[Frontiers in Bioscience 9, 201-215, January 1, 2004]

GLUCOSE TRANSPORT IN THE HEART

E. Dale Abel

Division of Endocrinology, Metabolism and Diabetes and Program in Human Molecular Biology and Genetics, University of Utah, Salt Lake City, Utah, USA

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1. ABSTRACT

The heart is a unique organ in many ways. It consists of specialized muscle cells (cardiomyocytes), which are adapted to contract constantly in a coordinated fashion. This is vital to the survival of the organism given the central role of the heart in the maintenance of the cardiovascular system that delivers oxygen, metabolic substrates and hormones to the rest of the body. In order for the heart to maintain its function it must receive a constant supply of metabolic substrates, to generate ATP to maintain contractile function, without fatigue. Thus the heart is capable of utilizing a variety of metabolic substrates and is able to rapidly adapt its substrate utilization in the face of changes in substrate supply. The major metabolic substrate for the heart is fatty acids. However, up to 30% of myocardial ATP is generated by glucose and lactate, with smaller contributions from ketones and amino acids. Although glucose is not the major metabolic substrate in the heart at rest, there are many circumstances in which it assumes greater importance such as during ischemia, increased workload and pressure overload hypertrophy. Like all other cells, glucose is transported into cardiac myocytes by members of the family of facilitative glucose transporters (GLUTs). In this regard, cardiomyocytes bear many similarities to skeletal muscle, but there are also important differences. For example, the most abundant glucose transporter in the heart is the GLUT4 transporter, in which translocation to the plasma membrane represents an important mechanism by which the net flux of glucose into the cell is regulated. Because cardiomyocytes are constantly contracting it is likely that contraction mediated GLUT4 translocation represents an important mechanism that governs the entry of glucose into the heart. While this is also true in skeletal muscle, because many muscles are often at rest, insulin mediated GLUT4 translocation represents a quantitatively more important mechanism regulating skeletal muscle glucose uptake than is the case in the heart. In contrast to skeletal muscle, where most GLUT1 is in perineural sheaths (1), in the heart there is significant expression of GLUT1 (2), which under certain circumstances is

responsible for a significant component of basal cardiac glucose uptake. This review will summarize the current state of knowledge regarding the regulation of glucose transporter expression, and the regulation of glucose transport into myocardial cells.

2. GLUCOSE TRANSPORTER EXPRESSION IN THE HEART

The predominant glucose transport isoforms expressed in the heart are GLUT1 and GLUT4. The GLUT1/GLUT4 ratio in rat hearts varies from 0.1-0.6 (3). Each of these isoforms is subjected to developmental and hormonal regulation, and their expression is altered in various pathophysiological states. Significant advances have also been made towards understanding the transcriptional mechanisms that regulate their expression (see below). Other members of the glucose transporter family have been reported to have variable expression in the heart. GLUT3 protein was reported to be present in adult and fetal human hearts. GLUT3 expression was first noted in 10-week fetal hearts, peaking at 15 weeks and then declining (4). This contrasts with an earlier western blot study showing GLUT3 in testis and brain but not in the human heart (5). In rat cardiomyoblasts GLUT1, 3 and 4 were noted to be present but GLUT3 contributed more to high affinity hexose transport than GLUT1 (6). Taken together, these data suggest a more predominant role for GLUT3 during cardiac development. Four recently described members of the glucose transporter family have also been noted in cardiac cells. GLUT12 exhibits 29% homology with GLUT4 and shares similar carboxyl and amino terminal dileucine motifs. It is expressed in insulin sensitive tissues such as adipose tissue and muscle and the mRNA is highly expressed in adult human hearts (7). Immunohistochemical analysis of developing rats, reveal that GLUT12 expression occurs in the heart at embryonic day 15, and persists thereafter (8). GLUT12 may translocate in response to long-term insulin in MCF-7 breast cancer cells. Thus GLUT12 may represent a second important insulin-responsive glucose transporter in the heart. GLUT11 is expressed in the heart and skeletal muscle, and is predominantly located in the plasma membrane and mapped to human chromosome 22q11.2 (9). It is mainly homologous to GLUT5 (a fructose transporter) and unlike GLUT4 the ability to transport glucose is markedly inhibited by fructose (10). Thus GLUT11 might be functioning as a dual transporter of fructose and glucose in the heart. GLUT8 is 29.4% identical to GLUT1 and is mapped to human chromosome 9. It is mainly expressed in the testis but lower level mRNA expression was observed in the heart, skeletal muscle, brain and small intestines (11). Human GLUT 10 mRNA is widely expressed (lung, brain, liver, skeletal muscle, pancreas, placenta and kidney) and is also expressed in the human heart (12). The subcellular distribution and protein content in the heart remain to be elucidated.

