlled by metabolic cues, i.e., nutrients, cellular energy, and growth factors such as insulin or IGF (Wullschleger et al., 2006). Although mTORC1 is present in essentially all tissues, the findings that it is controlled by metabolic signals and is implicated in metabolic disorders suggest that it plays a particularly important role in metabolic tissues. Thus, we have focused on studying the role of the mTORC1 signaling pathway in a metabolic tissue, in particular, adipose tissue.

The traditional role of white adipose tissue (WAT) is as a long-term fat storage depot for the body. However, it is also an important endocrine organ that secretes hormones such as leptin, adiponectin, TNF-α, and many others (Gimeno and Klaman, 2005; Kershaw and Flier, 2004; Shi and Burn, 2004) that regulate energy homeostasis, lipid metabolism, appetite, fertility, and immune and stress responses. An excess or deficiency of adipose tissue can lead to severe metabolic diseases such as type 2 diabetes, cardiovascular disorders, and cancer.

Adipose tissue also participates in the regulation of energy homeostasis through adaptive thermogenesis. This occurs via a type of adipose tissue known as brown adipose tissue (BAT), which oxidizes fatty acids and dissipates energy as heat. Heat production in BAT is achieved by short-circuiting, or uncoupling, the mitochondrial proton gradient that is generated by the respiratory chain. Uncoupling of the proton gradient from ATP production in BAT is mediated by the BAT-specific uncoupling protein 1 (UCP1). Two UCP1 homologs termed UCP2 and UCP3 are also expressed in BAT, but these proteins are expressed at very low levels and it is less clear whether they transfer protons and are, thus, indeed uncoupling proteins (Brand and Esteves, 2005). Trans-differentiation between WAT and BAT can be achieved, at least partly, via expression of specific transcription factors. For example, expression of the transcription factor PRDM16 (Seale et al., 2007), PGC1α (Puigserver et al., 1998), RIP140 (Christian et al., 2005; Kiskinis et al., 2007; Leonardsson et al., 2004), FOXC2 (Cederberg et al., 2001), p107 (Scime et al., 2005), or retinoblastoma (Hansen et al., 2004;
Scime et al., 2005) causes WAT cells to exhibit properties characteristic of brown fat.

Several studies have suggested that mTORC1 is essential for the differentiation and maintenance of adipocytes in vitro (Bell et al., 2000; Cho et al., 2004; El-Chaar et al., 2004; Gagnon et al., 2001; Kim and Chen, 2004; Yeh et al., 1995). To examine the role of mTORC1 in adipose tissue in vivo, we generated mice lacking the specific and essential mTORC1 component raptor. Since a nonconditional, full-body knockout of raptor in mice is embryonic lethal (Guertin et al., 2006), we generated conditional raptor knockout mice using the cre/loxP system. These mice were used to delete raptor specifically in adipose tissue. We demonstrate that adipose raptor controls adipocyte metabolism and, thereby, full-body energy homeostasis.

RESULTS

mTORC1 Is Required for Adipogenesis and Adipose Maintenance In Vitro

As shown previously, long-term rapamycin treatment prevents adipogenic differentiation of 3T3-L1 cells in culture (Bell et al., 2000; Cho et al., 2004; El-Chaar et al., 2004; Gagnon et al., 2001; Kim and Chen, 2004; Yeh et al., 1995). Since long-term rapamycin treatment can disrupt mTORC2 as well as inhibit mTORC1 (Sarbassov et al., 2006), we genetically knocked down raptor in undifferentiated 3T3-L1 preadipocytes to determine the role specifically of mTORC1 in adipogenesis (Figure 1A). The cells in which rapamycin was knocked down were then treated to induce adipogenesis and examined for lipid accumulation, indicating that mTORC1 is indeed required for adipogenesis. To test further the requirement for mTORC1 in adipogenesis, we differentiated heterozygous raptor+/− mouse embryonic fibroblasts (MEFs) derived from mice lacking one copy of the raptor gene (see Supplemental Experimental Procedures). Heterozygous MEFs were used for this experiment because homozygous raptor knockout MEFs could not be obtained due to very early embryonic lethality, as observed previously (Guertin et al., 2006). The raptor+/− MEFs differentiated into adipocyte-like cells but with significantly reduced efficiency compared to MEFs from wild-type littermates (Figure 1C), providing further support for a role of mTORC1 in adipogenesis.

