Transcriptional regulation of the insulin-responsive glucose transporter GLUT4 gene: from physiology to pathology

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Submitted 20 March 2008; accepted in final form 16 May 2008

The insulin-responsive glucose transporter 4 (GLUT4) plays a key role in glucose uptake and metabolism in insulin target tissues. Being a rate-limiting step in glucose metabolism, the expression and function of the GLUT4 isoform has been extensively studied and found to be tightly regulated at both mRNA and protein levels. Adaptation to states of enhanced metabolic demand is associated with increased glucose metabolism and GLUT4 gene expression, whereas states of insulin resistance such as type 2 diabetes mellitus (DM2), obesity, and aging are associated with impaired regulation of GLUT4 gene expression and function. The present review focuses on the interplay among hormonal, nutritional, and transcription factors in the regulation of GLUT4 transcription in health and sickness.

Transcription Factors Regulating GLUT4 Gene Expression

Although data supporting the presence of a unique insulin-responsive glucose transporter protein had already emerged in the beginning of the 80’s (48), concrete evidence became available only years later, when the first GLUT4 cDNA was cloned from human (31) and rat (43) tissues. Later, when the mouse (45) and human (15) GLUT4 genes and their 5'-flanking transcriptional regulatory regions had been isolated and characterized, studies on GLUT4 regulation at promoter level flourished. Studies characterizing GLUT4 promoter showed that it contains binding sites for several nuclear transcription factors, such as Sp1 and C/EBP (45), and that 2,400 bp of the 5'-flanking region of the GLUT4 gene are sufficient for tissue-specific and hormonal/metabolic regulation of the human GLUT4 gene in transgenic mice (58, 76). The following section reviews the main transcription factors that regulate expression of the GLUT4 protein in muscle and adipose tissues.

Myocyte enhancer factor 2. In an effort to identify the cis-DNA elements that account for muscle-specific GLUT4 expression, C2C12 and L6 myotubes were used as a model for skeletal muscle differentiation. Liu et al. (57) identified a 103-bp region within the GLUT4 promoter that includes a functional myocyte enhancer factor 2 (MEF2) binding site, and this cis-element was found necessary (although insufficient) for GLUT4 myotube-specific expression. Studies using transgenic mice demonstrated that MEF2 binding activity is also necessary for the regulation of the GLUT4 gene promoter in adipose tissue as well as in cardiac muscle, and reduction in MEF2 expression correlated with reduced GLUT4 expression (70, 94). Furthermore, it was shown that although MEF2 mRNA is ubiquitously expressed, it preferentially accumulates in skeletal muscle, heart, and brain, and MEF2 protein and its binding activity are exclusively detected in muscle tissues and differentiated muscle cell lines (70).

The MEF2 family of transcription factors includes several isoforms, and studies designed to identify the specific MEF2 isoform of diabetes and obesity. The present review focuses on the interplay between factors that govern GLUT4 gene expression in health and sickness. This information will hopefully assist in understanding the molecular processes regulating glucose homeostasis and shed light on potential targets for therapy that are associated with the GLUT4 transcriptional machinery, aiming at improving insulin sensitivity.
isoform(s) responsible for GLUT4 expression point to a tissue-specific pattern. Studying the pattern of expression of the MEF2 isoforms in insulin-sensitive tissues, Mora and Pessin (70) found that whereas both heart and skeletal muscle express the MEF2A, MEF2C, and MEF2D isoforms but not the MEF2B isoform, only the MEF2A-MEF2D heterodimer was selectively decreased in insulin-deficient diabetes, thus suggesting that the MEF2A isoform is required for striated muscle-specific expression of GLUT4. In an attempt to resolve the question of why virtually all cultured muscle cells express little or no GLUT4, Michael et al. (66) found that overexpression of peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1; see below) results in an increase in GLUT4 expression that is largely mediated by PGC-1 binding to and coactivating the muscle-selective transcription factor MEF2C, thus indicating that it is the MEF2C isoform that controls the level of GLUT4 in the muscle. It is now apparent that different isoforms of MEF2 regulate GLUT4 expression under various conditions (for an extensive review describing the mechanisms that control GLUT4 expression in muscle, see Ref. 114).

