Acute Inhibition of Fatty Acid Import Inhibits GLUT4 Transcription in Adipose Tissue, but Not Skeletal or Cardiac Muscle Tissue, Partly Through Liver X Receptor (LXR) Signaling

Beth A. Griesel,1 Juston Weems,1 Robert A. Russell,1 E. Dale Abel,2 Kenneth Humphries,3 and Ann Louise Olson1

OBJECTIVE—Insulin-mediated glucose uptake is highly sensitive to the levels of the facilitative GLUT protein GLUT4. Transcription of the GLUT4 gene is repressed in states of insulin deficiency and insulin resistance and can be induced by states of enhanced energy output, such as exercise. The cellular signals that regulate GLUT4 transcription are not well understood. We hypothesized that changes in energy substrate flux regulate GLUT4 transcription.

RESEARCH DESIGN AND METHODS—To test this hypothesis, we used transgenic mice in which expression of the chloramphenicol acetyltransferase (CAT) gene is driven by a functional 895-bp fragment of the human GLUT4 promoter, thereby acting as a reporter for transcriptional activity. Mice were treated with a single dose of etomoxir, which inhibits the transport of long-chain fatty acids into mitochondria and increases basal, but not insulin-mediated, glucose flux. GLUT4 and transgenic CAT mRNA were measured.

RESULTS—Etomoxir treatment significantly reduced CAT and GLUT4 mRNA transcription in adipose tissue, but did not change transcription in heart and skeletal muscle. Downregulation of GLUT4 transcription was cell autonomous, since etomoxir treatment of 3T3-L1 adipocytes resulted in a similar downregulation of GLUT4 mRNA. GLUT4 transcriptional downregulation required the putative liver X receptor (LXR) binding site in the human GLUT4 gene promoter in adipose tissue and 3T3-L1 adipocytes. Treatment of 3T3-L1 adipocytes with the LXR agonist, TO901317, partially restored GLUT4 expression in etomoxir-treated cells.

CONCLUSIONS—Our data suggest that long-chain fatty acid import into mitochondria in adipose tissue may produce ligands that regulate expression of metabolic genes. Diabetes 59: 800–807, 2010

GLUT4 is the major insulin-responsive GLUT responsible for insulin-dependent glucose disposal. Insulin-mediated glucose homeostasis is exquisitely sensitive to the level of GLUT4 protein. Mice that express exogenous GLUT4 gene in the major GLUT4-expressing tissues, or in skeletal muscle or adipose tissue alone, displayed enhanced insulin sensitivity and peripheral glucose utilization (1–4), whereas genetic ablation of one or both alleles of the GLUT4 gene result in insulin-resistant glucose disposal (5,6).

Expression of GLUT4 mRNA is subject to tissue-specific, hormonal, and/or metabolic regulation. GLUT4 mRNA expression is largely restricted to brown adipose, white adipose, skeletal muscle, and cardiac muscle tissues. Changes in GLUT4 gene expression are observed in pathophysiological states of altered glucose homeostasis. In general, GLUT4 mRNA expression is reduced in states of relative insulin deficiency such as streptozotocin-induced diabetes and chronic fasting (rev. in 7,8). Interestingly, the regulated steady-state levels of GLUT4 mRNA differ in various GLUT4-expressing tissues. For example, streptozotocin-dependent changes in GLUT4 mRNA expression occur much more rapidly in adipose tissue than skeletal muscle (9). Chronic fasting markedly reduces GLUT4 mRNA levels in adipose tissue, but has little or no effect on GLUT4 mRNA in skeletal muscle (10). Changes in steady-state levels of GLUT4 mRNA most likely reflect changes in the rate of mRNA synthesis (gene transcription) as opposed to changes in mRNA half-life, as evidenced by nuclear run-on assays showing that transcription is decreased in both adipose tissue and skeletal muscle in streptozotocin-induced diabetic animals (11,12), while the rate of the GLUT4 gene transcription in skeletal muscle of fasted animals is increased (12).

Regulation of GLUT4 gene expression within a narrow range (two to threefold) has a significant effect on whole body glucose homeostasis, suggesting that a fine control of GLUT4 levels is important for adaptation to extreme physiologic states such as prolonged fasting or to pathologic states such as chronic over nutrition. Because this gene is under complex metabolic control, the molecular basis for regulation of GLUT4 gene expression in altered physiologic states has been difficult to solve.

We hypothesize that changes in substrate flux may importantly contribute to the regulation of GLUT4 expression. These adaptive changes may emanate directly from specific substrate utilization or may result from hormonal
or neuronal signals that respond to changes in fuel sources or fuels.

In this article, transgenic mice carrying the chloramphenicol acetyltransferase (CAT) gene under the control of 895 bp of human GLUT4 promoter (hGLUT4-CAT) were treated with etomoxir, an irreversible inhibitor of carnitine palmitoyl transferase (CPT)-1. This treatment acutely inhibits mitochondrial long-chain fatty acid (LCFA) import and oxidation, increases glucose oxidation in tissues that normally oxidize fatty acids, and lowers circulating levels of glucose (13–15). Etomoxir treatment caused decreased GLUT4 expression in adipose tissue but not in the heart or hindquarter skeletal muscle. The effect on GLUT4 expression in adipose tissue was recapitulated in 3T3-L1 adipocytes and was mediated in part through a liver X receptor element (LXRE site) in the GLUT4 promoter. Our data support the hypothesis that GLUT4 expression is regulated by cell-autonomous tissue-specific metabolic signals.