It has also been shown previously that culturing differentiated 3T3-L1 cells in the presence of rapamycin causes loss of fat, suggesting that mTORC1 is also required for maintenance of adipocytes (Kim and Chen, 2004). We confirmed this observation...
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by measuring the triglyceride content of differentiated 3T3-L1 cells that were treated with rapamycin for 6 days after differentiation. Differentiated 3T3-L1 cells that were incubated with rapamycin for 6 days lost on average 46% of their triglyceride content (Figure 1D). Rapamycin treatment or siRNA-mediated raptor knockdown in differentiated 3T3-L1 adipocytes also caused loss of lipid stores as visualized by oil red O staining (Figure 1E). The above results confirm that mTORC1 is required in the maintenance of mature adipocytes, but not in adipogenesis.

**Generation and Validation of Adipose-Specific raptor Knockout Mice**

To elucidate the role of mTORC1 in adipose tissue, we utilized the cre/loxP system to generate mice in which raptor, an essential and specific component of mTORC1, is deleted exclusively in adipose tissue. A “floxed” raptor allele was created in embryonic stem cells of the 129S1/SvImJ mouse strain by introducing a loxP site into the introns flanking raptor exon 6, using a neo cassette (G418 resistance) flanked by frt sites as a selectable marker (Figure 2A). The targeted stem cells were injected into blastocysts combination was confirmed by Southern blot analysis (data not shown). Thus, the generated raptorfl/fl mice could be used to study the role of mTORC1 in the maintenance of mature adipocytes, but not in adipogenesis. raptor protein and S6K phosphorylation (T389) were absent in differentiated adipocytes, but not in other tissues, of raptorfl/fl mice (Figures 2B, 2C, and data not shown). We also examined, by RT-PCR, expression of the cre recombinase and confirmed that cre was expressed in white and brown adipose tissue, but not in other tissues, of raptorfl/fl mice (Figures 2B, 2C, and data not shown). Thus, raptorfl/fl mice lack raptor and are defective for mTORC1 signaling specifically in adipose tissue.

**raptorfl/fl Mice Are Lean and Resistant to Diet-Induced Obesity**

As a first analysis of the effect of raptorfl/fl on adipose tissue, we monitored the weight of mice. raptorfl/fl females showed reduced fertility, giving birth on average to 3.9 ± 0.5 pups per litter, as compared to 6.79 ± 0.6 and 6.8 ± 0.5 pups per litter for floxed females that did not express cre (raptorfl/fl) and for wild-type C57BL/6J females, respectively. The time between litters was also slightly longer for raptorfl/fl females. raptorfl/fl males showed similar fertility to that of raptorfl/fl and C57BL/6J males.

Figure 2. Generation and Validation of raptor Knockout
(A) Scheme describing the generation of raptor knockout.
(B) Western blot on epididymal WAT with raptor, S6K, mTOR, or actin antibodies as indicated, showing that the knockout was efficient.
(C) Western blots on epididymal WAT, inguinal WAT, BAT, liver, and soleus muscle with raptor or actin antibodies as indicated, showing that the knockout was specific to adipose tissue.
raptor mice on an HFD (Figures 3C and 3D). HFD-fed raptor mice had 70% less WAT than similarly fed ad/mice (Figure 3C). The adipocytes from knockout and control mice on a chow diet were similar in size, suggesting that the lower amount of WAT in raptor mice is due to fewer adipocytes. This finding was confirmed by FACS analysis, which showed that epididymal WAT of raptor mice contained approximately three times fewer cells than WAT of raptor mice (data not shown). Adipocytes from raptor mice on a high-fat diet displayed an expected ~2-fold increase in size (Kubota et al., 1999; Lemonnier, 1972), whereas adipocytes from raptor mice on an HFD displayed only a slight increase in size. Calculating and comparing the volumes of adipocytes from raptor and raptor mice on an HFD indicated that raptor adipocytes were approximately half the size of the raptor adipocytes. Although raptor adipocytes were significantly smaller, this decrease was not sufficient to account for the observed overall reduction in adipose tissue (Figure 3C), indicating again that a raptor knockout also reduces adipocyte number. The reduced weight of raptor knockout mouse is due to a combination of smaller and fewer WAT cells. There was no obvious morphological difference in BAT due to either diet or raptor knockout (data not shown), although the raptor mice contained less overall BAT. Thus, mTORC1 is required to maintain adipose tissue.

