Endocrine, *vol. 29, no. 1, 73–80, February 2006* 0969–711X/06/29:73–80/$30.00
ENDO (Online) ISSN 1559-0100

Insulin Resistance and Improvements in Signal Transduction

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Type 2 diabetes and obesity are common metabolic disorders characterized by resistance to the actions of insulin to stimulate skeletal muscle glucose disposal. Insulin-resistant muscle has defects at several steps of the insulin-signaling pathway, including decreases in insulin-stimulated insulin receptor and insulin receptor substrate-1 tyrosine phosphorylation, and phosphatidylinositol 3-kinase (PI 3-kinase) activation. One approach to increase muscle glucose disposal is to reverse/improve these insulin-signaling defects. Weight loss and thiazolidinediones (TZDs) improve glucose disposal, in part, by increasing insulin-stimulated insulin receptor and IRS-1 tyrosine phosphorylation and PI 3-kinase activity. In contrast, physical training and metformin improve whole-body glucose disposal but have minimal effects on proximal insulin-signaling steps. A novel approach to reverse insulin resistance involves inhibition of the stress-activated protein kinase Jun N-terminal kinase (JNK) and the protein tyrosine phosphatases (PTPs). A different strategy to increase muscle glucose disposal is by stimulating insulin-independent glucose transport. AMP-activated protein kinase (AMPK) is an enzyme that works as a fuel gauge and becomes activated in situations of energy consumption, such as muscle contraction. Several studies have shown that pharmacologic activation of AMPK increases glucose transport in muscle, independent of the actions of insulin. AMPK activation is also involved in the mechanism of action of metformin and adiponectin. Moreover, in the hypothalamus, AMPK regulates appetite and body weight. The effect of AMPK to stimulate muscle glucose disposal and to control appetite makes it an important pharmacologic target for the treatment of type 2 diabetes and obesity.

Key Words: Exercise; glucose transport; type 2 diabetes mellitus; obesity; JNK; PTP1B; AMPK.

Insulin Resistance

Skeletal muscle is the main site responsible for insulin-stimulated glucose disposal in the body *(1)*. The effect of insulin to promote disposal of circulating glucose into skeletal muscle depends on the translocation of the GLUT4 glucose transporter from an intracellular compartment to the cell surface. Muscle insulin resistance refers to a state of decreased responsiveness to circulating concentrations of insulin to stimulate muscle glucose transport. Insulin resistance is one of the earliest and more consistent findings in people with type 2 diabetes and obesity. Insulin action is initiated through the binding of the hormone to the extracellular α subunits of the insulin receptor. This leads to a conformational change and subsequent activation of the intracellular tyrosine kinase domain of the insulin receptor β subunit. The receptor then undergoes a series of transphosphorylation reactions that lead to tyrosine phosphorylation of different substrates, including insulin receptor substrate-1 (IRS-1). When tyrosine residues are phosphorylated, they serve as docking and activating sites for phosphatidylinositol 3-kinase (PI 3-kinase). Insulin-stimulated activation of PI 3-kinase leads to GLUT4 translocation and increased glucose transport. The precise mechanism by which activation of PI 3-kinase causes GLUT4 translocation is still unknown, although activation of phosphoinositide-dependent kinase 1 (PDK1) *(2,3)*, Akt *(4,5)*, protein kinase C (PKC) λ/ζ *(6,7)*, and the RabGAP protein AS160 *(8)* have been implicated. A detailed description of the insulin transduction pathway can be found elsewhere *(9)*.

