Amino acid-sensing mTOR signaling is involved in modulation of lipolysis by chronic insulin treatment in adipocytes

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Zhang C, Yoon MS, Chen J. Amino acid-sensing mTOR signaling is involved in modulation of lipolysis by chronic insulin treatment in adipocytes. Am J Physiol Endocrinol Metab 296: E862–E868, 2009. First published February 3, 2009; doi:10.1152/ajpendo.90651.2008.—Chronically high insulin levels and increased circulating free fatty acids released from adipose tissue through lipolysis are two features associated with insulin resistance. The relationship between chronic insulin exposure and adipocyte lipolysis has been unclear. In the present study we found that chronic insulin exposure in 3T3-L1 adipocytes, as well as in mouse primary adipocytes, increased basal lipolysis rates. This effect of insulin on lipolysis was only observed when the mammalian target of rapamycin (mTOR) pathway was inhibited by rapamycin in the adipocytes. In addition, amino acid deprivation in adipocytes phenocopied the effect of rapamycin in permitting the stimulation of lipolysis by chronic insulin exposure. The phosphatidylinositol 3-kinase-Akt pathway does not appear to be involved in stimulation of lipolysis by chronic insulin exposure. The phosphatidylinositol 3-kinase-Akt pathway does not appear to be involved in this insulin effect. Furthermore, we found that triacylglycerol hydrolase (TGH) activity was required for the stimulation of lipolysis by combined exposure to insulin and rapamycin. Therefore, we propose that nutrient sufficiency, mediated by an mTOR pathway, suppresses TGH-dependent lipolysis stimulated by chronic insulin exposure in adipocytes.

mamalian target of rapamycin; insulin resistance; 3T3-L1 cells; nutrient sufficiency; triacylglycerol hydrolase

IN FAT CELLS of mammalian adipose tissue, lipolysis is the process of triacylglycerol breakdown to yield free fatty acids (FFA) and glycerol. While the released FFA through lipolysis provides an energy source for the body, high plasma concentrations of FFA are believed to contribute to the metabolic syndrome including obesity, insulin resistance, and type 2 diabetes (5, 42). Two features are clinically associated with insulin resistance: high circulating insulin levels caused by the decrease of insulin sensitivity and high plasma FFA concentrations resulting from increased adipocyte lipolysis (21, 32, 44). Hence, the relationship between chronic insulin exposure and lipolysis rate in adipocytes is of significant interest for the understanding of mechanisms underlying the metabolic syndrome.

To date, three lipases have been identified to be responsible for lipolysis in adipocytes: hormone-sensitive lipase (HSL) (20), adipose triacylglycerol lipase (ATGL) (46), and triacylglycerol hydrolase (TGH) (6). Lipolytic hormones, such as catecholamines, activate lipolysis through the production of cAMP and subsequent activation of protein kinase A (PKA). PKA phosphorylation of HSL and perilipin A allows the recruitment of HSL to perilipin-coated lipid droplets, leading to increased lipolysis (20, 35). While HSL appears to make greater contributions to hormone-stimulated lipolysis than basal lipolysis (24), the more recently identified lipase, ATGL, has been shown to regulate both basal and hormone-stimulated lipolysis (17). Meanwhile, the third lipase, TGH (6), has been found in 3T3-L1 adipocytes (7) as well as adipose tissue (34) and is proposed to mediate HSL-independent lipolysis in adipocytes. Most recently, TGH has been shown to contribute to basal, but not catecholamine-stimulated, lipolysis in 3T3-L1 adipocytes (43).

It is well established that acute insulin treatment inhibits hormone-stimulated lipolysis by activating a phosphodiesterase (PDE) that hydrolyzes cAMP (8, 15, 33), whereas basal lipolysis rates are not affected by similar treatments. On the other hand, the effects of chronic insulin treatment on basal or hormone-stimulated lipolysis have been controversial. Some have reported inhibition, and others have reported activation or no change, of lipolysis rates by chronic insulin treatment at various insulin concentrations (3, 4, 16, 25, 26, 45). Further investigation is warranted to dissect the relationship between chronic insulin treatment and lipolysis.