**raptor** Mice Have Smaller and Fewer WAT Cells To determine whether the reduced amount of fat in raptor mice is due to fewer fat cells and/or smaller fat cells, we measured the circumference of individual adipocytes in epididymal WAT of raptor and raptor mice (Figure 3E). The adipocytes from knockout and control mice on a chow diet were similar in size, suggesting that the lower amount of WAT in raptor mice is due to fewer adipocytes. This finding was confirmed by FACS analysis, which showed that epididymal WAT of raptor mice contained approximately three times fewer cells than WAT of raptor mice (data not shown). Adipocytes from raptor mice on a high-fat diet displayed an expected ~2-fold increase in size (Kubota et al., 1999; Lemonnier, 1972), whereas adipocytes from raptor mice on an HFD displayed only a slight increase in size. Calculating and comparing the volumes of adipocytes from raptor and raptor mice on an HFD indicated that raptor adipocytes were approximately half the size of the raptor adipocytes. Although raptor adipocytes were significantly smaller, this decrease was not sufficient to account for the observed overall reduction in adipose tissue (Figure 3C), indicating again that a raptor knockout also reduces adipocyte number. The reduced weight of a raptor knockout mouse is due to a combination of smaller and fewer WAT cells. There was no obvious morphological difference in BAT due to either diet or raptor knockout (data not shown), although the raptor mice contained less overall BAT. Thus, mTORC1 is required to maintain adipose tissue.

**raptor** Mice Have Better Metabolic Parameters We next investigated the effect of the raptor knockout on whole-animal metabolism. First, we performed an IP glucose tolerance test (Figure 3F). The glucose tolerance of raptor mice was better than that of control mice, indicating that the raptor knockout reduces liver glucose production. To determine whether the improved glucose tolerance was due to decreased circulating insulin levels, we measured plasma insulin levels before and after glucose injection (Figure 3G). The insulin levels of raptor mice were significantly lower than those of control mice, indicating that the raptor knockout reduces insulin release from the pancreas. Together, these results suggest that the raptor knockout improves whole-animal metabolism by reducing both liver glucose production and insulin release.
test to determine the ability of the mice to clear glucose from the blood (Figure 4A). On a chow diet, the basal glucose level of the raptor<sup>fl/fl</sup> mice was lower than that of raptor<sup>ad−/−</sup> mice (4.72 ± 0.3 versus 6.36 ± 0.52 mM, respectively). Upon IP injection of glucose, the knockout mice had a normal glucose response and clearance rate. On the HFD, basal glucose levels were, as expected, higher than on the chow diet but slightly lower in raptor<sup>ad−/−</sup> mice compared to raptor<sup>fl/fl</sup> mice. HFD-fed raptor<sup>ad−/−</sup> and raptor<sup>fl/fl</sup> mice both exhibited reduced rates of glucose clearance. However, the glucose clearance rate of the raptor<sup>ad−/−</sup> mice was markedly better compared to the raptor<sup>fl/fl</sup> mice, indicating that the knockout mice were more glucose tolerant than control mice. We then determined plasma insulin levels. The fasting insulin levels were lower in raptor<sup>ad−/−</sup> mice compared to raptor<sup>fl/fl</sup> mice (Figure 4B). The lower insulin level combined with the observed lower basal glucose level and better glucose clearance rate suggest that raptor<sup>ad−/−</sup> mice are more insulin sensitive, despite lower adiponectin plasma levels (Figure 4C).