In obesity *(10)* and type 2 diabetes *(11,12)* there is reduced insulin-stimulated muscle glucose disposal, and studies using nuclear magnetic resonance have shown that glucose transport is the main step at which insulin action fails *(13)*. GLUT4 protein content is normal in muscle from insulin-resistant people; however, insulin-stimulated GLUT4 translocation is impaired *(14)*. At the cellular level, insulin resistance involves decreases in insulin receptor tyrosine kinase activity, IRS-1–associated tyrosine phosphorylation, and insulin-stimulated PI 3-kinase activation *(15–19)*. Other defects reported in muscle from obese and type 2 diabetic muscle include decreases in insulin-stimulated PKC λ/ζ *(7)* and glycogen synthase *(20–23)* activities, and in AS160 phosphorylation *(24)*. The underlying cause of these insulin-signaling defects is not clear. Recent studies have demonstrated that excessive intramyocellular accumulation of

Received October 20, 2005; Accepted October 20, 2005.

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lipid metabolites, such as fatty acyl-CoA, ceramides, and diacylglycerol *(25,26)*, activate kinases that in turn serine phosphorylate IRS-1, resulting in decreased activation of PI-3 kinase *(27–29)*.

Improvements in Insulin Signal Transduction

In view of the critical role that insulin resistance plays in the pathogenesis of obesity, type 2 diabetes, and other metabolic disorders, strategies aimed at restoring insulin-signaling defects is a priority in diabetes prevention and management. Indeed, pharmacologic and non-pharmacologic interventions have been shown to improve insulin-stimulated glucose disposal in humans. For example, lifestyle interventions such as exercise and weight reduction, as well as the antidiabetic drugs metformin and thiazolidinediones (TZDs), have been shown to increase glucose disposal. While all these interventions increase peripheral glucose disposal, there is significant variation regarding the effect that they have on insulin signaling.

Acute Exercise and Physical Training

The effects of exercise on insulin sensitivity and signaling are complex. These effects can be divided in three. One involves the acute insulin-independent increase in muscle glucose transport that occurs during a single bout of exercise; second, is the enhancement in insulin sensitivity during the immediate postexercise period; and third, refers to numerous adaptations that take place in the muscle after physical training. Certainly, in real life, these effects overlap and they all contribute to the improvement in glycemic control attributed to exercise. Acute exercise results in a decline in plasma insulin levels and does not increase insulin receptor or IRS-1 tyrosine phosphorylation nor enhance PI-3 kinase activity *(30–32)*. Moreover, blockade of the insulin signaling cascade does not affect exercise-stimulated muscle glucose transport *(33–36)*. These observations clearly establish that acute exercise increases muscle glucose transport through an insulin-independent pathway.

It is well established that skeletal muscle insulin sensitivity is enhanced after exercise *(11,37,38)* and affects insulin-sensitive processes such as glucose transport *(31,39, 40)* and glycogen synthesis *(40,41)*. However, the underlying mechanism mediating the increase in muscle insulin sensitivity after exercise is unclear. While acute exercise does not activate proximal insulin-signaling events, in the period immediately after exercise, insulin causes a marked increase in phosphotyrosine-associated PI 3-kinase activity, as compared with the effect of insulin alone *(36,42)*. Yet, in the period after contraction or exercise, insulin-stimulated IRS-1-associated PI 3-kinase activity actually decreases *(30, 41)* or remains unchanged *(36)*. This suggests that there is another insulin-stimulated tyrosine phosphoprotein that binds and activates PI 3-kinase after exercise. We found that prior exercise in mice increased insulin-stimulated IRS-2-associated PI 3 kinase activity more than insulin alone, and that genetic ablation of IRS-2 attenuated insulin-stimulated phosphotyrosine-associated PI 3-kinase activity *(43)*. This suggests that IRS-2 can partially account for the increase in phosphotyrosine-associated PI 3-kinase activity after exercise. Whether exercise affects the ability of insulin to alter an event distal to PI 3-kinase or a PI 3-kinase-independent signaling mechanism remains to be determined.