MEF2-associated transcription factors. As mentioned above, the region encompassing a MEF2 binding site in the GLUT4 gene is necessary but insufficient for its transcriptional activation, thus indicating the involvement of other factors. Indeed, MEF2 activity was found associated with the transcriptional repressor histone deacetylase 5 (HDAC5), and overexpression of HDAC5 repressed GLUT4 reporter gene expression in primary human myotubes, whereas its inhibition increased endogenous GLUT4 gene expression. The phosphorylation of HDAC5 by AMP kinase leads to nuclear export of HDAC5 and its reduced association with the GLUT4 promoter, hence resulting in increased GLUT4 gene expression (64). It was recently shown that nuclear regulatory factors NRF1 and NRF2, which regulate the expression of genes required for mitochondrial genome transcription, protein import, and numerous respiratory chain subunits, also regulate GLUT4 expression, probably via the induction of MEF2 (83). These pathways link cellular energy changes to gene expression so that both cellular and whole body energy balance are restored.

Another important MEF-associated activity, described by Olson and colleagues (51, 78), is the GLUT4 enhancer factor (Glut4EF, or GEF), a zinc-finger transcription factor that physically associates with MEF2 to activate GLUT4 transcription. GEF proteins are well conserved from Drosophila to humans (109). GEF also shares homology with a segment of a larger protein known as the Huntington’s disease gene regulatory region-binding protein 1 (HDBP1), which regulates the transcription of the Huntington polypeptide in brain (93). More data are needed to elucidate GEF function in insulin resistance in human diseases.

PGC-1. PGC-1 genes code for two distinct isoforms, PGC-1α and PGC-1β, which serve as coactivators of several nuclear receptors and other transcription factors shown to be involved in the regulation of mitochondrial biogenesis, adaptive thermogenesis, and enzymes involved in oxidative metabolism. Both isoforms are highly expressed in skeletal muscle, having overlapping and differing effects in regulating skeletal muscle fiber transition and metabolism (71). Both PGC-1α and PGC-1β are downregulated in skeletal muscle in type 2 diabetes (79), whereas only PGC-1α is regulated by endurance exercise (21, 65) and regulates glucose transport (for review, see Ref. 82).

Studies by Spiegelman and colleagues (66, 82) suggest that effects of PGC-1α on glucose uptake and metabolism are of particular interest in diabetes because the rates of mitochondrial oxidation can affect glucose uptake (82). Beyond the effects of PGC-1α on mitochondrial respiration in skeletal muscle cells, it also induces GLUT4 gene expression and increases glucose uptake (66). The relative insulin sensitivity of this transport process, however, does not increase. The authors suggest that this effect on GLUT4 gene expression is partially mediated through PGC-1α binding to and coactivation of MEF2F, probably the MEF2C isoform. p38 MAPK was also suggested to induce GLUT4 and mitochondrial biogenesis via the activation PGC-1α and MEF2, leading to subsequent increases in the content of these proteins and enhanced Ca2+--induced GLUT4 synthesis (105). Since decreases in mitochondrial and GLUT4 contents are associated with skeletal muscle insulin resistance, understanding the mechanisms by which these processes can be normalized will aid in the prevention and treatment of DM2.

C/EBP. Peroxisome proliferator-activated receptor-γ (PPARγ) and C/EBPs are main transcription factors that play critical roles in adipocyte differentiation, and coordinated expression of both is required for the acquisition of insulin-sensitive glucose transport (for a detailed discussion on C/EBP in adipocytes, see review in Ref. 59).

Traditionally, the C/EBPα isoform is looked on as the main adipogenic isoform. Studies characterizing the GLUT4 promoter showed that it contains binding sites for C/EBP (45). It is known that C/EBPα transactivates the GLUT4 gene and that its reduction is associated with decreased GLUT4 mRNA (45). However, it remains uncertain whether other C/EBP isoforms are involved in the acquisition of the insulin-responsive phenotype. With the use of C/EBPβ/δ-double-deficient mouse embryonic fibroblasts, it was found that both C/EBPβ and C/EBPδ are involved in insulin receptor substrate (IRS)-2 and GLUT4 expression, as well as in insulin-sensitive glucose uptake during adipocyte differentiation (106).