To determine whether insulin signaling is indeed enhanced in raptor<sup>ad−/−</sup> mice, we examined the effect of insulin stimulation on Akt phosphorylation (T308 and S473) in WAT, muscle, and liver of raptor<sup>fl/fl</sup> and raptor<sup>ad−/−</sup> mice. As expected, upon insulin stimulation, Akt was phosphorylated in all tissues (Figures 4D–4F). Akt phosphorylation was similar in WAT and liver of raptor<sup>fl/fl</sup> and raptor<sup>ad−/−</sup> mice, indicating that insulin signaling...
is not enhanced in these tissues in raptor<sup>ad−/−</sup> mice. In contrast, Akt was hyperphosphorylated in muscle in raptor<sup>ad−/−</sup> mice compared to raptor<sup>fl/fl</sup> mice, indicating that insulin signaling is enhanced in muscle of raptor<sup>ad−/−</sup> mice. Thus, the lower basal glucose level and better glucose clearance rate of the knockout mice are likely due to improved insulin signaling and glucose uptake by muscle. In this context, it is important to note that muscle is the major insulin-stimulated glucose-consuming tissue.

Prolonged stimulation of mTORC1 and S6K inhibits upstream signaling by the insulin pathway (including Akt), thereby forming a negative feedback loop (Dowling et al., 2007; Harrington et al., 2004; Krebs et al., 2007; Shah et al., 2004; Tzatsos and Kandror, 2006; Ueno et al., 2005; Um et al., 2004). To investigate the effect of raptor<sup>ad−/−</sup> on this negative feedback loop, we examined Akt and S6K phosphorylation in WAT of HFD-fed raptor<sup>ad−/−</sup> and raptor<sup>fl/fl</sup> mice. HFD-fed control mice (raptor<sup>fl/fl</sup>) exhibited S6K hyperphosphorylation and Akt hypophosphorylation, indicating that an HFD activates the negative feedback loop in WAT (Figure 4G). raptor<sup>ad−/−</sup> eliminated the HFD-induced negative feedback loop, as indicated by loss of S6K hyperphosphorylation and restoration of Akt phosphorylation (Figure 4G). Thus, the enhanced insulin sensitivity of the raptor<sup>ad−/−</sup> mice on HFD could also be due to loss of negative feedback in adipose tissue. In agreement with previous data (Aguilar et al., 2007), we also observed increased AMPK phosphorylation in WAT of raptor<sup>ad−/−</sup> mice compared to raptor<sup>fl/fl</sup> mice (Figure 4G), which could also contribute to higher insulin sensitivity. Taken together, the above results suggest that raptor<sup>ad−/−</sup> mice are more insulin sensitive due to enhanced insulin signaling in muscle and, on an HFD, in WAT.

Plasma levels of cholesterol showed a similar trend as observed for glucose tolerance (Figure 4H). Levels were similar for the raptor<sup>ad−/−</sup> and raptor<sup>fl/fl</sup> mice on the chow diet and were substantially increased for raptor<sup>fl/fl</sup> mice on the HFD. However, raptor<sup>ad−/−</sup> mice maintained normal cholesterol levels even on the HFD. The raptor knockout had no effect on plasma levels of triglycerides and free fatty acids on either the chow or high-fat diet (data not shown). Taken together, the above results suggest that inactivation of mTORC1 in adipose tissue improves whole-animal metabolism, in particular when mTORC1-deficient mice are challenged by an HFD.

As described above, the raptor<sup>ad−/−</sup> mice displayed reduced adiposity. Since an alteration in fat metabolism often results in aberrant accumulation of fat in the liver (steatosis), we examined the livers of the knockout and control mice by oil red O staining (Figures 4I and 4J). On the chow diet, there was a small but significant reduction in the amount of triglycerides stored in livers of raptor<sup>ad−/−</sup> compared to raptor<sup>fl/fl</sup> mice. The HFD caused extensive and similar hepatic steatosis in raptor<sup>ad−/−</sup> and raptor<sup>fl/fl</sup> mice.