There is substantial evidence that exercise training improves insulin-stimulated glucose disposal in people with insulin-resistance *(44)* and type 2 diabetes *(45)*. Whether this is due in part or entirely to reversal of impairments in insulin signaling is unclear. In healthy, insulin-sensitive people, insulin-stimulated PI 3-kinase activity increased after exercise training *(38)*. However, while short-term training in insulin-resistant people *(44)* and long-term training in people with type 2 diabetes *(45)* improved insulin-stimulated glucose disposal, these training programs had no effect on IRS-1 tyrosine phosphorylation, or the activities of PI 3-kinase and Akt. These results indicate that training does not reverse abnormalities in proximal insulin signaling. Training, however, causes several adaptations in muscle, including increases in GLUT4 protein expression *(37,46)* and in the activity of glycogen synthase *(47)* and hexokinase *(48)*. These training-induced adaptations likely contribute to the effect of training to increase insulin-stimulated peripheral glucose disposal. Still, more research is required to fully elucidate how training improves insulin sensitivity in obesity and type 2 diabetes.

Weight Reduction

As mentioned above, obesity is characterized by several insulin-signaling abnormalities including decreased insulin receptor tyrosine kinase activity, IRS-1-associated tyrosine phosphorylation and PI 3-kinase activation *(15,16,18)*. Several studies have shown that weight reduction improves peripheral insulin sensitivity in obese people *(49–51)*. While acute exercise and training do not improve proximal events, weight loss appears to have a more pronounced effect to reverse abnormalities in insulin signaling. Kim et al. found that a very low calorie diet in obese insulin-resistant people with a mean body mass index (BMI) of 34 kg/m^2, which resulted in a mean weight loss of approx 9 kg, significantly improved insulin-stimulated glucose disposal by 24% measured during an insulin clamp study *(7)*. This improvement in insulin sensitivity was accompanied by increases in insulin-stimulated IRS-1 tyrosine phosphorylation, IRS-1 associated PI-3 kinase activity, and PKC λ/ζ activity *(7)*. Pender et al. examined the effect of major weight loss in severely obese people with a mean BMI of 47 kg/m^2, brought about by bariatric surgery *(52)*. One year after surgery, these people lost 38 kg (35% decrease in body weight), leading to a fivefold increase in insulin sensitivity measured using the minimal model. Weight loss also increased insulin receptor con-

tent and insulin receptor tyrosine phosphorylation in skeletal muscle. Collectively, these results suggest that weight loss has a robust effect to improve insulin sensitivity and signaling, although future studies will be necessary to corroborate these recent findings.

Effects of Metformin on Insulin Signaling

Metformin is the most commonly prescribed oral antidiabetic drug in the US. Despite years of research, the effects of metformin on glucose transport in skeletal muscle remain controversial. Metformin increases glucose transport in myotubes *(53)*, muscles from streptozotocin-treated rodents *(54, 55)*, and muscle strips from diabetic people *(56)*. The increase in muscle glucose transport caused by metformin has been associated with translocation of glucose transporters to the plasma membrane in L6 cells *(57)* and adipocytes *(58)*. There is also some evidence that metformin might enhance insulin receptor signaling. For example, in erythrocytes *(59, 60)*, monocytes *(61)*, fat cells *(62)*, and *Xenopus* oocytes *(63)*, metformin increased insulin receptor binding and tyrosine kinase activity. However, these cells are not the main target of metformin's action. A study done in people with type 2 diabetes found that treatment with maximal doses of metformin for 3–4 mo increased insulin-stimulated whole-body glucose disposal but did not increase IRS-1-associated PI 3-kinase or Akt activity in muscle *(64)*. Thus, at least in muscle from type 2 diabetic people, it is unlikely that metformin upregulates proximal steps in insulin receptor signaling.