**RESEARCH DESIGN AND METHODS**

**Animals.** Mice carrying a randomly integrated transgenic construct of the CAT gene driven by 895 bp of the human GLUT4 promoter DNA (895-hGLUT4-CAT) were obtained as previously described (16). A loss of function mutation in the putative LXRE site of the human GLUT4 promoter was engineered into the 895-HG4-CAT fragment and renamed ΔLXR-CAT. The mutation was identical to that reported by Dalen et al. (17), changing human GLUT4 LXRE (shown in bold) to GGGTTA

In this article, transgenic mice carrying the chloramphenicol acetyltransferase (CAT) gene under the control of 895 bp of human GLUT4 promoter (hGLUT4-CAT) were treated with etomoxir, an irreversible inhibitor of carnitine palmitoyl transferase (CPT)-1. This treatment acutely inhibits mitochondrial long-chain fatty acid (LCFA) import and oxidation, increases glucose oxidation in tissues that normally oxidize fatty acids, and lowers circulating levels of glucose (13–15). Etomoxir treatment caused decreased GLUT4 expression in adipose tissue but not in the heart or hindquarter skeletal muscle. The effect on GLUT4 expression in adipose tissue was recapitulated in 3T3-L1 adipocytes and was mediated in part through a liver X receptor element (LXRE site) in the GLUT4 promoter. Our data support the hypothesis that GLUT4 expression is regulated by cell-autonomous tissue-specific metabolic signals.

**RESEARCH DESIGN AND METHODS**

**Animals.** Mice carrying a randomly integrated transgenic construct of the CAT gene driven by 895 bp of the human GLUT4 promoter DNA (895-hGLUT4-CAT) were obtained as previously described (16). A loss of function mutation in the putative LXRE site of the human GLUT4 promoter was engineered into the 895-HG4-CAT fragment and renamed ΔLXR-CAT. The mutation was identical to that reported by Dalen et al. (17), changing human GLUT4 LXRE (shown in bold) to GGGTTA

In this article, transgenic mice carrying the chloramphenicol acetyltransferase (CAT) gene under the control of 895 bp of human GLUT4 promoter (hGLUT4-CAT) were treated with etomoxir, an irreversible inhibitor of carnitine palmitoyl transferase (CPT)-1. This treatment acutely inhibits mitochondrial long-chain fatty acid (LCFA) import and oxidation, increases glucose oxidation in tissues that normally oxidize fatty acids, and lowers circulating levels of glucose (13–15). Etomoxir treatment caused decreased GLUT4 expression in adipose tissue but not in the heart or hindquarter skeletal muscle. The effect on GLUT4 expression in adipose tissue was recapitulated in 3T3-L1 adipocytes and was mediated in part through a liver X receptor element (LXRE site) in the GLUT4 promoter. Our data support the hypothesis that GLUT4 expression is regulated by cell-autonomous tissue-specific metabolic signals.

**RESEARCH DESIGN AND METHODS**

**Animals.** Mice carrying a randomly integrated transgenic construct of the CAT gene driven by 895 bp of the human GLUT4 promoter DNA (895-hGLUT4-CAT) were obtained as previously described (16). A loss of function mutation in the putative LXRE site of the human GLUT4 promoter was engineered into the 895-HG4-CAT fragment and renamed ΔLXR-CAT. The mutation was identical to that reported by Dalen et al. (17), changing human GLUT4 LXRE (shown in bold) to GGGTTA

In this article, transgenic mice carrying the chloramphenicol acetyltransferase (CAT) gene under the control of 895 bp of human GLUT4 promoter (hGLUT4-CAT) were treated with etomoxir, an irreversible inhibitor of carnitine palmitoyl transferase (CPT)-1. This treatment acutely inhibits mitochondrial long-chain fatty acid (LCFA) import and oxidation, increases glucose oxidation in tissues that normally oxidize fatty acids, and lowers circulating levels of glucose (13–15). Etomoxir treatment caused decreased GLUT4 expression in adipose tissue but not in the heart or hindquarter skeletal muscle. The effect on GLUT4 expression in adipose tissue was recapitulated in 3T3-L1 adipocytes and was mediated in part through a liver X receptor element (LXRE site) in the GLUT4 promoter. Our data support the hypothesis that GLUT4 expression is regulated by cell-autonomous tissue-specific metabolic signals.

**RESEARCH DESIGN AND METHODS**

**Animals.** Mice carrying a randomly integrated transgenic construct of the CAT gene driven by 895 bp of the human GLUT4 promoter DNA (895-hGLUT4-CAT) were obtained as previously described (16). A loss of function mutation in the putative LXRE site of the human GLUT4 promoter was engineered into the 895-HG4-CAT fragment and renamed ΔLXR-CAT. The mutation was identical to that reported by Dalen et al. (17), changing human GLUT4 LXRE (shown in bold) to GGGTTA

In this article, transgenic mice carrying the chloramphenicol acetyltransferase (CAT) gene under the control of 895 bp of human GLUT4 promoter (hGLUT4-CAT) were treated with etomoxir, an irreversible inhibitor of carnitine palmitoyl transferase (CPT)-1. This treatment acutely inhibits mitochondrial long-chain fatty acid (LCFA) import and oxidation, increases glucose oxidation in tissues that normally oxidize fatty acids, and lowers circulating levels of glucose (13–15). Etomoxir treatment caused decreased GLUT4 expression in adipose tissue but not in the heart or hindquarter skeletal muscle. The effect on GLUT4 expression in adipose tissue was recapitulated in 3T3-L1 adipocytes and was mediated in part through a liver X receptor element (LXRE site) in the GLUT4 promoter. Our data support the hypothesis that GLUT4 expression is regulated by cell-autonomous tissue-specific metabolic signals.