**Food Intake or Absorption, Physical Activity, Adipose-Specific Lipolysis, or Expression of Fat Genes Do Not Account for the Leanness of raptor<sup>ad−/−</sup> Mice**

What is the underlying mechanism that causes raptor<sup>ad−/−</sup> mice to be lean and resistant to diet-induced obesity? Adipose tissue is an endocrine organ that interacts with other metabolic organs to regulate behavior and whole-body metabolism. The observed leanness could, therefore, be due to the interaction of adipose tissue with other organs. To examine this possibility, we first measured feeding behavior. raptor<sup>ad−/−</sup> and raptor<sup>fl/fl</sup> mice consumed similar amounts of calories per day on both the chow and high-fat diets (Figure 5A). Similar food intake occurred despite markedly reduced plasma leptin levels in raptor<sup>ad−/−</sup> mice, suggesting enhanced leptin sensitivity (Figure 5B). The reduced leptin levels in raptor<sup>ad−/−</sup> mice are in agreement with the observed reduction in WAT mass (Figure 3C), as leptin levels correlate with WAT mass (Considine et al., 1996). Second, we investigated voluntary locomotor activity of raptor<sup>ad−/−</sup> and raptor<sup>fl/fl</sup> mice. Surprisingly, raptor<sup>ad−/−</sup> mice were less active than raptor<sup>fl/fl</sup> mice, as measured by activity on running wheels. On average, raptor<sup>ad−/−</sup> mice ran ~2000 m/day, whereas raptor<sup>fl/fl</sup> mice ran ~4500 m/day (Figure 5C). Third, we examined fecal lipid content to determine whether the observed leanness could be due to a defect in food absorption. There was no difference in the fecal lipid content of raptor<sup>ad−/−</sup> and raptor<sup>fl/fl</sup> mice (Figure 5D), indicating that raptor<sup>ad−/−</sup> mice did not have a defect in absorption of lipid from food. Thus, the leanness of raptor<sup>ad−/−</sup> mice is not due to decreased food intake, increased physical activity, or reduced lipid absorption.

An adipose maintenance defect could also be due to increased lipolysis or dedifferentiation resulting from decreased expression of adipose-specific fat storage genes. To test these possibilities, we first measured lipolysis in differentiated 3T3-L1 cells treated or untreated with rapamycin (Figure 6A) and in epididymal WAT pads of raptor<sup>ad−/−</sup> and raptor<sup>fl/fl</sup> mice (Figure 6B). In neither system did we detect a significant difference.
Finally, we determined the respiratory exchange ratio of raptor$^{ad/C0}$ and raptor$^{fl/fl}$ mice as a measure of the utilized energy source. As expected, HFD-fed mice had a lower ratio than chow-fed mice, indicating greater utilization of fat versus carbohydrates as an energy source. However, the raptor$^{ad/C0}$ and raptor$^{fl/fl}$ mice had a similar ratio (Figure 6C), indicating similar utilization of carbohydrates and fat as energy sources. These results suggest that the leanness of raptor$^{ad/C0}$ mice is not due to enhanced lipolysis.

We then analyzed the expression pattern of several well-known adipose-specific genes that mediate fat storage, in particular the genes encoding the transcription factors PPAR$\gamma$, CEBP$\alpha$, and SREBP, and the genes encoding fatty acid-binding protein aP2, fatty acid synthase (FAS), lipoprotein lipase (LPL), resistin, and hormone-sensitive lipase (HSL). In 3T3-L1 cells, inhibition of mTORC1, either by rapamycin treatment or by raptor siRNA, caused a significant decrease in all genes examined (Figure 6D), suggesting that inactivation of mTORC1 causes mature adipocytes to “dedifferentiate” due to lack of expression of the genes necessary for lipid production and storage. These results suggest that the leanness of raptor$^{ad/-}$ mice is not due to enhanced lipolysis.