2.1. GLUT1

2.1.1. Transcriptional and Developmental Regulation

In the embryonic heart, GLUT1 is highly expressed and is more abundant than GLUT4. However shortly after birth there is a developmental change in expression so that levels of GLUT1 fall as levels of GLUT4 increase. In the embryonic heart GLUT1 expression (mRNA and protein) peaks at E 9.5 and gradually declines to E13.5. It is the major glucose transporter in the neonatal heart and even in utero its expression can be regulated; for example GLUT1 protein content in the heart is increased by maternal/fetal hypoglycemia (13). The most extensively characterized transcriptional regulators of GLUT1 expression in the heart have been the SP1and SP3 transcription factors. SP1 is a positive regulator and SP3 a repressor of GLUT1 expression in the heart. GLUT1 promoter sequences between -99/-33 of the GLUT1 gene are sufficient to drive transcriptional activity in neonatal rat cardiomyocytes. SP1 binds to the –102/-82 region of the promoter during late fetal life. There is marked downregulation of SP1 expression during early neonatal life and minimal expression/binding during adulthood (14). The activity of SP3 is dominant over that of SP1in the regulation of GLUT1 expression. Thus the increased ratio of SP3/SP1 during myogenesis is associated with decreased expression of GLUT1. A similar increase in this ratio is also seen following forced expression of MyoD (15). MyoD promotes GLUT4 expression. These findings therefore provide mechanistic insight into the divergent regulation of GLUT1 and GLUT4 in cardiomyocytes.

2.1.2. Hormonal Regulation

Fasting is associated with decreased levels of glucose and insulin and increased levels of free fatty acids (FFA). Cardiac utilization of fatty acids increases and glucose utilization falls. This is associated with a decline in GLUT1 expression. After 48-hours of fasting, basal glucose uptake and GLUT1 expression are decreased by 90 and 60% respectively in rat hearts. However GLUT 4 expression and insulin mediated glucose uptake did not change, indicating that GLUT4 is the major regulator of insulin stimulated glucose uptake in the heart (3). Preventing the fasting-induced decline in insulin levels by hyperinsulinemic clamp blocks the fall in GLUT1. Hyperinsulinemic euglycemia leads to upregulated GLUT1 content that was blocked either by raising glucose concentration or increasing FFA concentration (16). An important role for insulin in the regulation of myocardial GLUT1 content is also supported by the observation that cardiac content is of GLUT1 and basal glucose uptake in isolated cardiomyocytes is significantly reduced in hearts from mice with cardiomyocyte-restricted deletion of the insulin receptor (17).

2.1.3. Changes in Pathophysiologic States

Despite the developmental downregulation of GLUT1 expression in the heart, there are a variety of pathophysiological circumstances in which GLUT1 expression is reinduced in the heart. Chronic left ventricular hypertrophy (LVH) in rats is associated with increased total GLUT1 and decreased total GLUT4 and is associated with increased distribution of both transporters to the plasma membrane and increased activity of AMP Kinase (alpha 1 and alpha 2). Basal glucose uptake was increased but insulin-mediated glucose uptake was similar in banded and control hearts (18). Activation of the GLUT 1 promoter in response to hypertrophic stimuli in cardiomyocytes is mediated in part via the ras-mapk pathway.

There is partial inhibition by wortmannin (19), indicating involvement of some components of the PI3-Kinase pathway that cross-talks with the MAP Kinase pathway. There are important species differences however in the transcriptional response of hearts to LVH, and differences that depend on whether or not hearts are in a state of compensated hypertrophy or have transitioned to heart failure. Thus, in neonatal pressure overload hypertrophy in rabbits there is reduced glucose uptake without any change in the expression of GLUTs (20). In a post-myocardial infarction (MI) rat model, early LVH (compensated) was not associated with any change in the expression of GLUT1, although there is downregulation of medium chain acyl CoA dehydrogenase (MCAD) expression. After the onset of heart failure upregulation of GLUT1 expression occurs (21). Finally, in severely failing human hearts there is downregulation of GLUT1, GLUT4, muscle carnitine palmitate transferase –1 (mCPT-1) and uncoupling protein 3 (UCP3). Only UCP3 expression was reversed by attempts to increase cardiac function with left ventricle assist devices (LVAD) (22).