PPARs. PPARs are ligand-activated transcription factors from the nuclear receptor family that function as regulators of lipid and lipoprotein metabolism and glucose homeostasis and that influence cellular proliferation, differentiation, and apoptosis (see detailed reviews, Refs. 29, 91). PPARs include three isoforms that display tissue-specific expression: 1) PPARα is highly expressed in liver, muscle, kidney, and heart, where it stimulates the β-oxidative degradation of fatty acids; 2) PPARβ (also referred to as PPARδ) is expressed in a wide variety of tissues, with high levels in skeletal muscle; and 3) PPARγ is predominantly expressed in intestine and adipose tissue and promotes lipid storage and expression of adipose-specific genes.

Recent studies point at a crucial role for PPARδ in skeletal muscle glucose metabolism and insulin action. Studies by Kramer et al. (53) in primary human myotubes showed that activation of PPARδ results in a direct activation of glucose transport. Barish et al. (9) used muscle-specific PPARδ transgenic mice to establish the role of PPARδ in whole body glucose homeostasis. Interestingly, these mice not only had enzymatic and gene expression profiles that promote oxidative metabolism in skeletal muscle but also had a reduced body fat mass due to a reduction of adipocyte cell size. PPARδ enhances
Perspectives

TRANSCRIPTIONAL REGULATION OF GLUT4

fatty acid catabolism and energy uncoupling in adipose tissue and muscle, and it suppresses macrophage-derived inflammation (for extensive review, see Ref. 9). Transgenic mice with cardiac-specific expression of PPARδ (MHC-PPARδ) had increased myocardial glucose utilization, did not accumulate myocardial lipid, and had normal cardiac function compared with MHC-PPARα mice. Furthermore, in vitro reporter assays supported the notion that PPARδ and PPARα exert differential transcriptional control of the GLUT4 promoter, which may explain the observed isotype-specific effects on glucose uptake (14). This can partially explain the underlying pathophysiological mechanism for induction of cardiomyopathy frequently observed in diabetic patients; i.e., chronic activation of the PPARα pathway drives excessive fatty acid (FA) oxidation, lipid accumulation, and reduced glucose utilization. This also is consistent with our previous observation of the role of increased free fatty acid (FFA) and lipotoxicity in repressing GLUT4 promoter in cardiomyocytes and reduced GLUT4 protein in human cardiac muscle biopsies (5). Together, these data provide biological validation of PPARδ as a potential target for antidiabetic therapy, highlighting its broad potential in the treatment of metabolic disease. Its combined activities in these and other tissues makes it a multifaceted therapeutic target for the metabolic syndrome with the potential to control weight gain, enhance physical endurance, improve insulin sensitivity, and ameliorate atherosclerosis.

PPARγ is a main adipogenic factor that triggers adipocyte differentiation. There are two PPARγ isotypes, γ1 and γ2, that arise from the use of different promoters and alternative splicing: PPARγ2 is adipose-specific, whereas both PPARγ isotypes are expressed in muscle (28). Rosiglitazone and pioglitazone, two synthetic hypoglycemic drugs from the thiazolidinedione (TZD) family, are potent ligands of PPARγ that regulate lipid metabolism and glucose homeostasis, increase insulin responsiveness, correct hyperglycemia, hyperinsulinemia, and hypertriglyceridemia in DM2 patients, enhance adipocyte differentiation, and increase GLUT4 mRNA in DM2 muscle tissue (75). Counterintuitively, however, PPARγ knockout mice fed a high-fat diet exhibited improved insulin sensitivity and were protected from development of insulin resistance caused by adipocyte hypertrophy (54, 67). This “counterintuitivity” was resolved when we (7) recently demonstrated a novel mechanism for the beneficial action of TZDs in enhancing insulin sensitivity in rat and human adipocytes. We have shown that in its unliganded form (i.e., in the absence of any ligand), PPARγ binds to cis-elements on the GLUT4 promoter, keeping it in a repressed state; once TZD is introduced, it binds to PPARγ, causing the detachment of corepressors and the attachment of coactivators, which subsequently leads to the detachment of PPARγ from the GLUT4 gene promoter. This leads to the alleviation of the repression, increased GLUT4 expression, and, subsequently, enhanced insulin responsiveness. It remains to be discovered whether this mechanism also holds true for GLUT4 regulation by PPARγ in the muscle.