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Macrophage infiltration into WAT increases with obesity (Weisberg et al., 2003) and contributes to the secretion of inflammatory cytokines that are associated with changes in insulin sensitivity and other metabolic parameters (Weisberg et al., 2006; Xu et al., 2003). To determine the extent of macrophage infiltration into the epididymal WAT of raptor$^{ad/C0}$ and raptor$^{fl/fl}$ mice, we measured the expression level of mac1, a macrophage marker, in cDNA extracts made from WAT (including any infiltrated macrophages). As expected, mac1 expression was increased in response to an HFD and the resulting obesity. However, there was little to no difference in mac1 expression between raptor$^{ad/C0}$ and raptor$^{fl/fl}$ mice on either a chow or high-fat diet (data not shown). Thus, leanness was not due to a change in metabolism resulting from a difference in macrophage infiltration.

raptor$^{ad/-}$ Mice Have Higher Energy Expenditure Due to an Increase in Uncoupled Respiration in WAT

Leanness of the raptor$^{ad/-}$ mice could be due to increased energy expenditure. To investigate energy expenditure, we measured oxygen consumption by indirect calorimetry. raptor$^{ad/-}$ mice displayed an increase in oxygen consumption as compared...
to raptor^{fl/fl} mice (Figure 7A), suggesting that the knockout mice indeed expend more energy. We then analyzed, using a Clark electrode, the oxygen consumption by isolated white adipocytes derived from raptor^{ad+/C0} and raptor^{fl/fl} mice. Adipocytes from raptor^{ad+/C0} mice displayed a significant increase in oxygen consumption compared to control adipocytes (Figure 7B). Thus, raptor^{ad+/C0} causes an increase in mitochondrial respiration in WAT.

Why is respiration increased in WAT of raptor^{ad+/C0} mice? The observed increase in oxygen consumption by isolated adipocytes, as described above, was abolished by the addition of ADP (data not shown). This effect of ADP, which stimulates coupled respiration and inhibits uncoupled respiration (Nicholls, 2001), suggested that the increase in oxygen consumption could be due to uncoupled mitochondrial respiration. To investigate this possibility further, we examined expression of mitochondrial uncoupling genes in WAT from raptor^{ad+/C0} and raptor^{fl/fl} mice. We observed a strong increase (25-fold) in the expression of the uncoupling protein 1 (UCP1) gene and, to a lesser extent, in expression of UCP2 and other brown fat markers such as type 2 deiodinase (dio2) and cidea in WAT of raptor^{ad+/C0} mice (Figure 7C). Thus, the leanness of raptor^{ad+/C0} mice appears to be due to increased energy expenditure resulting from mitochondrial uncoupling in WAT. Surprisingly, we also detected a small increase (3-fold) in UCP1 expression in skeletal muscle of raptor^{ad+/C0} mice. This increase might also contribute to the leanness of raptor^{ad+/C0} mice.

No difference in oxygen consumption was detected in brown adipocytes isolated from raptor^{ad+/C0} and raptor^{fl/fl} mice (data not shown). In agreement with this finding, the level of UCP1 expression in BAT was unaffected by raptor knockout (Figure 7D), although expression of PPARγ, as well as UCP2 and UCP3, was reduced (Figure 7D). It remains to be determined why BAT mass is reduced in raptor^{ad+/C0} mice.

DISCUSSION

Here, we describe the generation and phenotypic characterization of conditional raptor knockout mice. Knockout of raptor specifically in adipose tissue (raptor^{ad+/C0}) results in mice that are lean and resistant to diet-induced obesity. raptor^{ad+/C0} mice also exhibit an overall better metabolic profile than control
mice, including enhanced glucose tolerance and resistance to diet-induced hypercholesterolemia. Furthermore, we present evidence that the leanness of rapto<sup>ad</sup>/C0 mice is due to elevated energy expenditure as a consequence of uncoupled mitochondria in white adipose tissue. These results suggest that mTORC1 in adipose tissue plays an important role in the control of both adipose metabolism and whole-body energy homeostasis.