Chronic or intermittent myocardial ischemia is also associated with increased expression of GLUT1 in the heart. For example, chronic myocardial ischemia in a canine model was associated with increased GLUT1 mRNA and protein in both ischemic and non-ischemic portions of the heart with a trend towards higher GLUT1 in non-ischemic versus ischemic portions of the heart (23). In this model there was no effect of chronic ischemia on the expression levels of GLUT4. Similarly, low flow ischemia/reflow in pig hearts leads to a 2-fold increase in GLUT1 expression in contrast to a more modest 1.5 fold increase in GLUT4 (24). Chronic hypobaric hypoxia induces right ventricular (RV) hypertrophy but not LV hypertrophy in the rat heart. This is associated with a 3-fold increase in GLUT1 in the RV and a 1.5 fold increase in the LV. In the RV, GLUT4 mRNA and protein declined by 26% and 54% respectively (25). The mechanisms for the ischemia/hypoxia mediated increase in cardiac GLUT1 expression have not been elucidated, but candidate regulatory mechanisms could include AMP kinase that clearly plays a role in pressure overload hypertrophy and the hypoxia inducible factor (HIF-?lpha), that is known to bind to the GLUT1 promoter in other cell-types (26, 27).

Two other states that have been associated with induction of GLUT1 expression in the heart are inflammation/oxidative stress, and denervation or unloading of the heart. Oxidative stress induced by doxorubicin administration, increased GLUT1 expression and basal glucose uptake (28). In inflammatory (autoimmune) myocarditis there is increased glucose uptake on the basis of increased GLUT1 expression (29). Chronic unloading of the heart results in the re-expression of fetal genes such as myosin heavy chain –beta (beta-MHC) and GLUT1 in a fashion that is analogous to that seen with pressure overload hypertrophy (30) In the denervated heart in vivo, there are an upregulation of GLUT1 and a downregulation of PDH activity (31).

2.2. GLUT4

2.2.1. Transcriptional and Developmental Regulation

As noted above, GLUT4 is expressed predominantly in the post-natal and adult heart (32). GLUT4 protein expression is detectable on day 21 of gestation and increases progressively at birth to adult levels at day 15. mRNA was initially reported to be detectable at E 17, remaining relatively flat and then increasing at post natal day 10 (33). Using in-situ hybridization histochemistry, Vannuci et al demonstrated that GLUT 4 is transiently expressed in the developing heart between embryonic days 10-12, particularly in the endocardial cushions (34). Once adult levels of GLUT4 are achieved, whether or not GLUT4 levels decline with age depends on the models in which this has been examined. For example, a 4-5 fold increase in cardiac GLUT4 content was noted in the hearts of C57 Bl6 mice between mid-late adulthood (35). In contrast, there is a progressive decline in myocardial GLUT4 content in Fischer 344/Brown Norway F1 hybrid rats as they age (36, 37).

The increase in GLUT4 content that occurs in the transition from the embryonic to the neonatal heart is in part mediated at the level of gene transcription. MEF2 and TR-alpha binding are required for transcriptional activation of GLUT4 in cardiac muscle. MyoD may also play a cooperative role in skeletal muscle. In cells in which GLUT4 is not expressed, forced- expression of MEF2 and MyoD results in GLUT4 expression (38). In L6 cells that do not normally express GLUT4, forced expression of PGC-1 induced GLUT4 mRNA by co-activating MEF2-C (39). MEF2-A/MEF2-D were found to be the predominant heterodimers responsible for regulation of the GLUT4 promoter fragment that was responsive to insulin deficient diabetes in rats (40). In cardiomyocytes MEF 2A was reduced in diabetes while MEF-2D was reduced in adipocytes. However overexpression of MEF-2A rescued the downregulation that occurred in diabetic adipocytes indicating that the MEF2 transcription factors are functionally interchangeable (40).