Thiazolidinediones

Thiazolidinediones (TZDs), another widely used class of drugs for the treatment of type 2 diabetes, decrease blood glucose concentrations mainly by increasing insulin-stimulated muscle glucose transport *(65)*. TZDs are ligands for the peroxisome proliferator activated nuclear receptor γ (PPARγ), which is mainly expressed in adipose tissue *(66)*, although lesser PPARγ expression is found in liver and muscle *(67–69)*. Evidence indicates that the metabolic effects of TZDs are the result of enhanced fat cell sensitivity to insulin, leading to inhibition of the accelerated rates of lipolysis and a reduction in plasma free fatty acid concentrations *(70,71)*. TZDs also inhibit the release of adipokines from adipocytes including tumor necrosis factor α (TNFα), interleukin-6, and resistin, which promote muscle insulin resistance *(65)*. Another major effect of TZDs is to stimulate the secretion of the insulin-sensitizing hormone adiponectin from fat cells *(65)*. In contrast to metformin, most studies have shown that increases in peripheral insulin sensitivity caused by TZDs are accompanied by improvements in insulin signaling. Administration of troglitazone to people with type 2 diabetes for 3–4 mo lead to a significant increase in insulin-stimulated IRS-1-associated PI-3 kinase and Akt activities in muscle *(64)*. Rosiglitazone treatment also improved insulin-stimulated IRS-1 tyrosine phosphorylation *(72,73)* and p85 association with IRS-1 *(72)* in muscle from type 2 diabetic people. In contrast to these findings, Karlsson et al. reported that rosiglitazone treatment improved insulin-stimulated glucose disposal in muscle from people with recently diagnosed type 2 diabetes, but did not improve proximal insulin signaling events *(74)*. The longer duration, and possibly more severe, diabetes present in the people from the studies showing a positive effect of TZDs on insulin signaling *(64,72, 73)*, compared with the people with milder diabetes *(74)*, may help explain these discrepant findings. This also suggests that under certain experimental conditions, the effect of TZDs on peripheral glucose disposal and on proximal insulin signaling can be dissociated, and that other signaling mechanisms may contribute to the insulin sensitizing effect of TZDs.

Jun N-Terminal Kinase (JNK) and Insulin Resistance

Jun N-terminal kinase (JNK) belongs to the family of stress-activated protein kinases that also includes the p38 protein kinases. Jun N-terminal kinase (JNK) regulates the transcription factor AP-1, which is implicated in the controlled expression of many genes involved in inflammatory and immune responses. JNK is activated by inflammatory cytokines and free fatty acids, molecules that have been implicated in the development of type 2 diabetes. Three highly related but distinct gene products, JNK1, JNK2, and JNK3, are expressed *(75)*. Aguirre et al. found that IRS-1 Ser^{307} phosphorylation by JNK mediates the inhibitory effect of TNFα on insulin signaling *(76)* (Fig. 1). This finding suggests that JNK inhibition could improve insulin resistance. Indeed, Hirosumi et al. showed that JNK activity is abnormally elevated in obesity, and that genetic ablation of JNK1 in mice results in decreased adiposity, improved insulin sensitivity, and enhanced insulin receptor signaling *(77)*. Consistent with these findings, administration of a pharmacologic JNK inhibitor to *db/db* mice significantly improved glucose levels *(78)*, further supporting the notion that JNK inhibition could prove useful in treating insulin resistance and diabetes.

Protein Tyrosine Phosphatases

The mechanism that leads to insulin signal termination is not well understood. One hypothesis involves dephosphorylation of key tyrosine residues by protein tyrosine phosphatases (PTPs). The level of receptor activation is determined by the opposing actions of receptor phosphorylation, and dephosphorylation by PTPs. Several PTPs have been studied, including LAR, SHP2, CD45, PTPα, and PTPε. However, the most consistent results regarding a role in insulin-sensitivity regulation involve the tyrosine phosphatase PTP1B. PTP1B overexpression inhibits insulin signaling in cultured cells *(79–82)* and mice *(83)* (Fig. 1). PTP1B-