The mammalian target of rapamycin (mTOR), a large protein kinase conserved from yeast to human, assembles signaling networks that are essential for cellular processes ranging from cell growth and proliferation to survival and differentiation (9, 10, 14). Two functionally distinct protein complexes containing mTOR have been characterized, namely, mTORC1 and mTORC2, which mediate the rapamycin-sensitive and rapamycin-insensitive signaling of mTOR, respectively (29). mTORC1 transduces multiple upstream signals including mitogens, nutrient (e.g., amino acid) availability, and cellular energy levels (9, 10, 14). The two best-characterized downstream targets of mTORC1 are ribosomal S6 kinase 1 (S6K1) and eukaryotic initiation factor-4E (eIF-4E)-binding protein 1 (4E-BP1), both of which regulate translation initiation (14). Rapamycin, the specific inhibitor of mTORC1, is a potent immunosuppressant used clinically for antitumor rejection. A major side effect of rapamycin treatment in patients of organ transplantation is hyperlipidemia, characterized by elevated triacylglycerol and FFA levels in the blood (22, 23), providing the first hint that rapamycin may stimulate lipolysis. More direct evidence for mTOR involvement in lipolysis has come from studies of S6K1-knockout mice, which show increased basal lipolysis rates in the adipocytes in vitro (41). Our previous observation (18) that rapamycin treatment of fully differentiated 3T3-L1 adipocytes leads to decreased lipid droplet size is also consistent with a role of mTORC1 in lipolysis. However, a contribution of mTOR signaling to insulin regula-

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tion of lipolysis has never been examined before, and the mechanism by which rapamycin presumably enhances lipolysis is not clear. Here we report that chronic exposure to insulin leads to enhanced basal lipolysis in both differentiated 3T3-L1 cells and mouse primary adipocytes, but this only occurs in the absence of, or upon inhibition of, amino acids, suggesting that amino acid sufficiency, mediated by mTORC1 signaling, plays an important role in the modulation of lipolysis by insulin. Furthermore, pharmacological inhibition of TGH blocks this stimulation of lipolysis, implicating TGH as a potential target of the combined action of insulin and rapamycin.

MATERIALS AND METHODS

Antibodies and other reagents. Antibodies for the following proteins were all from Cell Signaling Technology: S6K1, phospho-S6K1 (Thr389), 4E-BP1, phospho-4E-BP1 (Ser65), mTOR, aP2, HSL, phospho-HSL (Ser660), phospho-Akt (Ser473), and ATGL. Anti-α-tubulin antibody was from Abcam. The following were all purchased from Sigma: BSA (fatty acid free), insulin (human recombinant), 3-isobutyl-1-methylxanthine (IBMX), dexamethasone (Dex), glycerol assay kit, and diethyl-p-nitrophenylphosphate (E600). The nonesterified fatty acids (NEFA) assay kit was purchased from Wako. 4,4,4-Trifluoro-2-[2-(3-methylphenyl)hydrazono]-1-(2-thienyl) butane-1,3-dione [TGH-specific inhibitor (TGH)] was purchased from Maybridge. Collagenase D was purchased from Roche. All cell culture media and reagents were from Invitrogen.

Cell culture. 3T3-L1 preadipocytes were maintained at 37°C in 5% CO2 in DMEM containing 4.5 g/l glucose, 4 mM glutamine, 1 mM sodium pyruvate, 1.5 g/l sodium bicarbonate, and 10% fetal bovine serum (M-1). For differentiation, the cells were grown to confluence in M-1, and then maintained in M-1 for 2 more days before switching to M-2 medium (M-1/H11001 250 nM insulin, 0.5 mM IBMX, and 1.0 μM dexamethasone). The time of switching from M-1 to M-2 was designated day 0 of differentiation. After 2 days in M-2, the medium was

Fig. 1. Rapamycin enhances basal lipolysis in the presence of insulin. A: fully differentiated 3T3-L1 adipocytes were incubated in DMEM containing 10% serum and 250 nM insulin for 23 h with or without 100 nM rapamycin. Medium was then replaced with fresh DMEM (same as above) with or without 1 μM epinephrine and with or without 100 nM rapamycin, followed by measurements of glycerol release and nonesterified fatty acid (NEFA) release in the medium. B: 3T3-L1 adipocytes were incubated in plain DMEM overnight, followed by stimulation with either 10 nM insulin or 10% serum for 24 h, with or without 100 nM rapamycin. Glycerol and NEFA release were measured subsequently. All data shown are the average of 3 independent experiments (each experiment having triplicate samples for each condition); error bars represent SD. *P < 0.05, **P < 0.01 compared with control samples (no stimulation or treatment) by Student’s t-test.