Other transcription factors are involved in the regulation of GLUT4 expression in the heart. Thus, a GLUT4 promoter minigene (-423) that lacks the MEF2 binding site is still expressed in heart and muscle (41). However, expression of a similar construct (-412) was associated with ectopic expression of GLUT4 in the liver and brain and weak expression in cardiac muscle. The –730 construct (which contains a MEF2 biding site) though not expressed in the liver, had persistent brain expression (42). Moreover, the 730 bp GLUT4 promoter fragment although still expressed in muscle lacked the characteristic negative regulation observed with the native gene after withdrawal of insulin, and did not achieve wildtype expression levels in the heart. However, an 895 bp promoter fragment exhibited normal tissue specific and regulated expression as long as the proximal MEF2 binding site was intact (43). These observations argue for the presence of alternative regulatory mechanisms that act in a synergy with MEF2 binding to result in normal levels of cardiac GLUT4 expression. A novel binding protein that binds between –712 and –772 (domain I) has been identified and may act cooperatively with the adjacent MEF 2 binding site (44). Recently, the Kruppel-like factor KLF15 was shown to regulate the insulin-sensitive glucose transporter GLUT4 by interacting with MEF2A (45). Its

role in the regulation of GLUT4 expression in the heart remains to be fully elucidated.

Exercise and denervation are also associated with transcriptional regulation of GLUT4 in the heart and the mechanisms responsible for this might be distinct from those that govern the regulation of GLUT4 expression in the heart by insulin. Thus in studies by Tsunoda et al., the exercise responsive elements are located between -551 and -442 of the GLUT4 gene, and the element responsible for denervation induced downregulation is located downstream of –423 (41) or -730 (46). These data were supported by studies by Dohm et al, showing that the exercise element lies within the first 895 base pairs of the promoter (47), and that the increase in GLUT4 expression induced by AMPK administration required sites between –895 and –730. When a response was seen there also appeared to be increased binding to the MEF2 consensus sequence between –473 and –464 suggesting cooperativity (48).

2.2.2. Hormonal/Nutrient Regulation

GLUT4 expression in the heart is subject to regulation by nutrient flux and by thyroid hormone. Insulin withdrawal *in vivo* leads to dramatic reductions in the expression of GLUT4 in the heart that is reversed when insulin is administered. It is not yet certain if this represents a direct effect of insulin on GLUT4 gene transcription, or if these changes are secondary to the systemic metabolic effects of insulin withdrawal such as hyperglycemia and increased concentrations of free fatty acids. Increased fatty acid delivery (24 hr. intralipid), leads to reduced insulin-stimulated glucose uptake into hearts *in vivo* and decreased GLUT4 mRNA (49). Transgenic expression of PPAR-alpha in the heart leads to increase cardiac FFA metabolism and repression of GLUT4 protein and mRNA (50). 46 hours of fasting in rats induced genes involved in FA metabolism and at the same time reduced the expression of GLUT4 (despite concomitant decreases in the expression of PPAR-alpha. Interestingly, treatment with nicotinic acid during the final 8-hours of fasting normalized FFA levels, but did not change the transcriptional profile in the hearts with the exception of UCP3 (31). Taken together, these data suggest that increased fatty acid flux may contribute to the changes in cardiac GLUT4 expression that occur in the context of systemic reductions in insulin concentrations, but may not necessarily be the sole regulatory factor. A direct role for insulin deficiency per se is less certain given the preservation of GLUT4 protein in 48 hour fasted rats and the absence of any changes when euglycemic hyperinsulinemia is maintained during the fast (16). Indeed, in fetal sheep, 24 hours of euglycemic hyperinsulinemia decreased GLUT4 protein content (51). Chronic undernutrition reduces total cardiac content of GLUT1, GLUT4 and GLUT3, but there is increased distribution of GLUT1 to the plasma membrane and enhanced insulin signaling that leads to increased basal and insulin-stimulated glucose uptake (*in vivo*) (52).

Thyroid hormone is an important regulator of cardiac glucose utilization and cardiac GLUT4 expression. Thyroid hormone is believed to play an important role in the developmental increase in GLUT4 expression that occurs shortly after birth. Perinatal and neonatal hypothyroidism are both associated with persistent expression of GLUT1 and decreased expression of GLUT4 in the heart (53). T_4 and retinoid administration to hypothyroid neonatal rats leads to a brisk increase in GLUT4 expression within 1 hour of T_3 administration and a reduction in GLUT1 expression (54). Similarly, in long-term cultured adult rat cardiomyocytes (cultured in the absence of T_3), T_3 administration increases the expression of GLUT4 mRNA (2.8 fold), increases basal glucose uptake by 1.95 fold and insulin-mediated glucose uptake by 1.75 fold. Basal and insulin stimulated glycogen synthesis was also increased (55). The downregulaion of GLUT4 expression in hypothyroidism appears to be a characteristic of neonatal or cultured cardiac cells, as GLUT4 expression in adult cardiomyocytes has not been shown to consistently respond to changes in thyroid hormone status (37, 56, 57). The direction of the response of GLUT4 in immature cardiac cells to hypothyroidism is subject to tissue specific differences in regulation as exemplified by the concomitant induction of GLUT4 expression by hypothyroidism in brown adipocytes (54).