Similar to adipose-specific raptor knockout, whole-body knockout of S6K1, which is positively regulated by mTORC1, results in lean mice that are resistant to diet-induced obesity due to increased energy expenditure (Pende et al., 2000; Um et al., 2004). Furthermore, S6K1 knockout mice exhibit normal food intake despite lower leptin levels, smaller WAT cells, and enhanced insulin sensitivity. Conversely, knockout of 4E-BP1 and 4E-BP2, which are negatively regulated by mTORC1, results in increased obesity and hypersensitivity to diet-induced obesity due to lower energy expenditure (Le Bacquer et al., 2007). This remarkable correlation between adipose-specific knockout of mTORC1 and full-body knockout of its direct effectors S6K1 and 4E-BP1/2 suggests that at least part of the effects of the S6K1 and 4E-BP1/2 knockouts are via adipose tissue. This, in turn, provides further evidence that mTORC1 signaling in adipose tissue plays a central role in controlling whole-animal metabolism.

raptor<sup>ad</sup>/C0 mice have lower basal glucose levels, improved glucose clearance, and reduced plasma insulin levels compared to control raptor<sup>fl/fl</sup> mice, indicating that raptor<sup>ad</sup>/C0 mice have enhanced insulin sensitivity. We find that this enhanced insulin sensitivity is due, at least in part, to increased insulin signaling in muscle and, on a high-fat diet, in adipose tissue. This underscores the role of adipose and, in particular, adipose mTORC1 in controlling whole-body energy homeostasis. The increased insulin signaling in adipose tissue is due to loss of the negative feedback loop within the mTORC1 pathway. It remains to be determined how mTORC1 signaling in adipose tissue controls insulin sensitivity in other tissues.

Previous studies on the role of mTORC1 in mitochondrial function have provided seemingly conflicting results. Observed changes in respiration by full-body S6K1 or 4E-BP1/2 knockout mice suggest, in line with our results, that mTORC1 negatively controls mitochondrial respiration (or uncoupling) (Le Bacquer et al., 2007; Um et al., 2004). In contrast, muscle-specific knock-out of raptor results in decreased oxidative capacity and mitochondrial gene expression (Bentzinger et al., 2008 [this issue of Cell Metabolism]). Furthermore, rapamycin treatment or knock-down of mTOR or raptor in muscle cells or in TSC knockout MEFs decreases mitochondrial gene expression and oxygen consumption (Cunningham et al., 2007). These latter effects are via downregulation of PGC1α and the transcription factor YY1 and do not involve S6K1 or Akt (Cunningham et al., 2007; Schieke et al., 2006). Taken together, our data and the above findings suggest that mTORC1 controls mitochondrial respiration either negatively or positively depending on the mTORC1 effectors that might be found in a particular tissue.

Impaired TORC1 signaling in yeast, flies, and worms extends lifespan (Kaebelrein et al., 2005; Kapahi et al., 2004; Powers et al., 2006; Vellai et al., 2003). Inhibition of TOR signaling mimics nutrient deprivation, and the extension of lifespan by TOR inhibition might, thus, be equivalent to the extension of lifespan by dietary restriction. In this context, it is interesting to note that rapto<sup>ad</sup>/C0 mice have improved metabolic parameters that could lead to extended lifespan. Furthermore, rapto<sup>ad</sup>/C0 enhances respiration, and, at least as shown in budding yeast, impaired TORC1 signaling extends lifespan by increasing mitochondrial respiration among other effects (Bishop and Guarente, 2007; Bonawitz et al., 2007; Kaebelrein and Powers, 2007). It would be of interest to determine whether mTORC1 in mammals and, in particular, in adipose tissue controls lifespan.