**RESEARCH DESIGN AND METHODS**

**Animals.** Mice carrying a randomly integrated transgenic construct of the CAT gene driven by 895 bp of the human GLUT4 promoter DNA (895-hGLUT4-CAT) were obtained as previously described (16). A loss of function mutation in the putative LXRE site of the human GLUT4 promoter was engineered into the 895-HG4-CAT fragment and renamed ΔLXR-CAT. The mutation was identical to that reported by Dalen et al. (17), changing human GLUT4 LXRE (shown in bold) to GGGTTA

In this article, transgenic mice carrying the chloramphenicol acetyltransferase (CAT) gene under the control of 895 bp of human GLUT4 promoter (hGLUT4-CAT) were treated with etomoxir, an irreversible inhibitor of carnitine palmitoyl transferase (CPT)-1. This treatment acutely inhibits mitochondrial long-chain fatty acid (LCFA) import and oxidation, increases glucose oxidation in tissues that normally oxidize fatty acids, and lowers circulating levels of glucose (13–15). Etomoxir treatment caused decreased GLUT4 expression in adipose tissue but not in the heart or hindquarter skeletal muscle. The effect on GLUT4 expression in adipose tissue was recapitulated in 3T3-L1 adipocytes and was mediated in part through a liver X receptor element (LXRE site) in the GLUT4 promoter. Our data support the hypothesis that GLUT4 expression is regulated by cell-autonomous tissue-specific metabolic signals.

**RESEARCH DESIGN AND METHODS**

**Animals.** Mice carrying a randomly integrated transgenic construct of the CAT gene driven by 895 bp of the human GLUT4 promoter DNA (895-hGLUT4-CAT) were obtained as previously described (16). A loss of function mutation in the putative LXRE site of the human GLUT4 promoter was engineered into the 895-HG4-CAT fragment and renamed ΔLXR-CAT. The mutation was identical to that reported by Dalen et al. (17), changing human GLUT4 LXRE (shown in bold) to GGGTTA

In this article, transgenic mice carrying the chloramphenicol acetyltransferase (CAT) gene under the control of 895 bp of human GLUT4 promoter (hGLUT4-CAT) were treated with etomoxir, an irreversible inhibitor of carnitine palmitoyl transferase (CPT)-1. This treatment acutely inhibits mitochondrial long-chain fatty acid (LCFA) import and oxidation, increases glucose oxidation in tissues that normally oxidize fatty acids, and lowers circulating levels of glucose (13–15). Etomoxir treatment caused decreased GLUT4 expression in adipose tissue but not in the heart or hindquarter skeletal muscle. The effect on GLUT4 expression in adipose tissue was recapitulated in 3T3-L1 adipocytes and was mediated in part through a liver X receptor element (LXRE site) in the GLUT4 promoter. Our data support the hypothesis that GLUT4 expression is regulated by cell-autonomous tissue-specific metabolic signals.

**RESULTS**

Insulin resistance and obesity is associated with incomplete fatty acid oxidation (22,23). To explore the consequences of decreased fatty acid oxidation on GLUT4 translocation, we acutely inhibited CPT-1 and thus LCFA entry into the mitochondria. Two-month-old male mice were given one intraperitoneal injection of etomoxir (50 mg/kg body wt). Etomoxir inhibits CPT-1 through an irreversible modification of the active site of CPT-A (24,25). To ensure that etomoxir inhibited CPT-1 activity, we performed a CPT assay in crude mitochondrial preparations from hindquarter skeletal muscle, heart, and liver. CPT-1 activity was inhibited in all tissues tested (Fig. 1A). Fed glucose levels were decreased nearly 50% (P < 0.05) after treatment with etomoxir (Fig. 1B). Wet weight of perigonadal adipose tissue and subscapular brown adipose tissue were unchanged by etomoxir treatment, suggesting that lipids were not mobilized from these tissues (Fig. 1C). Intracellular triacylglycerides were estimated by measuring glycerol content of a total lipid extract after treatment with lipase enzyme. Overnight treatment with etomoxir significantly increased intracellular triacylglycerides in liver tissue and heart (P < 0.05) (Fig. 1D). By contrast, triacylglycerol content of hindquarter skeletal muscle was significantly decreased in etomoxir-treated mice (Fig. 1D).