2.2.3. Changes in Pathophysiologic States

Diabetes, cardiac hypertrophy and heart failure are the pathophysiological states in which altered expression of cardiac GLUT4 have been most widely examined.

A consistent observation in most models of diabetes (type 1 or type 2) is reduced expression of GLUT1 and GLUT4 in the heart. The decline in mRNA and protein have been reported to occur as early as 3.5 days after the onset of streptozotocin (STZ) induced diabetes (58) and the reduction persists as long as the diabetes remains untreated and is associated with up to a 70% decline in the levels of these transporters and a coordinate decrease in myocardial glucose utilization (59). In models of type 2 diabetes, a reduction in glucose transporter expression also occurs, but the decline takes place over a longer period of time. Thus, in 5-week-old db/db mice very shortly after diabetes develops (5wks), GLUT4 levels in the heart are not changed (60). However by 10-weeks of age, glucose utilization is markedly impaired (61). A similar progressive phenotype was seen in studies of Zucker (fa/fa) rats. In 5-week-old rats basal glucose uptake is normal and the increase in glucose uptake following insulin or increased workload was reduced. However, when both stimuli were simultaneously applied the response matched controls. However at 15 weeks, the response of obese mice to any stimulus was significantly blunted relative to controls (62). These findings reflect the combined effect of altered transporter expression, diminished insulin signaling (63, 64) and impaired trafficking (see below). In models that do not progress to diabetes such as during the evolution of obesity in the MSG treated mouse, there is no change in cardiac GLUT4 protein after the onset of obesity. GLUT4 expression only falls after 7-months of age (65).

The mechanism for the decline in glucose transporter expression in the hearts of diabetic animals is not known. One possibility is a direct effect of loss of

insulin signaling. This is based on the observation that treatment of animals with insulin deficient diabetes at early time points after the onset of diabetes results in normalization of the altered expression of glucose transporters. However, treatment with insulin will normalize hyperglycemia and the other metabolic defects such as increased FFA and ketones that are associated with diabetes. Indeed, in mice with cardiomyocyte-selective deletion of the insulin receptor, GLUT4 protein content is increased and GLUT1 content is decreased. These observations suggest that insulin may have divergent effects on the regulation of cardiac glucose transporter content (17). A number of interventions have been shown to reverse the decline in GLUT4 expression that occurs in diabetes. Exercise ameliorated the decline in sarcolemmal GLUT4 in STZ rats from 84% decrease to 50%, and prevented the decline in GLUT4 mRNA (vs. 58% reduction in non-trained diabetic rats) (66). In a similar study, exercise partially ameliorated the decline in GLUT4 and partially reduced glucose levels. A correlation between GLUT4 levels and blood glucose concentrations was observed. Intriguingly, GLUT1 levels fell further with exercise (67). It is also of interest to note that exercise does not alter cardiac GLUT4 expression in non-diabetic animals (68). A single dose of vanadium normalizes blood glucose (24-hours later). This apparently is sufficient to normalize the levels of GLUT4 protein (69). Similarly, 10-weeks of vanadyl sulphate administered to STZ rats prevented the decline in GLUT4 protein and mRNA (70). 2 weeks of recombinant insulin like growth factor-1 (rIGF-1) administered to rats with STZ diabetes reverses/normalizes the reduced expression of GLUT4 protein and mRNA. It should be noted rIGF-1 normalized blood glucose in these studies (71). Finally, treatment of diabetic rats with an angiotensin-1 (AT-1) receptor antagonist but not an angiotensin converting enzyme inhibitor, reverses the diabetes induced decline in cardiac GLUT4 protein by unknown mechanisms (72). Taken together, these observations indicate that the reduction in cardiac GLUT4 content might be a consequence of the systemic metabolic disturbances induced by diabetes (such as hyperglycemia or increased myocardial FFA delivery). While it is clear that altered binding of transcription factors that regulate the GLUT4 promoter such as MEF2 are involved, the specific signaling mechanisms linking diabetes and downregulation of GLUT4 expression are unknown.