Forkhead box O1. Forkhead box O1 (FOXO1; previously known as FKHR) is an important member of the winged-helix/forkhead box family of transcription factors called forkhead box (FOX). FOXO1 is the most abundant isoform in insulin-responsive tissues such as hepatic, adipose, and pancreatic cells, where it regulates the transcription of target genes involved in glucose and energy metabolism (98). FOXO1 is negatively regulated by the serine/threonine protein kinase B (PKB/Akt, which lies downstream of phosphatidylinositol 3-kinase in the insulin signaling cascade. Accili (2) documented a hierarchical phosphorylation of FOXO1 on T24, S256, and S319 that leads to its nuclear exclusion and subsequent inhibition of its transcriptional activity. We found in primary adipocytes that FOXO1 upregulates GLUT4 gene expression at both transcriptional and posttranscriptional levels and both directly, via physical binding to the GLUT4 promoter, and indirectly, via repressing PPARγ expression (4), the latter being a repressor of GLUT4. This effect was either partially or completely curtailed by insulin in a tissue-specific manner (for extensive review, see Ref. 6). Accordingly, Cooke and Lane (22) showed in 3T3-L1 adipocytes that expression of the murine GLUT4 gene is decreased by insulin and that this repression is mediated by an insulin-response element (IRE) residing in the murine GLUT4 promoter. In liver and pancreatic β-cells, Foxo1 (the murine homolog of FOXO1) was demonstrated as a negative regulator of insulin sensitivity (72). Indeed, we have found that FOXO1 can repress GLUT4 expression in some tissues while upregulating it in others. Although the reason for this is still illusive, it underscores the tissue-specific nature of FOXO1 contribution to glucose metabolism.

Other transcription factors. Other transcription factors such as MyoD (86) and Kruppel-like factor 15 (35) were shown to be involved in GLUT4 transcription (see review, Ref. 114). GLUT4 promoter has a thyroid hormone receptor α1 (TRα1)-sensitive region (84, 86, 97). Euthyroidism is important for physiological muscle GLUT4 content. For example, congenital hypothyroidism is associated with reduced skeletal and cardiac muscle GLUT4 content (18). In parallel, long-term T3 administration stimulates GLUT4 expression and glucose transport in rat skeletal muscle (17, 102). Treating diabetic and/or obese rats with T3 resulted in an increase of GLUT4 expression in both red and white skeletal muscle (96).

Ca2+ has an important role in regulating cellular events as well as GLUT4 gene expression. Increased cytosolic levels of Ca2+, which activate Ca2+/calmodulin-dependent protein kinase (CaMK), lead to increases in GLUT4 and mitochondrial protein contents. This effect is likely controlled through CaMK and the p38 mitogen-activated protein kinase (MAPK) pathway by activating transcription factors such as MEF2 and PGC-1α. Subsequent increases in the content of these proteins enhance Ca2+-induced GLUT4 and mitochondrial biogenesis (73, 74, 105).

Metabolic States That Modulate GLUT4 Gene Expression

Exercise and physical training. Physical training and fitness contribute to health status such that a further increase in physical activity and fitness will lead to additional improvements in health status (see review, Ref. 100). Skeletal muscle is the primary target for the stimulation of glucose uptake and utilization by various factors. Muscle contraction increases insulin-mediated glucose disposal. However, exercise- and insulin-stimulated muscle glucose transports are regulated via distinct mechanisms (49). Indeed, GLUT4 overexpression in skeletal muscle was shown to increase insulin- and contraction-stimulated glucose transport activity and glucose metabolism (37).
GLUT4 levels in skeletal muscle are largely regulated at the level of transcription as well as posttranslational. By examining the effects of exercise on GLUT4 gene transcription in several lines of transgenic mice expressing various lengths of the human GLUT4 promoter, endogenous GLUT4 mRNA was shown to increase in response to exercise between 20 and 90%. These effects of exercise on GLUT4 expression are probably mediated by AMP-activated protein kinase (AMPK) pathway (60). In addition, cellular nitric oxide (NO) increase activates cGMP- and AMPK-dependent mechanisms, thus enhancing GLUT4 expression. This is consistent with a role for NO in the regulation of AMPK (56). As mentioned, AMPK also regulates GLUT4 transcription through the phosphorylation of the HDAC5 (62, 64). Interestingly, adiponectin, which is secreted from adipose cells and is important for insulin action (107), as well as the anti-diabetic drug metformin can both activate AMPK (113) and thus indirectly regulate GLUT4 gene expression.