Fig. 2. Chronic insulin treatment stimulates basal lipolysis in adipocytes in the presence of rapamycin. A: 3T3-L1 adipocytes were incubated in plain DMEM overnight and then stimulated with 10 nM insulin for various lengths of time with or without rapamycin, followed by measurements of glycerol release in the medium. Data are presented as fold increase (left) and absolute glycerol concentrations (right). B and C: adipocytes treated as in A for 8 h were subjected to Oil Red O staining (B) or Western analysis for aP2 (C). D: mouse primary adipocytes were treated with 100 nM rapamycin, 10 nM insulin, or both for 8 h, followed by lipolysis assays. All data shown are the average of 3 independent experiments (each experiment having triplicate samples for each condition); error bars represent SD. *P < 0.05, **P < 0.01 compared with control samples (no stimulation or treatment) by Student’s t-test.
replaced by M-3 (M-1 + 250 nM insulin), and differentiation was allowed to proceed in M-3 until days 10–12.

**Lipolysis assays.** 3T3-L1 cells were differentiated in 10-cm plates as described above. After 10–12 days of differentiation, the adipocytes were trypsinized and seeded into 48-well plates at 1 × 10⁵ cells/well in M-3 and incubated for 24 h. The cells were then incubated at 37°C in 5% CO₂ in DMEM containing 1% BSA, for various durations and under various conditions as described in the text and Figs. 1–6. Cell media were collected for measurements of glycerol and NEFA with the Glyceral Assay Kit (Sigma) and the NEFA Assay Kit (Wako), respectively, according to the manufacturers’ manuals. Cell treatments and measurements were always done with triplicate wells. Freshly isolated mouse primary adipocytes (see below) were resuspended in DMEM containing 1% BSA at a density of 1.0 × 10⁶ cells/ml and seeded in 48-well plates at 0.2 ml of cell suspension per well. The cells were incubated at 37°C in 5% CO₂ with shaking at 120 rpm (model SSM1, Stuart) and treated as described in Figs. 1–6 before glycerol and FFA release assays.

**Isolation of mouse primary adipocytes.** Mouse primary adipocytes were isolated from epididymal fat pads of 8- to 12-wk-old male C57BL/6J mice based on a previously reported protocol (27) with some modifications. All handling of animals was performed under protocols approved by the Institutional Animal Care and Use Committee of the University of Illinois. The epididymal fat pads were removed and minced in a small amount of KRBS buffer (in mM: 120 NaCl, 4 KH₂PO₄, 1 MgSO₄, 1 CaCl₂, 10 NaHCO₃, and 30 HEPES, pH 7.4, containing 1% BSA, 2.5 mM D-glucose, and 200 mM adenosine). The minced tissues were incubated in 2 ml of KRBS buffer containing 1 mg/ml collagenase D at 37°C with shaking at 120 rpm. Once the tissue suspension appeared creamy, it was filtered with a screen mesh of 100 μm (BD Falcon). The filtered cells were centrifuged at 400 g for 30 s, and primary adipocytes at the top layer were carefully collected. The cells were washed twice with KRBS buffer and then resuspended in DMEM containing 1% BSA at the desired cell density.

**Western blotting analysis.** Cells were harvested in lysis buffer containing (in mM) 20 Tris·HCl (pH 7.5), 0.1 Na₃VO₄, 25 NaF, 25 glycerophosphate, 2 EGTA, 1 dithiothreitol, and 0.5 phenylmethylsulfonyl fluoride, with 0.3% Triton X-100. The lysates were mixed 1:1 with Laemmli sample buffer and boiled before loading on SDS-PAGE. Western blotting was carried out by following procedures recommended by suppliers of the antibodies. Detection of horseradish peroxidase-conjugated secondary antibodies was performed with Western Lightning Chemiluminescence Reagent Plus (PerkinElmer Life Sciences) and exposure on X-ray films. The results were then scanned with an Epson scanner (Perfection 2400) using Photoshop CS2.