The question then arises regarding the functional consequence of decreased GLUT4 and GLUT1 expression in the diabetic heart. One of the difficulties in evaluating this arises from the fact that in addition to impaired glucose uptake, there are other defects that exist in the diabetic heart such as altered glucose and fatty acid oxidation, that may influence cardiac performance independently of changes in glucose transport. For example, in diabetic pigs, there is a 50-65% reduction in GLUT1 and GLUT4, was associated with decreased glycolysis and decreased lactate uptake. Following dobutamine induced increase in workload there was an increase in glycolysis in diabetics and non-diabetics, but a marked defect in glucose oxidation was seen in diabetics under inotropic stress (73). However, glucose uptake during ischemia was diminished (74). A more direct demonstration of the consequences of decreased cardiac GLUT4 expression was obtained in studies performed in mice with cardiomyocyte-restricted deletion of GLUT4. These hearts demonstrated impaired functional recovery after ischemia that may be related to the inability to maintain glycolysis (75). Thus one consequence of reduced expression of GLUT4 in the heart is impaired recovery from ischemia. This is supported by findings from other models such as transgenic mice with overexpression of A1 adenosine receptors whose enhanced tolerance to ischemia is associated with increased expression of GLUT4, genes involved in anti-apoptosis and ion channels (76). The transfer of activated Akt to rat hearts using adenoviral gene transfer also leads to increased sarcolemmal GLUT4 expression (77) and diminished susceptibility of the myocardium to ischemic injury.

In hypertrophied hearts from SHR rats, basal glucose uptake is increased 3-fold but insulin-mediated glucose uptake is reduced (1.4 vs. 5-fold in controls), and is associated with decreased GLUT4 mRNA (78). In humans with compensated LV hypertrophy, secondary to aortic stenosis basal glucose uptake is normal but insulin-mediated glucose uptake was impaired. This was associated with a reduction in the ratio of GLUT4/GLUT1 protein (79). In failing hearts from humans, expression of a variety of genes involved in substrate utilization such as PPAR-alpha, MCAD (medium chain acyl CoA dehydrogenase) and UCP3 (uncoupling protein 3) and alpha-MHC are repressed, but GLUT4 expression was not decreased. In failing hearts from diabetics there was additional repression of PPAR-alpha, MCAD and alpha-MHC and in these hearts the expression of GLUT4, and SERCA2 (sarcoplasmic endoplasmic reticulum Ca^{2+}/Mg^{2+} ATPase) were also reduced. Interestingly, PDK4 (pyruvate dehydrogenase kinase) expression was increased most markedly in the diabetics. GLUT4, SERCA2 and ? lpha-MHC are MEF2C regulated genes and indeed MEF2 protein levels were depressed to the greatest extent in the failing-diabetic hearts (80). In fetal and failing human hearts, the expression patterns of myosin isoforms and metabolic genes are regulated in a similar fashion. Thus both GLUT4 and GLUT1 expression are repressed in fetal and failing human hearts relative to normal hearts (81).

3. REGULATION OF GLUCOSE TRANSPORT IN THE HEART

3.1. Glucose Transporter Trafficking and Insulin Mediated Glucose Uptake

In many ways, the trafficking and translocation of GLUT4 in cardiac muscle is similar to that observed in skeletal muscle. There are some differences that are unique to the heart, and these will be highlighted in this section. Early studies suggested that very similar protein patterns were observed in immunoabsorbed GLUT4 vesicles obtained from heart, skeletal muscle, primary adipocytes and 3T3L1 adipocytes (82). As in skeletal muscle and adipose tissue, GLUT4 and IRAP (insulin-regulated aminopeptidase) are co-localized in the same vesicles in cardiomyocytes that reside in tubulovesicular structures that are close to the plasma membrane. Approximately 50%

of this intracellular GLUT4 translocates from tubulovesicular structures close to the sarcolemma, T-tubules and trans-golgi to the plasma membrane and T-tubules in equal proportions. The non-translocated GLUT4 remains in these structures as well as near to intercalated disks (83). Like skeletal muscle, insulin causes GLUT4 translocation and increases the activation of GLUT4 transporters in the heart (84). In addition, insulin stimulates the translocation of GLUT1 in cardiomyocytes and the translocation of both transporters by insulin in blocked my wortmannin (85). Increased workload also increased GLUT4 translocation (86). Moreover, GLUT4 vesicles from cardiomyocytes express the v-snare protein cellubrevin/VAMP3, and the t-SNARE proteins SNAP 25, Syntaxin 1 A and Syntaxin 1 B (87), indicating similarities between skeletal muscle and adipocytes in the cellular machinery that could regulate GLUT4 vesicle fusion to the plasma membrane. Recent data in VAMP3 null mice indicate however that VAMP3 is dispensable for GLUT4 vesicle recycling in adipocytes and muscle cells (88).