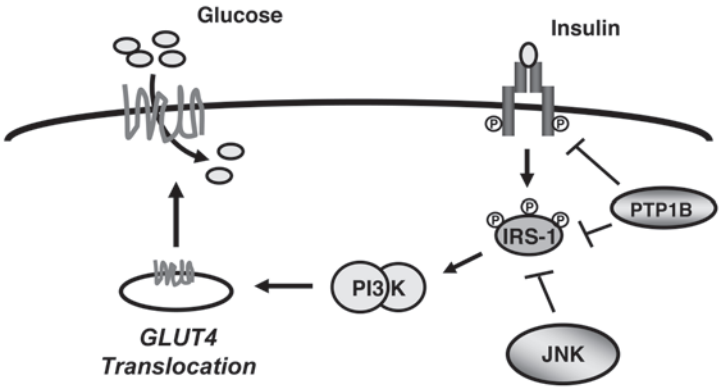

Fig. 1. Role of JNK and PTP1B in insulin resistance. The insulin-resistant skeletal muscle is characterized by numerous insulin-signaling abnormalities, including decreased insulin-stimulated insulin receptor tyrosine kinase activity, IRS-1 tyrosine phosphorylation, and insulin-stimulated PI 3-kinase activity. The protein tyrosine phosphatase PTP1B impairs insulin signaling by dephosporylating the insulin receptor and IRS-1 at tyrosine residues, while the stress-activated protein kinase JNK, inhibits signaling by serine phosphorylating IRS-1 at Ser^{307}. Inhibitors of PTP1B and JNK are therefore attractive targets for the treatment of insulin-resistant disorders, such as obesity and type 2 diabetes.

deficient mice have enhanced insulin-stimulated whole body glucose disposal *(84)*, in association with improved insulin signaling in muscle and liver *(85)*. PTP1B-deficient mice also have reduced adiposity and are protected from diet-induced obesity *(84,85)*. Moreover, antisense inhibition of PTP1B enhances insulin signaling *(86)* and improves insulin sensitivity *(87)* in insulin-resistant mice. In conclusion, there is compelling evidence that PTP1B negatively regulates insulin signaling and sensitivity, and that PTP1B inhibition is an attractive target for the pharmacologic treatment of insulin resistance.

AMP-Activated Protein Kinase (AMPK)

Most of the research aimed at increasing glucose disposal in skeletal muscle has focused on trying to reverse the defects in insulin signaling. Another appealing approach would be to stimulate glucose disposal through an insulin-independent pathway, thus bypassing these defects in insulin signaling. As mentioned above, there is significant evidence that exercise stimulates muscle glucose transport through an insulin-independent mechanism. Unraveling the molecular mechanisms by which exercise functions could lead to the development of new drugs that would have an exercise-like effect to improve blood glucose concentrations. Investigations performed by several groups have suggested that AMPK may be an important mediator of contraction-stimulated glucose transport. AMPK is a heterotrimeric protein formed by an α subunit, which contains the catalytic activity, and by the β and γ regulatory subunits important to maintain stability of the heterotrimer complex *(88,89)*. AMPK is an energy-sensing enzyme that functions as a fuel gauge, and responds to changes in cellular energy stores *(88,89)*. When AMPK senses a decrease in high-energy phosphate levels, it switches off ATP-consuming pathways and switches on pathways for ATP synthesis. AMPK activity increases during conditions of ATP such as muscle contraction, hypoxia, and ischemia, and by inhibitors of glycolysis and uncouplers of oxidative phosphorylation *(90–92)*. AMPK is activated by increases in the AMP/ATP ratio, through mechanisms involving phosphorylation by one or more upstream kinases (AMPKKs) and by allosteric modification *(88,89)*. There are two isoforms of the α subunit *(88,89)*. AMPK α1 is widely expressed, whereas the α2 subunit isoform is mainly found in the heart, skeletal muscle, and liver *(93)*. The main phosphorylation site responsible for activation of AMPK is Thr^{172} within the catalytic domain of the α subunit and phosphorylation of this site is required for activation of the enzyme. The upstream kinase(s) responsible for AMPK activation have not been well characterized. Recently, it was discovered that the serine/threonine kinase LKB1 mediates the phosphorylation of AMPK *(94–96)*. LKB1 is a tumor suppressor, and LKB1 mutations are the cause of the Peutz–Jeghers syndrome, a dominantly inherited disease characterized by predisposition to hamartomatous intestinal polyposis, mucocutaneous pigmentation, and tumor formation. LKB1, complexed with two other subunits, STRAD α/β and MO25α/β, phosphorylates AMPK at site Thr172 of the α subunit *(94)*. Current investigations are aimed at establishing the role of LKB1 as regulator of the AMPK cascade in vivo.