Metformin is a widely prescribed antidiabetic drug that increases insulin sensitivity and lowers blood glucose and lipid levels. It functions through activation of the LKB1-AMPK pathway in liver (Shaw et al., 2005; Tzatsos and Kandror, 2006). Metformin also inactivates mTORC1 downstream of AMPK (Dowling et al., 2007; Gwinn et al., 2008; Shaw et al., 2004; Tzatsos and Kandror, 2006). Given that adipose-specific knockout of mTORC1 increases insulin sensitivity and lowers blood glucose and cholesterol levels, metformin might also exert part of its therapeutic effect via activation of AMPK and inhibition of mTORC1 in adipose tissue. It is also interesting to note that rapamycin treatment in humans and rats prevents weight gain (Rovira et al., 2008). Our results suggest that this effect might also be mediated via inhibition of mTORC1 in adipose tissue.

raptor<sup>ad</sup>/C0 mice are lean due to a reduction in the size and number of adipocytes. A previous study in 3T3-L1 cells demonstrated that rapamycin blocks adipogenesis via inhibition of the key adipogenic transcription factor PPARγ (Kim and Chen, 2004). Kim and Chen (2004) also reported that rapamycin strongly inhibits PPARγ activity in differentiated 3T3-L1 and that incubation of differentiated 3T3-L1 with rapamycin induces loss of fat. Our results in 3T3-L1 cells support these findings (Figures 1 and 6D). However, surprisingly, raptor<sup>ad</sup>/C0 mice are lean despite the absence of an effect on expression of PPARγ and PPARγ targets (Figure 6E, unpublished for PPARγ protein level). Thus, in animals, there must be a mechanism(s) in addition to mTORC1 signaling that maintains PPARγ. It remains to be determined what this mechanism might be. mTORC1-deficient mice lose fat due to increased UCP1 expression leading to enhanced energy expenditure and not due to a PPARγ deficiency. Rapamycin treatment of differentiated 3T3-L1 cells did not lead to increased UCP1 expression (data not shown), suggesting that the effect of raptor knockout on UCP1 expression might not be cell autonomous.

Close examination of the weight curves shows that raptor<sup>ad</sup>/C0 mice reached a maximal weight of ~30 g, regardless of being on a chow or high-fat diet (Figure 3A). This weight was reached at a slow, constant rate on the chow diet or very rapidly on the high-fat diet. This observed maximal weight suggests that there is a limit to the amount of fat that raptor<sup>ad</sup>/C0 mice can accumulate. This is also supported by the findings that the size of individual adipocytes did not increase upon HFD and that the number of adipocytes was reduced in raptor<sup>ad</sup>/C0 mice on both the chow and high-fat diet (Figure 3E). The reason for such a fixed limit on the amount of fat that raptor<sup>ad</sup>/C0 mice can accumulate is unknown.

In conclusion, mTORC1 in adipose tissue regulates whole-body metabolism and energy homeostasis. Inhibition of mTORC1 results in metabolically healthier mice, with no apparent adverse effects. Thus, adipose mTORC1 is a potential target for antiobesity and antidiabetes drugs.
EXPERIMENTAL PROCEDURES

Materials
Adenoviruses encoding RNAi against raptor were created by cloning the previously described RNAi sequence and H1 promoter (Jacinto et al., 2004) from pSuper into pAd-DEST (Invitrogen). Empty pAd-DEST vector was used as control. For raptor knockdown, differentiated or undifferentiated 3T3-L1 were incubated with viruses for 2 days. Antibodies for raptor were from Bethyl. Antibodies for actin were from Chemicon. Antibodies for Akt, pAkt, AMPK, and AMPK pT172 were from Cell Signaling. Rapamycin was from LC Laboratories and was used at a concentration of 100 nM. Isopropenol was from Sigma and was used at a concentration of 10 μM. Insulin, dexamethasone, and IBMX were from Sigma.

Supplemental Experimental Procedures can be found in the Supplemental Data online.

SUPPLEMENTAL DATA

The Supplemental Data for this article include Supplemental Experimental Procedures and can be found with this article at http://www.cellmetabolism.org/S1550-4131(08)00287-8.

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