Evidence exists for the presence of two pools of GLUT4 transporters in cardiomyocytes. The large insulin responsive or storage pool (pool 1) contains predominantly GLUT4 and the second pool (pool 2), is believed to be an endosomal pool that is enriched for GLUT1 and SCAMP 39 but also contains some GLUT4. Rotenone recruits GLUT1 and GLUT4 from pool 2, but does not recruit GLUT4 from pool 1. In contrast, insulin recruits all of GLUT1 from pool 2 and GLUT4 from pool 1 but a second insulin challenge (at a time when pool 1 is depleted) mobilizes GLUT4 from pool 2, raising the possibility that a proportion of GLUT4 from the insulin responsive pool may transit through the endosomal compartment. Insulin withdrawal leads to reentry of GLUT1 and GLUT4 transporters to the respective pools from which they translocated (2, 89). Similar findings have been reported in skeletal muscle (87). Additional evidence for the existence of distinct pools from which GLUT4 can be translocated comes from studies examining glucose uptake in response to insulin or alpha and beta-adrenergic stimulation in perfused hearts. Perfusion of hearts with the golgi inhibitor brefeldin A, increased basal glucose uptake and blocked the increase in glucose uptake induced by insulin or alpha-adrenergic stimulation. In contrast, the ability of beta-adrenergic stimuli to increase glucose uptake remained intact implying differences in signaling or in the nature of the pools from which glucose transporters are translocated (90). In atrial cardiomyocytes there is a small pool of GLUT4 that is associated with secretory granules (specifically ANF granules) (91). In addition, these GLUT4 molecules enter the ANF secretory granule in the trans-golgi reticulum arriving there via the recycling as opposed to the biosynthetic pathway (92).

3.2. Glucose Transporter Trafficking and Glucose Transport in Diabetes and Insulin Resistant States

In section 2.2.3., data addressing changes in the expression of glucose transporters in diabetes was presented. In this section evidence for altered trafficking of glucose transporters in insulin resistant states will be presented. Impaired recruitment of GLUT4 from microsomal membrane fractions to the plasma membrane has been demonstrated in the hearts of Zucker fa/fa rats. This is associated with impaired or absent insulin-mediated recruitment of various low molecular weight GTP binding proteins such as rab4A from cytosolic fractions and rab11 and rab3C from microsomal fractions respectively to the plasma membrane (93-96). Because these GTP binding proteins may play a role in the regulation of GLUT4 trafficking (97), it has been postulated that insulin resistance may alter glucose transporter translocation in the heart by altering the trafficking of these molecules. In addition to defects in trafficking, insulin signaling is also perturbed in the hearts of insulin resistant mice as evidenced by impaired association of the IR-IRS1 complex with the P85-? eta subunit of PI-3 Kinase (64).

Some studies have sought to determine whether or not therapeutic agents that are used in the treatment of diabetes and insulin resistance may directly alter glucose transport in cardiomyocytes. PPAR-gamma expression has been demonstrated in cardiomyocytes and was ~ 30% as abundant as in adipocytes. Acute exposure of adult ventricular cardiomyocytes to thiazolidinediones (TZDs) MCC-555 and troglitazone leads to increased insulin stimulated glucose uptake without affecting basal glucose uptake, an effect that is blocked by inhibiting protein synthesis with cycloheximide. Exposure of insulin resistant cardiomyocytes obtained from obese Zucker rats to these TZDs also increased insulin sensitivity and restored glucose transport. This was associated with a reduction in the serine phosphorylation of IRS-1 (98). After 20 hours of incubation, there is a 3-fold increase in protein content of GLUT1, 1.5 fold increase in GLUT4 protein content and a 2-fold increase in GLUT4 abundance in plasma membranes that is associated with increased basal cardiac glucose uptake (99).