Exercise rapidly increases the AMP/ATP and Cr/PCr ratios in skeletal muscle, causing a robust increase in AMPK activity (reviewed in ref. *97*). This increase in AMPK activity directly correlates with contraction-stimulated muscle glucose transport *(90,98)*. Similar to the effects of contraction, chemical activation of AMPK with the compound 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) increases glucose transport via an insulin-independent mechanism *(99,100)*. This suggests that AMPK may play an important role in mediating the effects of contraction on muscle glucose transport.

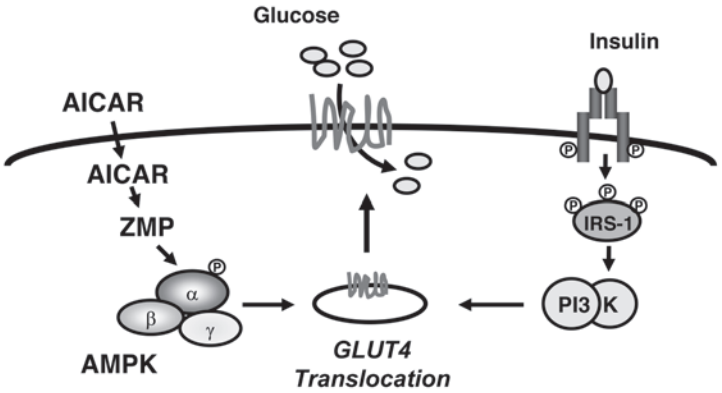

Fig. 2. Pathways involved in glucose transport in muscle. Most research aimed at increasing glucose transport in skeletal muscle has focused on trying to reverse the defects in insulin signaling. Another approach is to stimulate muscle glucose transport through an insulinin-dependent pathway, thus bypassing these defects in insulin signaling. Several studies have shown that pharmacologic activation of AMPK with AICAR effectively increases glucose transport in muscle, independent of the actions of insulin. After entering the cell, AICAR is converted to its monophosphorylated derivative ZMP, which activates AMPK by mimicking the effects of AMP on the enzyme. These findings highlight the importance of AMPK as a target for type 2 diabetes treatment.

The generation of knock out and transgenic mouse models has helped clarify the role of AMPK on muscle glucose transport. Insulin-stimulated glucose transport is normal in muscle from mice with genetic deletion of AMPK α1, AMPK α2 *(101)*. In contrast, inactivation (by overexpressing an inactive mutant) or deletion of AMPK α2, fully blocks the stimulation of muscle glucose transport caused by AICAR *(92,102,103)*, hypoxia *(102,103)*, and rotenone, a complex 1 inhibitor *(92)*. However, inactivation/deletion of AMPK α2 causes a small *(102)* or no *(92,103)* decrease in contraction-stimulated glucose uptake. This indicates that AMPK α2 is indispensable for AICAR and hypoxia to stimulate glucose transport, but it is not required for normal contraction-stimulated glucose transport. At any rate, these findings have established that AMPK pathway stimulation is an efficient method to increase insulin-independent glucose transport in muscle (Fig. 2).

Effect of Metformin and Adiponectin on AMPK

It is widely accepted that metformin lowers blood glucose concentrations mainly by suppressing hepatic glucose production *(104)*, but some studies suggest that increases in glucose disposal contribute to a lesser extent *(64,104,105)*. In view of the observation that metformin and the AMPK activator AICAR inhibit hepatic glucose production and stimulate muscle glucose transport, we hypothesized that AMPK mediates the metabolic effects of metformin *(106)*. Metformin increased AMPK activity in rat hepatocytes. Moreover, the inhibitory effect of metformin on hepatic glucose production was partially blocked using an AMPK inhibitor, indicating that metformin inhibits hepatic glucose production through an AMPK-dependent mechanism. We also examined the effect of metformin in people with type 2 diabetes. Metformin treatment for ten weeks caused a significant increase in muscle AMPK α2 activity and in peripheral glucose disposal *(107)*. These findings suggest that metformin lowers blood glucose concentrations, at least in part, by activating AMPK.