We then tested GLUT4 promoter function under etomoxir treatment using a line of transgenic animals that carry the CAT reporter gene expressed under the control of an 895-bp fragment of the human GLUT4 promoter (–895-HG4-CAT) (Fig. 2A). We have previously demonstrated that this fragment of the human GLUT4 promoter is sufficient to direct tissue-specific and regulated expression of the CAT gene in a manner that is identical to endogenous mouse GLUT4 (16). To this end, CAT and...
endogenous GLUT4 mRNA levels were measured in brown adipose tissue and white adipose tissue, hindquarter skeletal muscle, and heart tissue. The effects of etomoxir treatment on CAT gene expression and endogenous GLUT4 gene expression were highly tissue specific. CAT mRNA and GLUT4 mRNA levels in brown adipose tissue and white adipose tissue were decreased (P < 0.05) in both male and female mice (Fig. 2B). In contrast, CAT mRNA and GLUT4 mRNA in hindquarter skeletal muscle and heart were unaffected by etomoxir treatment (Fig. 2B).

Because blood glucose levels were decreased after etomoxir treatment, we reasoned that insulin levels would also be decreased, and this could lead to downregulation of GLUT4 gene transcription. Using conditional tissue-specific knockouts of the insulin receptor, several studies have shown that GLUT4 protein is not decreased in adipose tissue, skeletal muscle, or cardiac tissue (18,26,27). To check whether the transcriptional regulation of the GLUT4 gene was changed by the absence of insulin receptor signaling, we made use of the cardiac-specific receptor knockout of the insulin receptor (CIRKO) model. CIRKO mice were crossed with transgenic mice carrying the −895-HG4-CAT genotype. The CIRKO-HG4-CAT mice were evaluated for their level of CAT and endogenous GLUT4 mRNA expression using the RNAse protection assay. CAT and GLUT4 mRNA expression appear to be unchanged with the loss of an intact insulin receptor (Fig. 3).
Because etomoxir treatment inhibits fatty acid import into mitochondria, we reasoned that a signal to the GLUT4 promoter might be mediated through the putative LXRE on the human GLUT4 promoter. LXR signaling promotes fat storage; therefore, we reasoned that changes in fat oxidation could be sensed through this signaling pathway (28). To test this hypothesis, we generated a line of transgenic mice carrying a loss of function mutation in the LXRE site in the context of the −895-HG4-CAT transgenic construct. This new mutant construct (∆LXR-CAT) was used to generate two founder lines of mice. Both founder lines expressed transgenic mRNA in the GLUT4-expressing tissues, but restricted expression in tissues that do not normally express GLUT4 (data not shown). From this, we conclude that the LXRE site was not required for a tissue-specific expression pattern of the transgene. To determine the role of LXRE in mediating the downregulation of GLUT4 mRNA under etomoxir treatment, we calculated the ratio of CAT mRNA to GLUT4 mRNA in white and brown adipose tissue in both lines of transgenic mice (Fig. 4). In animals carrying −895-HG4-CAT, the ratio of CAT to GLUT4 remained the same because CAT and GLUT4 mRNA were downregulated to the same extent with etomoxir. In contrast, the ratio of CAT mRNA to GLUT4 mRNA doubled with etomoxir treatment in adipose tissues from animals carrying the ∆LXR-CAT construct (Fig. 4).

To determine if etomoxir downregulates GLUT4 through changes in the hormones or other systemic factors other than insulin, we treated 3T3-L1 adipocytes with etomoxir overnight and measured GLUT4 mRNA (Fig. 5A). Similar to adipose tissue in vivo, etomoxir treatment downregulates GLUT4 mRNA in 3T3-L1 adipocytes about fourfold. To determine if the etomoxir-induced changes were due to inhibition of long-chain fatty acid import, we sought to restore GLUT4 mRNA by providing palmitoyl-α-carnitine. Etomoxir-treated cells were further treated without or with 1 mmol/l palmitoyl-α-carnitine to bypass carnitine-palmitoyl-transferase activity. This treatment restored GLUT4 mRNA to 75% of control levels (Fig. 5B), confirming that the major effect of etomoxir was due to inhibited formation of acyl carnitines.

In transgenic animals, the signal to downregulate GLUT4 mRNA was mediated, in part, through the LXRE of the GLUT4 promoter. To further elucidate LXRE function, we expressed either a luciferase promoter driven by the human GLUT4 promoter (−895-HG4-LUC) or the construct carrying the loss-of-function mutation in the LXRE (Δ-LXR-HG4-Luc) in 3T3-L1 adipocytes. The constructs were transactivated by overexpression of MEF2A and GEF to boost the signal. Mutation of the LXRE site resulted in a highly reproducible and statistically significant decrease in reporter expression (Fig. 6A). When cells were treated with etomoxir, expression of −895-HG4-Luc was decreased ~50% (Fig. 6A). Expression of Δ-LXR-HG4-Luc decreased no further upon etomoxir treatment, suggesting that effects of etomoxir treatment are mediated, in part, through LXRE signaling. To determine if the LXRE element was bound by LXR protein, we performed chromatin immunoprecipitation using an antibody raised against LXR-α. LXR-α specifically bound the GLUT4 promoter in control 3T3-L1 adipocytes (Fig. 6B); however, LXR-α binding was reduced in etomoxir treatment. Quantification of three independent experiments revealed that etomoxir reduced binding by 84% compared with control cells (see Fig. 7C for quantification).
Recently, several laboratories have provided evidence that mitochondrial LCFA import into mitochondria results in a tissue-restricted downregulation of GLUT4 mRNA in liver and heart tissue, but not skeletal muscle or heart tissue (Fig. 2). This was unexpected, since previous studies showed that etomoxir treatment in rats led to increased triacylglycerol in skeletal muscle (31). The different findings in the rat and mouse models are likely due to the different tissue distribution of mitochondrial LCFA import in this tissue.