**RESULTS**

**Chronic insulin treatment stimulates basal lipolysis in presence of rapamycin.** Previously we observed that rapamycin treatment led to a decrease in the lipid droplet size in differentiated adipocytes (18) and that it was not a result of apoptosis (J. E. Kim and J. Chen, unpublished data). To test whether this was due to an increased lipolysis rate in the rapamycin-treated adipocytes, we set out to measure glycerol and NEFA release in those cells. 3T3-L1 preadipocytes were induced to differentiate with standard protocols (27) with some modifications. Fully differentiated adipocytes were subjected to treatment with 100 nM rapamycin for 24 h. As shown in Fig. 1A, rapamycin stimulated basal lipolysis rate by approximately twofold. As a control, treatment of the adipocytes with epinephrine for 1 h stimulated lipolysis by approximately fivefold, comparable to the degree of epinephrine stimulation reported by others (e.g., Ref. 4), but rapamycin had no significant effect on epinephrine-stimulated lipolysis. These data suggest that mTORC1 signaling may be involved in basal but not hormone-stimulated lipolysis.

However, when we performed the same experiment by incubating cells in a buffer (KRBS) that is commonly used for lipolysis assays (15, 25), the effect of rapamycin completely disappeared (data not shown). Since the adipocytes in the earlier experiments were exposed to 10% fetal bovine serum and 250 nM insulin (standard differentiation conditions) during the rapamycin treatment, we wondered whether the exposure to serum or insulin might have contributed to the stimulatory effect of rapamycin on lipolysis. Thus we compared the effect
of rapamycin on basal lipolysis with and without insulin or serum. Fully differentiated 3T3-L1 adipocytes were incubated in plain DMEM medium (without any additive) for 24 h and then subjected to various treatments for another 24 h before glycerol and NEFA release were measured in the medium. The results in Fig. 1B showed that neither rapamycin nor insulin alone had any effect on basal lipolysis but the combination of insulin and rapamycin significantly increased the lipolysis rate. Serum, on the other hand, did not synergize with rapamycin treatment to stimulate lipolysis, although on its own serum modestly increased the lipolysis rate (Fig. 1B). It should be noted that 10 nM insulin was used in these experiments (Fig. 1B), which better reflects physiological insulin concentrations than those typically used for 3T3-L1 differentiation (e.g., 250 nM; Fig. 1A). We found no difference in the influence of lipolysis by 10 nM and 250 nM insulin in the experiments described here (data not shown). From this point on, all experiments were performed with 10 nM insulin.

We went on to examine the synergistic effect of insulin and rapamycin on basal lipolysis in a time course. As shown in Fig. 2A, the effect of rapamycin was observed only when the cells were treated with insulin for 8 or 24 h, but not after up to 3 h of insulin exposure. The glycerol release data in Fig. 2A are presented as both fold increase (Fig. 2A, left) and absolute glycerol concentrations (Fig. 2A, right). Conventionally, 8 h or more of insulin treatment is considered chronic exposure for adipocytes in culture (3, 45). Therefore, our data imply that chronic, but not acute, insulin treatment leads to enhanced lipolysis in the presence of rapamycin.

Since both insulin and rapamycin could impact adipocyte differentiation, we wondered whether the change in lipolysis rates observed here could be a consequence of differentiation/dedifferentiation even within the 8-h window. As shown in Fig. 2B, neither insulin nor rapamycin affected the degree of lipid droplet formation visibly; the combined treatment by insulin and rapamycin for 8 h slightly decreased the amount of lipid droplets, consistent with increased lipolysis. The expression of the differentiation marker aP2 was not affected by any of these treatments (Fig. 2C), implying that the observed changes in lipolysis were not due to altered differentiation states.

To further confirm the physiological relevance of the lipolysis regulation, we extended the assay to mouse primary adipocytes. As shown in Fig. 2D, neither rapamycin nor insulin alone had any effect on lipolysis, but chronic insulin treatment in the presence of rapamycin significantly stimulated basal lipolysis in the primary cells. Together, our findings strongly suggest that chronic insulin exposure leads to increased lipolysis in adipocytes when mTORC1 signaling is inhibited.