Recent studies focusing on the transcriptional regulation of muscle contraction demonstrated rapid increase in GLUT4, MEF2A, MEF2D, and hypoxia-inducible factor-1α mRNAs with changes in the binding activity of nuclear proteins to consensus NF-κB, GLUT4-Ebox, and GLUT4-AT-rich element probes, parallel to the mRNA changes of their respective transcriptional factors NF-κB, HIF1-a, and MEF2s (90). (For a detailed review, see Ref. 114).

As expected, muscle training in humans induces a local contraction-dependent increase in GLUT4 protein, which enhances the effect of insulin on glucose uptake (25). Similarly, an acute bout of exercise increased the DNA-binding activities of MEF2 heterodimer and Glut4EF-GEF in human skeletal muscle without changing their nuclear content (63). The role of exercise protocols in upregulating muscle GLUT4 also is important. For example, an exercise protocol designed to repeatedly induce a large dependence on carbohydrate and large increases in glycolytic flux rate resulted in rapid increases in the levels of GLUT4 and monocarboxylate transporter (MCT)-4 proteins in working muscle of normal volunteers (36). Whereas exercise enhances GLUT4 muscle content, immobilization leads to a significant 30-40% reduction in muscle GLUT4 mRNA and protein (26). Signals from neuronal cells in the form of neuregulins may mediate some of these effects (92).

Nutrition and diet. Prolonged fasting resembles insulin resistance in that it leads to reduced cellular glucose transport rates (46). Prolonged fasting repressed GLUT4 gene expression and protein function in brown and white adipose tissues and soleus muscle, with no effect on the gastrocnemius muscle, whereas refeeding induced a rapid overexpression of GLUT4 mRNA in the various tissues (112). Refeeding was shown to increase GLUT4 mRNA levels concomitantly with enhanced levels of sterol regulatory element binding protein (SREBP)-1c (40). Studies including the use of dominant negative SREBP and chromatin immunoprecipitation assay further support the hypothesis that SREBP-1c activates GLUT4 transcription in adipocytes (40, 41). It was thus proposed that feeding behavior influences GLUT4 gene expression pattern through changes in sympathetic activity, especially during long-term starvation periods (112).

Regulation of gene expression by dietary fats has a significant impact on the development of insulin resistance and its related pathophysiology. Beyond their obvious metabolic role, FFAs modulate gene transcription by exerting a direct, membrane-independent influence on molecular events that govern gene expression, mRNA stability, and cellular differentiation (27, 80, 88). The current paradigm suggests that high levels of FFAs cause insulin resistance by interfering with serine kinase cascade(s). This is consistent with the fact that activation of protein kinase-Cδ leads to serine/threonine phosphorylation, resulting in decreased phosphorylation and reduced activity of major components essential for muscle insulin action (50). FFAs also attenuate insulin signaling and GLUT4 translocation through activation of the IkB kinase (IKK) pathway (111).

Low-fat diet improves glycemic control and reduces cardiovascular risk factors (10). However, less is known about FFA-induced regulation of GLUT4 at the level of gene transcription. Since normal cardiac function is dependent on a continuous supply of nutrients such as long-chain FFAs and glucose (27), we examined the effects of hyperlipidemia on GLUT4 gene expression in human cardiac muscle (3). On the basis of data gathered both in vivo and in vitro, we suggested a potential mechanism for GLUT4 gene regulation in cardiac muscle, where arachidonic acid reduces GLUT4 gene expression at the transcription level via binding of FFA mediator proteins to three newly identified response elements on the GLUT4 promoter. FFAs also decrease GLUT4 expression at the protein level via an unknown posttranslational process(s) and decrease transcriptional activity from the PPARY promoter, thus leading to reduced levels of PPARY mRNA and protein. Teleologically, since increased levels of PPARY lead to reduced expression of GLUT4 gene (see above and Ref. 7), this mechanism of FFA-induced repression of PPARY may serve to protect the GLUT4 gene from further dampening of its own expression. In accordance, an increasing body of evidence suggests that the regulation of gene transcription by fatty acids is caused by changes in the activity or abundance of several main transcription factors, the most prominent among them being members of the PPARY family, hepatic nuclear factor 4, SREBP, and nuclear factor Y (80).