To understand the nature of the signaling pathway coupled with long-chain acyl carnitine formation, we sought to determine the signaling pathway to activate the transcription of mitochondrial LCFA inhibited GLUT4 gene expression in adipose tissue and heart, but not in skeletal muscle or heart tissue (Fig. 2). Recently, several laboratories have provided evidence supporting a model for dysregulation of lipid metabolism as a cause of insulin resistance (23,29,30). These papers invoke several mechanisms including increased levels of ceramide (30), increased levels of the signaling and biosynthetic intermediate diacylglycerol (29), and reduced capacity for mitochondrial oxidation of fatty acids (23) as the cause of insulin resistance. Because the level of GLUT4 expression may play a role in chronic insulin resistance, it seems likely that changes in lipid metabolism could regulate GLUT4 transcription and contribute to downregulation of GLUT4 mRNA. We reasoned that inhibition of mitochondrial LCFA import might also play a role in generating a metabolic signal leading to regulation of GLUT4 transcription. We observed that the acute inhibition of mitochondrial LCFA inhibited GLUT4 transcription in white and brown adipose tissue, but did not alter transcription in skeletal muscle or heart (Fig. 2). This suggests that inhibition of LCFA import, per se, does not directly inhibit GLUT4 transcription, but rather the fate of the imported LCFA in a given tissue may generate a signal to the GLUT4 promoter. Therefore, the effect of etomoxir on adipose tissue reveals a unique function for mitochondrial LCFA import in this tissue.

In our model, intracellular triacylglycerol accumulated in liver and heart tissue, but not skeletal muscle (Fig. 1). This was unexpected, since previous studies showed that etomoxir treatment in rats led to increased triacylglycerol in skeletal muscle (31). The different findings in the C57BL/6 mice used in this study compared with rats...
are consistent with the notion that GLUT4 transcription is regulated by decreased insulin signaling, increased counterregulatory hormone secretion, or the concomitant changes in substrate flux that accompany both insulin resistance and fasting (34,36). For example, during insulin resistance and fasting, insulin signaling is reduced, insulin-mediated glucose flux is low, and circulating fatty acids are high. Insulin resistance and fasting have similar effects on adipose tissue metabolism, including increased lipolysis with release of fatty acids and glycerol that is used for hepatic gluconeogenesis (37). On the other hand, insulin resistance states and fasting have different effects on substrate utilization in skeletal muscle. Fatty acid oxidation is inhibited in insulin resistance, while it is quite active in fasting (22,23,38). In muscles of obese and type 2 diabetics, glucose oxidation in the fasted state in much higher than lean controls, and there is little or no difference in fatty acid oxidation between the fed and fasted states (38).

We have now used a metabolic paradigm that incorporates aspects of both enhanced glucose utilization of plasma glucose levels and decreased lipid utilization to examine the effects on GLUT4 expression. Etomoxir-treatment inhibited β-oxidation by inhibiting CPT-1 activity and lowered blood glucose levels (Fig. 1). Previous studies in rats have shown that etomoxir treatment lowers insulin levels, but does not necessarily lead to lipolysis, since the fat mass was reported to be increased and circulating free fatty acids not elevated (31). In the current study, we found that neither adipose tissue wet weight nor cAMP levels (data not shown) were altered under etomoxir treatment. Nevertheless, the GLUT4 transcription levels in both brown and white adipose tissue were reduced under etomoxir treatment consistent with a shift away from glucose utilization in adipose tissue, thereby increasing its availability for other organs.

Although we have not shown it directly, it is unlikely that the downregulation of GLUT4 transcription in adipose tissue is due to low levels of signaling through the insulin receptor. First, the adipose-specific knockout of the insulin receptor does not alter GLUT4 protein expression (18). Second, we demonstrated that GLUT4 transcription in heart is not altered by cardiac-specific knockout of the insulin receptor (Fig. 3). While we did not find that etomoxir led to changes in cardiac GLUT4 gene expression, the findings that insulin receptor signaling is not required for GLUT4 transcription in the cardiac tissue is evidence that other signals likely regulate the GLUT4 promoter.

GLUT4 transcription is differentially regulated in adipose tissue compared with muscle tissue. The differences in transcription likely reflect differences in the physiologic and metabolic roles that each of these tissues play. In adipose tissue, GLUT4 may be playing a role in energy sensing, whereas in muscle tissue, GLUT4 may play a more important role in fuel selection. This is supported by evidence that GLUT4 knockout mice oxidize fatty acids at a significantly higher rate than wild-type mice (39,40). Moreover, skeletal muscle and adipose tissue respond differently to activation of AMP-activated protein kinase (AMPK). In skeletal muscle, AMPK activation leads to increased glucose uptake and fatty acid oxidation presumably to increase ATP levels, while in adipose tissue, AMPK activation inhibits these processes, presumably allowing free fatty acids to be released from adipocytes for use in other tissues (41,42). In part, the effects of AMPK on

suggest a species or strain-specific difference in fatty acid metabolism. For instance, the C57BL/6 mice may have increased secretion of free fatty acids and triacylglycerol from skeletal muscle or decreased uptake of free fatty acids from adipose tissue and liver. Nonetheless, the accumulation of triacylglycerol in heart tissue did not correlate with a downregulation of GLUT4 transcription, suggesting that these events are not directly linked. This is somewhat surprising to us, since increased triacylglycerol levels in hearts of mice with a cardiac-specific overexpression of lipoprotein lipase correlated with decreased GLUT4 mRNA levels (32). That study might reflect a chronic adaptation to continued high levels of triacylglycerol in those transgenic mice.