Amino acid deprivation potentiates effect of insulin on basal lipolysis. It is well established that the mTOR pathway senses nutrient availability, especially amino acid sufficiency, to regulate cellular functions. As the logical next question, we asked whether the availability of amino acids plays any role in the effect of chronic insulin exposure on basal lipolysis. We measured glycerol release (Fig. 3A) and NEFA release (Fig. 3B) in differentiated 3T3-L1 adipocytes incubated in medium free of amino acids. Interestingly, in the absence of amino acids, insulin alone significantly stimulated basal lipolysis, to a similar extent as combined insulin and rapamycin treatment in the presence of amino acids (Fig. 3, A and B). Eight hours of amino acid treatment or deprivation did not have any detectable effect on adipocyte differentiation, as demonstrated by the unchanged levels of aP2 (Fig. 3C).

mTOR downstream signaling was also examined under the conditions described above. As expected, insulin treatment enhanced the phosphorylation of both mTORC1 targets, 4E-BP1 (at Ser65) and S6K1 (at Thr389), and both were inhibited by rapamycin treatment, although 4E-BP1 was less sensitive to rapamycin than S6K1 (Fig. 4). Amino acid deprivation also led to dephosphorylation of S6K1 and 4E-BP1, and adding back
amino acids restored the phosphorylation of both proteins (Fig. 4). Thus the activity and regulation of mTORC1 signaling in 3T3-L1 adipocytes appeared normal. Together, our observations suggest that amino acid deficiency, via inhibition of mTOR signaling, allows insulin stimulation of basal lipolysis.

Because the phosphatidylinositol 3-kinase (PI3K)-Akt pathway is one of the major mediators of insulin receptor signaling and it can be connected to mTOR at multiple levels, we asked whether PI3K signaling might be involved in the regulation of lipolysis by chronic insulin and rapamycin treatment. Wortmannin, a specific inhibitor of PI3K, was employed to probe the functional requirement of PI3K. As shown in Fig. 5A, wortmannin treatment had no effect on basal lipolysis and did not perturb the upregulation of lipolysis in the presence of insulin and rapamycin, while insulin-stimulated Akt and S6K1 phosphorylation at Ser473 and Thr389, respectively, was completely blocked by wortmannin (Fig. 5B). Hence, the PI3K-Akt pathway does not appear to be required for the regulation of lipolysis by chronic insulin and rapamycin cotreatment. As expected, rapamycin abolished S6K1 phosphorylation but had no effect on Akt phosphorylation (Fig. 5B).

Inhibition of TGH activity blocks stimulation of basal lipolysis by insulin. Because adipogenic differentiation appeared unaffected by chronic insulin and rapamycin cotreatment (see Fig. 2C), we hypothesized that the signaling pathways described above might directly regulate the enzyme(s) involved in lipolysis. Three lipases are known to modulate lipolysis in adipocytes: HSL, ATGL, and TGH. We first examined the protein expression levels of HSL and ATGL. A functional TGH antibody was not available. As shown in Fig. 6A, neither HSL nor ATGL was affected at its protein level by cotreatment with insulin and rapamycin. The phosphorylation of HSL at Ser660, a PKA site known to be associated with HSL activity (1), was also unaffected by chronic insulin and rapamycin treatments. Hence, at the present time no evidence implicates either HSL or ATGL in the chronic insulin effect on lipolysis. However, to definitively exclude their involvement approaches such as RNA interference (RNAi) would be necessary, because specific inhibitors for these lipases are not available.

Since TGH has been reported to contribute to basal, but not hormone-stimulated, lipolysis in 3T3-L1 adipocytes (43), we considered TGH a strong candidate regulator in the process under present investigation. To examine a possible involvement of TGH, we took advantage of a TGH-specific inhibitor (TGHi), which was reported to inhibit the lipase activity of TGH both in vitro and in vivo (11). As shown in Fig. 6B, TGHi modestly inhibited basal lipolysis in control cells, but it completely blocked the insulin-stimulated lipolysis in the presence of rapamycin. The broad-spectrum lipase inhibitor E600, previously reported also to inhibit TGH (11), abolished lipolysis in both control and insulin-stimulated/rapamycin-inhibited cells (Fig. 6B). Hence, we propose that TGH is a potential target of two converging signaling pathways in the regulation of lipolysis by nutrients and chronic insulin treatment (Fig. 7; further discussed in DISCUSSION).

**DISCUSSION**

Our studies have revealed that prolonged exposure of mouse adipocytes to insulin results in increased basal lipolysis and that this process requires suppression of nutrient- and energysensing mTOR signaling and is dependent on TGH activity. The adipose tissue is now recognized as an important metabolic and endocrine organ (31), and its dysfunction plays prominent roles in the development of insulin resistance (13, 31) featured in hyperinsulinemia and increased circulating FFA concentrations. Our in vitro findings here are mechanistically consistent with the notion that chronically high insulin levels can potentially enhance the release of FFA from adipocytes in vivo, thus forming a vicious cycle in insulin resistance and type 2 diabetes.