GLUT4 Gene Regulation in Pathophysiological States

The critical importance of tissue-specific regulation of GLUT4 for maintaining normal glucose homeostasis is amplified in altered metabolic states. Insulin resistance in DM2, obesity, and aging is associated with a marked reduction in the intracellular pool of GLUT4 protein in adipose cells, which in turn impairs insulin stimulation of glucose transport (see review, Ref. 5). Impaired insulin stimulation of glucose uptake in adipose tissue and skeletal muscle is one of the earliest defects detected in insulin-resistant states (5, 32, 47, 89). Surprisingly enough, whereas the reduction of GLUT4 gene expression is prominent in adipose tissues in human and animal models, it has rarely been documented in muscle tissue (32), pointing at the tissue-specific nature of GLUT4 gene regulation in sickness and in health.

Obesity: inflammation leading to type 2 diabetes. The current paradigm is that GLUT4 muscle content in diabetic patients is not decreased. Yet when more detailed study was performed, GLUT4 density in slow muscle fibers from diabetic patients was found to be reduced by 9% compared with the
weight-matched obese subjects and by 18% compared with the lean control group (33). It has been proposed that a reduction in the fraction of slow-twitch fibers, combined with a reduction in GLUT4 expression in these fibers, may reduce the insulin-sensitive GLUT4 pool in type 2 diabetes and thus contribute to skeletal muscle insulin resistance (33).

Unveiling the molecular mechanism(s) leading to reduced GLUT4 expression in adipocytes and its impact on overall glucose homeostasis in diabetes and obesity has been the object of many studies. Epidemiological studies confirm that obesity and DM2 are closely linked. Life style modification, diet, and exercise were shown to prevent the development of diabetes in overweight patients with impaired glucose tolerance (52). Furthermore, increased levels of markers and mediators of inflammation and oxidative stress components correlate with impaired insulin action (38, 104). In DM2 and obesity, macrophage migration to and accumulation in omental fat tissue (103), together with overproduction of macrophage-derived cytokines and activation of the IKK-NF-κB pathways, reduce GLUT4 expression and cause insulin resistance (111). We have recently obtained preliminary data suggesting that NF-κB subunits can also directly bind to the GLUT4 promoter and repress its activity.

Oxidative stress. Oxidative stress is presently accepted as a likely causative factor in the development of insulin resistance. Prolonged exposure to ROS affects transcription of glucose transporters, leading to increased levels of GLUT1 while concomitantly reducing GLUT4 gene expression (see review, Ref. 12). Studies indicate that the endoplasmic reticulum (ER) participates in the oxidative stress, and activation of ER stress response results in decreased GLUT4 transcription. Efforts to understand this mechanism have revealed that activation of the ER stress response leads to increased expression of CHOP10, an inhibitor of the activity and expression of C/EBPα. Accordingly, decreased expression of C/EBPα as a result of increased CHOP10 levels is one potential mechanism for the repression of GLUT4 gene expression during stress (68). The proteasome system is also involved in the regulation of GLUT4 expression, given that treatment with proteasome inhibitors increases GLUT4 transcription. This suggests that proteasomal degradation of factors that regulate GLUT4 expression is an additional mechanism for the transcriptional regulation of GLUT4 gene (23). As can be expected, administration of antioxidants such as lipoic acid in oxidized cells in animal models of diabetes and in type 2 diabetes was shown to improve insulin sensitivity (81).