In humans and rodents, diabetes and obesity lead to downregulation of GLUT4 gene transcription in adipose tissue (19,33–35). The signaling pathways that are responsible remain elusive. GLUT4 expression in adipose tissue is also downregulated in response to prolonged fasting. Downregulation of adipose GLUT4 in diabetes and fasting
skeletal muscle metabolism may be mediated through increased GLUT4 gene transcription in muscle (43,44). GLUT4 transcription is regulated by metabolic changes that are associated with insulin resistance, but might not be directly regulated by insulin action per se. We demonstrated that the effects of etomoxir (in vivo and in vitro) are mediated, in part, by LXR signaling (Figs. 4, 6, and 7). The GLUT4 promoter regulatory region contains an LXRE site, which we have now confirmed binds to the LXR-α ligand both in adipose tissue, and 3T3-L1 adipocytes (Figs. 6 and 7). Similar to insulin, LXR signaling promotes the synthesis and storage of lipids (28). It is quite reasonable that LXR signaling would support production of GLUT4 mRNA during times when lipid storage in adipose tissue is required. Etomoxir treatment reduced LXR-α association with the GLUT4 promoter, consistent with the conclusion that LXR plays a positive role in GLUT4 gene transcription through the LXRE.

Replenishment of etomoxir-treated cells with either palmitoyl l-carnitine, the product of CPT-1 enzyme, and LXR agonist each restored GLUT4 expression to near-normal levels, suggesting that mitochondrial import of long-chain fatty acids may play in regulating LXR signaling in the adipocyte. This effect of LXR activation was present in cultured cells, suggesting that systemic factors are not involved. Thus, a metabolite of palmitate oxidation may generate the LXR ligand. Conversely, fatty acid oxidation may indirectly produce a LXR ligand. In hepatic cells, LXR may function as a glucose sensor by directly binding glucose or glucose-6-phosphate to induced LXR-dependent transcriptional activation (45). Finally, inhibition of fatty acid oxidation may lead to increased AMPK activation in adipose tissue, as is observed in fasted adipocytes (46), which in turn leads to decreased glucose uptake, lipogenesis, and fatty acid oxidation (41). In hepatic tissue, AMPK activation has been shown to suppress LXR-dependent gene transcription presumably through alterations in ligand production. It is possible that this mechanism also operates in adipose tissue (47). In fact, changes in LXR signaling may be part of the transition between the fed and fasted state in adipose tissue and show that LXR is responding to glucose flux in adipose tissue. LXR signaling may play a role in the adaptation to the metabolic stress that accompanies over-nutrition and diabetes. For example, treatment of obese insulin-resistant C57BL/6 mice with an LXR agonist improved glucose homeostasis (48). Future work is underway to determine the role of LXR signaling to the GLUT4 promoter in states of relative insulin deficiency.

Acknowledgments

This work was supported by grants from the National Institutes of Health (DK 062341 and DK 068438).

No potential conflicts of interest relevant to this article were reported.