The effect of insulin on basal lipolysis (as opposed to hormone-stimulated lipolysis) has been a controversial issue in the literature (3, 4, 16, 25, 26, 45). In light of our finding that
nutrient status and mTOR signaling modulate the response of adipocytes to insulin, it is reasonable to speculate that lipolysis may have been measured in adipocytes exposed to different medium or buffer conditions used by different groups, resulting in the discrepancy in the outcomes.

It was somewhat unexpected that the effect of chronic insulin exposure on basal lipolysis would be suppressed by normally active mTOR signaling, or sufficient nutrient (amino acids) levels. At first glance, this appears paradoxical to the well-recognized contribution of mTOR signaling to insulin resistance. Several conditions known to activate mTOR have been shown to lead to insulin resistance, including excessive amino acids (37–39), hyperinsulinemia (40), acute and chronic insulin stimulation (2, 36), deletion of TSC1/2 (12, 30), and inflammatory cytokine (interleukin-6) signaling (19); rapamycin restores insulin sensitivity in all cases. To reconcile our present observations with previous findings, we propose a dual role of mTOR in insulin resistance. As a nutrient and cellular energy sensor, mTOR signaling is critically involved in the homeostasis of various cellular processes. Hyperactive mTOR signaling, under the conditions described above including excess nutrients, directly contributes to insulin resistance. But normal mTOR signaling is necessary to safeguard cellular functions under pathological conditions such as chronic insulin exposure. A potential implication of this proposed model is that normal nutrient levels may be important for preventing some of the detrimental effects of chronically high insulin levels in insulin resistance and type 2 diabetes. Obviously, these cellular studies need to be extended to animal and human studies in future investigations.

Previously we observed a reduction in lipid droplet size induced by chronic rapamycin treatment of differentiated 3T3-L1 cells in differentiation medium that contained insulin (18). This lipid droplet size decrease can now be explained by the increased hormone-independent lipolysis stimulated by chronic cotreatment with insulin and rapamycin, most likely through the activity of the hormone-insensitive lipase TGH. Exactly which mTOR pathway is responsible for the modulation of the chronic insulin effect remains to be determined. The two best-characterized mTORC1 targets, S6K1 and 4E-BP1, are both regulators of protein synthesis, although S6K1 has additional functions. Cycloheximide, a general protein synthesis inhibitor, did not affect insulin-stimulated lipolysis in the presence of rapamycin (data not shown), suggesting that the insulin response, although requiring chronic treatment, does not involve new protein synthesis. The PI3K-Akt pathway, commonly found downstream of insulin receptor signaling, does not seem to be involved here either, because we found that the PI3K inhibitor wortmannin had no impact on lipolysis stimulated by chronic insulin and rapamycin treatment (Fig. 5).

While the underlying mechanism for the unusual relationship between chronic insulin treatment and mTOR inhibition remains an intriguing puzzle, it is reasonable to speculate that the ultimate target of this complex signaling interplay may be a lipase. Of the three lipases known to be involved in lipolysis in adipocytes, both ATGL and TGH are believed to contribute significantly to basal lipolysis (17, 43), while HSL makes a greater contribution to hormone-stimulated lipolysis (24). Our data (Fig. 6B) suggest that TGH may indeed be a player in the basal lipolysis that is regulated by nutrient signaling and chronic insulin exposure. The involvement of the other two lipases, especially ATGL, cannot be excluded at the present time; control of the lipase activity could occur by protein translocation rather than at the expression level, as exemplified by the regulation of HSL (35). Nevertheless, our finding highlights and extends the previously reported important role of TGH in modulating basal lipolysis in adipocytes (43) and reveals TGH as a potential target of insulin and nutrient regulation. Numerous possibilities exist for the molecular interaction between a nutrient-sensing mTOR pathway and a chronic insulin stimulatory pathway. The simplest model involves mTOR and insulin signaling converging on the regulation of TGH; either the two pathways work in parallel, or mTOR suppresses insulin signaling (Fig. 7). In either case, mTOR relays the status of nutrient availability to modulate insulin actions. Future investigations guided by this working model will likely provide important molecular insights into the regulation of lipid metabolism and its relationship with insulin resistance.

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