Tumorigenesis. Accelerated glucose metabolism has long been recognized as a hallmark feature of transformed cells, and activation of GLUT genes represents one of the earliest events in oncogenesis (11, 30). Although the accelerated glucose uptake observed in tumor cells has been ascribed mainly to GLUT1 overexpression, aberrant expression of other GLUT isoforms also has been implicated (11, 30, 85, 110). Investigating the role of GLUT4 in tumorigenesis, we have found an aberrant yet functional upregulation GLUT4 expression in alveolar rhabdomyosarcoma, associated with unique presence of the oncogenes FAX3/FKHR and RET/PTC1 (8). Going to the other side of the scope, we showed that the tumor suppressor p53 downregulates GLUT1 and GLUT4 gene expression, whereas p53 mutations that are usually associated with malignancy curtail this effect (87).

Teleologically, this aberrant GLUT4 regulation is of obvious benefit to the tumor cell, since it results in enhanced glucose metabolism that facilitates tumor growth. Although this enhancement may be of minor contribution in total glucose homeostasis, understanding modes of GLUT4 upregulation in aberrant states is very important. Beyond gaining deeper insight into GLUT4 regulation in general, this knowledge can contribute to the designing of novel modes of intervention for upregulating GLUT4 expression in insulin-resistant states.

Lessons From Transgenic Animals

Studies in transgenic animals, in which GLUT4 gene has been either overexpressed or knocked down, give deep insight into the role of GLUT4 in glucose homeostasis. The results of experiments using mouse models demonstrate that modifying the expression of GLUT4 profoundly affects whole body insulin action and, consequently, glucose and lipid metabolism (19, 69). Thus, mice that were genetically engineered to overexpress the GLUT4 gene, either systemically or specifically in skeletal muscle or adipose tissue, displayed enhanced insulin responsiveness and peripheral glucose utilization, and the overexpressed GLUT4 was able to enhance insulin responsiveness in experimental models of diabetes (20). Furthermore, data from adipose-specific GLUT4 knockout mice indicate that this particular reduction in cellular GLUT4 contributes to the pathogenesis of insulin resistance in obesity and diabetes, probably by altering the release of novel adipocyte-secreted molecules such as retinol binding protein-4 (34, 108). In accordance with these observations, breeding muscle GLUT4 knockout (MG4KO) mice to mice overexpressing GLUT4 in adipose tissue (AG4Tg) normalized the fasting hyperglycemia and overall glucose intolerance in MG4KO mice (16).

A Novel Paradigm to Increase Insulin Sensitivity

The GLUT4 gene is subject to complex tissue-specific and metabolic regulation that has a profound impact on insulin-mediated glucose disposal. This regulation is of special clinical interest, because insulin-mediated glucose homeostasis is highly sensitive to the levels of GLUT4 protein in muscle and adipose tissue. For this reason, the mechanisms regulating expression of the GLUT4 gene have been intensively studied over the past decade. On the basis of all the data gathered in our laboratory, we suggest a novel paradigm to increase insulin sensitivity in adipocytes, which connects together the regulatory effects of FOXO1 and PPARγ on their downstream targets in bona fide insulin-responsive adipocytes. According to this paradigm, FOXO1 represses transcription from both PPARγ1 and PPARγ2 promoters, leading to reduced expression of these transcription factors. Since PPARγ1 and PPARγ2 receptors repress GLUT4 transcription, this would lead to curtailing PPARγ transrepression effect. Thus, directly or indirectly, FOXO1 induces the derepression and/or activation of GLUT4 gene expression, which subsequently results in enhanced insulin sensitivity. We found in human cardiac muscle that FFA-induced repression of PPARγ gene expression might protect the GLUT4 gene from further dampening of its expression. Thus modulation of PPARγ gene expression by FFA and/or TZDs might, in addition, influence the inflammatory process through IKK/NF-κB signaling and subunit DNA binding.
We believe that further unveiling the mechanism(s) that regulate GLUT4 gene expression in diabetes and obesity will hopefully result in effective ways to improve overall insulin sensitivity and overcome insulin resistance.

GRANTS
This work was supported in part by Israel Science Foundation of the Israel Academy of Science and Humanities Grant 358/99-2, the L. R. Diamond Fund, D-Cure, the Russell Berrie Foundation, Israel-U.S. Binaional Fund Grant 2003238, and Technion-Israel Institute of Technology’s Vice President for Research Fund.

REFERENCES
Perspectives

TRANSCRIPTIONAL REGULATION OF GLUT4