References

1. Tsao TS, Burcelin R, Katz EB, Huang L, Charron MJ. Enhanced insulin action due to targeted GLUT4 overexpression exclusively in muscle. Diabesity 1996;45:28–36
2. Treadway JL, Hargrove DM, Nardone NA, McPherson RK, Russo JR, Milici AJ, Stukenbrok HA, Gibbs EM, Stevenson RW, Pessin JE. Enhanced peripheral glucose utilization in transgenic mice expressing the human GLUT4 gene. J Biol Chem 1994;269:29956–29961
3. Shepherd PR, Gnuadi L, Tozzolo E, Yang H, Leach F, Kahn BB. Adipose cell hyperplasia and enhanced glucose disposal in transgenic mice overexpressing GLUT4 selectively in adipose tissue. J Biol Chem 1993;268:22243–22246
4. Liu ML, Gibbs EM, McCoid SC, Milici AJ, Stukenbrok HA, McPherson RK, Treadway JL, Pessin JE. Transgenic mice expressing the human GLUT4/muscle-fat facilitator glucose transporter. Nature 1992;359:1196–1196
5. Katz EB, Stenbit AE, Hatton K, DePnaho R, Charron MJ. Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4. Nature 1993;377:151–195
6. Stenbit AE, Tsao TS, Li J, Burcelin R, Geenen DL, Factor SM, Houseknecht K, Katz EB, Charron MJ. GLUT4 heterozygous knockout mice develop muscular insulin resistance and diabetes. Nat Med 1995;1:1096–1101
7. Olson AL. Regulation of GLUT4 transcription and gene expression. Curr Diab Med Immun Endoc Metab Agents 2005;5:219–225
8. Olson AL, Knight JB. Regulation of GLUT4 expression in vivo and in vitro. Front Biosci 2003;8:401–400
9. Richardson JM, Balon TW, Treadway JL, Pessin JE. Differential regulation of glucose transporter activity and expression in red and white skeletal muscle. J Biol Chem 1991;266:12900–12904
10. Charron MJ, Kahn BB. Divergent molecular mechanisms for insulin-resistant glucose transport in muscle and adipose cells in vivo. J Biol Chem 1990;265:7994–8000
11. Gerrits PM, Olson AL, Pessin JE. Regulation of the GLUT4/muscle-fat transporter mRNA in adipose tissue of insulin-deficient diabetic rats. J Biol Chem 1995;268:6437–6444
12. Neufeld PD, Carey JO, Dohm GL. Transcriptional regulation of the gene for glucose transporter GLUT4 in skeletal muscle: effects of diabetes and fasting. J Biol Chem 1993;268:13824–13829
13. Foley JE. Rationale and application of fatty acid oxidation inhibitors in treatment of diabetes mellitus. Diabetes Care 1992;15:773–784
14. Hubinger A, Knode O, Susanto F, Reinauer H, Grues FA. Effects of the carnitine-acyltransferase inhibitor etomoxir on insulin sensitivity, energy expenditure and substrate oxidation in NIDDM. Horm Metab Res 1997;29:436–439
15. Rafaelos R, Patel R, Yip C, Goldfine ID, Hawley DM. Mutation of the high cysteine region of the human insulin receptor alpha-subunit increases insulin receptor binding affinity and transmembrane signalling. J Biol Chem 1989;264:15900–15904
16. Thai MV, Gurawasamy S, Cao KT, Pessin JE, Olson AL. Myocyte enhancer factor 2 (MEF2)-binding site is required for GLUT4 gene expression in transgenic mice: regulation of MEF2 DNA binding activity in insulin-deficient diabetes. J Biol Chem 1998;273:14285–14292
17. Dalen KT, Ulven SM, Bamberg K, Gustafsson JA, Nebb HI. Expression of the insulin-responsive glucose transporter GLUT4 in adipocytes is dependent on liver X receptor alpha. J Biol Chem 2003;278:48280–48291
18. Bellie DD, Betuing S, Tuttle MJ, Graveleau C, Young ME, Pharo M, Zhang D, Coolsey RC, McClain DA, Litwin SE, Taegtmeyer H, Severson D, Kahn CR, Abel ED. Insulin signaling coordinately regulates cardiac size, metabolism, and contractile protein isoform expression. J Clin Invest 2002;109:629–639
19. Olson AL, Pessin JE. Transcriptional regulation of the human GLUT4 promoter in diabetic transgenic mice. J Biol Chem 1995;270:23491–23495
20. Eyster CA, Duggins QS, Olson AL. Expression of constitutively active Akt/protein kinase B signals GLUT4 translocation in the absence of an intact actin cytoskeleton. J Biol Chem 2005;280:17978–17985
21. Sparling DP, Griesel BA, Weems J, Olson AL. GLUT4 enhancer factor (GEF) interacts with MEF2A and HDAC5 to regulate the GLUT4 promoter in adipocytes. J Biol Chem 2008;283:7429–7437
22. Kelley DE, Mardarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes 2009;58:677–838
23. Koves TR, Ussher JR, Noland RC, Szentiz D, Mosedale M, Ilgayeva O, Bain J, Stevens R, Dyck JR, Newgard CB, Lopashuk GD, Muoio DM. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab 2008;7:45–56
24. Korpeus TC, Hoer D, Ho W, Weaver LE, Insulin MG, Tutuiev GF. Identification of 2-tetradecylglycidyl coenzyme A as the active form of methyl 2-tetradecylglycidyl (methyl palmoxirate) and its characterization as an irreversible, active site-directed inhibitor of carnitine palmitoyltransferase A in isolated rat liver mitochondria. J Biol Chem 1984;259:9750–9755
25. Morillas M, Clotet J, Urra B, Serra D, Arin˜o J, Hegardt FG, Asins G. Identification of 2-tetradecylglycidyl coenzyme A as the active form of methyl 2-tetradecylglycidyl (methyl palmoxirate) and its characterization as an irreversible, active site-directed inhibitor of carnitine palmitoyltransferase A in isolated rat liver mitochondria. J Biol Chem 1995;270:23491–23495