Adiponectin is an adipokine secreted by the adipose tissue. This protein has the dual effects of increasing fatty acid oxidation and improving insulin sensitivity in skeletal muscle and liver *(108,109)*. Studies have shown an inverse relationship between circulating concentrations of adiponectin in blood and the severity of insulin resistance *(110–112)*. Therefore, understanding the mechanisms of adiponectin action could lead to novel ways to treat insulin resistance. Adiponectin increases AMPK activity in muscle *(109,113)*, liver *(109)*, and fat *(114)*. Consistent with the activation of AMPK by adiponectin, this adipokine stimulates glucose transport in skeletal muscle and fat cells, and increases fatty acid oxidation in muscle and liver. Furthermore, the metabolic effects of adiponectin are reversed by blocking AMPK activity with a dominant negative mutant *(109)* and a chemical inhibitor *(114)*. The findings from these studies strongly suggested that AMPK is an important cellular mediator of the metabolic effects of adiponectin.

Appetite Regulation by AMPK

Therapies aimed at reducing obesity will likely cause a decrease in the incidence of type 2 diabetes. Recent studies done in rodents have shown that AMPK responds to nutrient and hormonal signals in the hypothalamus and that the changes in food intake and body weight caused by these signals are mediated in part by AMPK *(115–117)*. Anorexigenic stimuli such as leptin *(115)*, melanocortin receptor agonists *(116)*, and fatty acid synthase inhibitors *(117)*, decrease AMPK activity in the hypothalamus. Accordingly, oroxigenic stimuli including ghrelin *(115)* and agouti-related protein *(116)*, increase AMPK activity. Stimulation of AMPK signaling in the hypothalamus either by AICAR *(115)*

or overexpression of constitutively active AMPK *(116)* enhances food intake and body weight, whereas blocking AMPK signaling in the hypothalamus using dominant-negative AMPK *(116)* or with a chemical inhibitor *(117)*, decreases food intake and body weight. These important findings clearly show that AMPK plays an important role in regulating appetite and body weight, and that blocking AMPK activity in the hypothalamus might be used for obesity treatment.

Summary

The muscle from obese and type 2 diabetic people is characterized by having defects at several steps of the insulin-signaling pathway, including decreases in insulin receptor and IRS-1 tyrosine phosphorylation, and phosphatidylinositol PI 3-kinase activity. One approach to increase muscle glucose disposal is to reverse defects in insulin signaling. Weight loss and thiazolidinediones (TZDs) are examples of interventions known to enhance glucose disposal by improving defects in insulin signaling. A novel approach to reverse insulin resistance involves blocking JNK, which serine phosphorylates IRS-1, and PTPs, which dephosphorylate tyrosine residues of key insulin-signaling molecules. A different strategy to increase muscle glucose disposal is by stimulating insulin-independent glucose transport. AMPK is an attractive pharmacologic target because its stimulation increases insulin-independent glucose transport in muscle and decreases hepatic glucose production, leading to improved blood glucose concentrations. Paradoxically, activation of AMPK in the hypothalamus increases appetite and body weight. Thus, the design of tissue-specific AMPK activators and inhibitors will be required in order to increase glucose disposal without increasing appetite, and to inhibit appetite without blocking muscle glucose transport and increasing hepatic glucose production.

Acknowledgments

This work was supported by grants AR42238 and AR45670 from the National Institutes of Health (L.J.G.), an American Diabetes Association Junior Faculty Award (N.M.), an Executive Research Committee Research Award from the University of Texas Health Science Center at San Antonio (N.M.), and a grant from the South Texas Health Research Center (N.M.).

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