26. Bluher M, Michael MD, Peroni OD, Ueki K, Carter N, Kahn BB, Kahn CR, Abel ED. Insulin signaling coordinately regulates cardiac size, metabolism, and contractile protein isoform expression. J Clin Invest 2002;109:629–639
27. Kelley DE, Mardarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes 2009;58:677–838
28. Koves TR, Ussher JR, Noland RC, Szentiz D, Mosedale M, Ilgayeva O, Bain J, Stevens R, Dyck JR, Newgard CB, Lopashuk GD, Muoio DM. Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab 2008;7:45–56
29. Korpeus TC, Hoer D, Ho W, Weaver LE, Insulin MG, Tutuiev GF. Identification of 2-tetradecylglycidyl coenzyme A as the active form of methyl 2-tetradecylglycidyl (methyl palmoxirate) and its characterization as an irreversible, active site-directed inhibitor of carnitine palmitoyltransferase A in isolated rat liver mitochondria. J Biol Chem 1984;259:9750–9755
30. Bluher M, Michael MD, Peroni OD, Ueki K, Carter N, Kahn BB, Kahn CR. Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. Dev Cell 2002;3:25–38
27. Brüning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D, Goodyear LJ, Kahn CR. A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. Mol Cell 1998;2:559–569
28. Kalaany NY, Gauthier KC, Zavacki AM, Mammen PP, Kitazume T, Peterson JA, Horton JD, Garry DJ, Bianco AC, Mangelsdorf DJ. LXRα regulates the balance between fat storage and oxidation. Cell Metab 2005;1:231–244
29. Chibalin AV, Leng Y, Vieira E, Krook A, Bjornholm M, Long YC, Kotova O, Zhong Z, Sakane F, Steiler T, Nylén C, Wang J, Laakso M, Topham MK, Gilbert M, Wallberg-Henriksson H, Zierath JR. Downregulation of diacylglycerol kinase delta contributes to hyperglycemia-induced insulin resistance. Cell 2008;132:375–386
30. Holland WL, Brozinick JT, Wang LP, Hawkins ED, Sargent KM, Liu Y, Narra K, Hoehn KL, Knotts TA, Siesky A, Nelson DH, Karathanasis SK, Fontenot GK, Birnbaum MJ, Summers SA. Inhibition of ceramide synthesis ameliorates rates glucocorticoid-, saturated-fat-, and obesity-induced insulin resistance. Cell Metab 2007;5:167–179
31. Dobbins RL, Szczepaniak LS, Bentley B, Esser V, Myhill J, McGarry JD. Prolonged inhibition of muscle carnitine palmitoyltransferase-1 promotes intramyocellular lipid accumulation and insulin resistance in rats. Diabetes 2001;50:123–130
32. Yagyu H, Chen G, Yokoyama M, Hirata K, Augustus A, Kako Y, Seo T, Hu Y, Lutz EP, Merkel M, Bensadoun A, Homma S, Goldberg DJ. Lipoprotein lipase (LpL) on the surface of cardiomycocytes increases lipid uptake and produces cardiomycopathy. J Clin Invest 2003;111:419–426
33. Garvey WT, Maianu L, Hueckstedt TP, Birnbaum MJ, Molina JM, Ciaramaldi TP. Pretranslational suppression of a glucose transporter protein causes insulin resistance in adipocytes from patients with non-insulin-dependent diabetes mellitus and obesity. J Clin Invest 1991;87:1072–1081
34. Murgia M, Jensen TE, Casinato M, Garcia M, Richter EA, Schiaffino S. Multiple signalling pathways redundantly control glut4 transporter GLUT4 gene transcription in skeletal muscle. Mol Cell 1998;2:559–569
35. McGee SL, van Denderen BJ, Howlett KF, Mollica J, Schertzer JD, Kemp BE, Hargreaves M. AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. Diabetes 2008;57:860–867
36. Sivitz WI, Bershadsky AD, Zavacki AM, Mammen PP, Kitazume T, Peterson JA, Horton JD, Garry DJ, Bianco AC, Mangelsdorf DJ. LXRα regulates the balance between fat storage and oxidation. Cell Metab 2005;1:231–244
37. Nurjhan N, Consoli A, Gerich J. Increased lipolysis and its consequences on gluconeogenesis in non-insulin-dependent diabetes mellitus. J Clin Invest 1992;89:169–175
38. Kelley DE, Goodpaster B, Wing RR, Simoneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol 1999;277:E1130–E1141
39. Ranalletta M, Jiang H, Li J, Tsao TS, Stenbit AE, Yokoyama M, Katz EB, Charron MJ. Altered hepatic and muscle substrate utilization provoked by GLUT4 ablation. Diabetes 2005;54:935–943
40. Ranalletta M, Du QX, Seki Y, Glenn AS, Kruse M, Fiallo A, Estrada I, Tsao TS, Stenbit AE, Katz EB, Charron MJ. Hepatic response to restoration of GLUT4 in skeletal muscle of GLUT4 null mice. Am J Physiol Endocrinol Metabol 2007;293:E1178–E1187
41. Gaidhu MP, Feducis C, Ceddia RB. 5-Aminomidazole-4-carboxamide-1-beta-D ribofuranoside-induced AMP-activated protein kinase phosphorylation inhibits basal and insulin-stimulated glucose uptake, lipid synthesis, and fatty acid oxidation in isolated rat adipocytes. J Biol Chem 2006;281:25965–25964
42. Kraemer DK, Al-Khalili L, Guigas B, Leng Y, Garcia-Roves PM, Krook A. Role of AMP kinase and PPARδ in the regulation of lipid and glucose metabolism in human skeletal muscle. J Biol Chem 2007;282:19313–19320
43. McGee SL, van Denderen BJ, Howlett KF, Mollica J, Schertzer JD, Kemp BE, Hargreaves M. AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. Diabetes 2008;57:860–867
44. Mitro N, Mak PA, Vargas L, Godio C, Hampton E, Molteni V, Kreusch A, Saiz E. The nuclear receptor LXR is a glucose sensor. Nature 2007;445:419–426
45. Li, Chao LC, Li J, Walczak R, Hummasti S, Joseph SB, Castrillo A, Wilpitz DC, Mangelsdorf DJ, Collins JL, Saez E, Tontonoz P. Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue. Proc Natl Acad Sci U S A 2003;100:5419–5424