Metformin administered *in vivo* does not alter (or increase already depressed rates of) basal or insulin-mediated glucose uptake in cardiomyocytes isolated from STZ diabetic rats. *In vitro* exposure to very high doses (1mM) did increase GLUT1 and GLUT4 content on the plasma membrane and increased basal glucose uptake. In non-diabetic myocytes the effect of metformin and submaximal insulin doses were additive. High dose metformin decreased MVO_2 and the content of high-energy phosphates implying that the mechanisms may be related to a “hypoxia mimicking” phenotype (100). At age 18 weeks Zucker (fa/fa) rats are hyperinsulinemic and have impaired glucose tolerance. GLUT4 translocation is largely absent in the hearts of these mice. Administration of acarbose for 12 weeks from the age of 6 weeks ameliorates the insulin resistance and impaired glucose tolerance (IGT), and this is associated with normalization of GLUT4 translocation and insulin-stimulated Akt-phosphorylation in the heart (101). Vanadate will stimulate glucose uptake in wildtype cardiomyocytes to about 50% of the levels seen with maximal insulin stimulation and is not additive with insulin. In Zucker fa/fa rats, basal, insulin or vanadate induced glucose uptake were reduced, as was the associated increase in Akt phosphorylation. In contrast to lean controls, in obese mice the effect of insulin and vanadate

were additive in terms of augmenting Akt phosphorylation and glucose transport to levels that exceeded, those observed with insulin alone (102). In perfused hearts glyburide increases cardiac glucose transport and phosphofructokinase activity and also augments the responsiveness of the heart to insulin (103, 104). Taken together these data suggest that therapeutic strategies used in the treatment of insulin resistance and type-2 diabetes will reverse some of the acquired defects in glucose transport and transporter trafficking that occurs in these models.

3.3. Catecholamines and Cardiac Glucose Transport

Activation of cardiac alpha and beta-adrenergic receptors by their respective ligands increases cardiac glucose uptake and the translocation of GLUT1 and GLUT4 in perfused hearts (105, 106). The increase in glucose uptake occurs independently of changes in contractility (107), and is also observed in isolated cardiomyocytes (108, 109). The activation of glucose transport by alpha-adrenergic stimulation is inhibited by wortmannin, but beta-adrenergic stimulation of glucose uptake occurs independently of PI-3 Kinase activation. The effects of alpha-adrenergic and insulin stimulation were additive suggesting activation of distinct PI-3 Kinase pools (107). *In vivo*, VMH stimulation acting via the sympathetic nervous system, increases cardiac glucose uptake independently of increasing GLUT translocation, and may act by increasing transporter intrinsic activity (110).

3.4. Myocardial Ischemia and Cardiac Glucose Transport

As is the case in skeletal muscle, ischemia will induce translocation of GLUT4 (and GLUT1) to the sarcolemma (85, 111). GLUT translocation is induced by global (no flow) as well as less severe low flow ischemia (112-114). This is associated with increased cardiac glucose transport, that if blocked by cytochalasin B will lead to more rapid functional deterioration (114), similar to what is observed in mice with cardiomyocyte deletion of GLUT4 (75). The increased glucose uptake may persist however during reperfusion even after the translocated GLUT4 transporters become internalized (112). The persistent increase in glucose uptake may be due to increased activity of hexokinase (115). In severe global ischemia, there is evidence that intrinsic activity or complete membrane insertion of the translocated GLUT4 may be severely impaired, as under these circumstances some workers have failed to detect an acute increase in glucose transport (116).

The mechanisms responsible for ischemia-mediated glucose transport are distinct from that regulate insulin-stimulated GLUT4 translocation in that it is not inhibited by wortmannin, and under some circumstances may be additive to insulin-mediated GLUT4 translocation (117). Activation of AMP Kinase (AMPK) is believed to be an important mediator of this response. This is based on the observations that hypoxia and administration of AICAR (5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside - an activator of AMPK), causes GLUT4 translocation and increases glucose uptake via PI3-Kinase independent pathways. Moreover, AMPK inhibition blocked hypoxia mediated GLUT translocation and glucose uptake (118). Studies in mice with transgenic inactivation of AMPK in the heart, also suggests that these mice are more susceptible to ischemic injury, which may be related to impaired GLUT4 translocation and reduced glucose uptake following ischemia (119). The signaling mechanisms responsible for the activation of AMPK in the ischemic heart remain to be completely elucidated, but given the fact that very similar effects are seen following inhibition of oxidative phosphorylation (120), it is likely that the major stimulus is an increase in the AMP/ATP ratio. There is some evidence that alpha-adrenergic blockade will inhibit ischemia mediated GLUT4 and GLUT1 translocation (105). However, another group reported that complete adrenergic blockade did not block the post-ischemic increase in glucose uptake (121). Because GLUT translocation was not determined in this latter study, it is unclear if the increase in glucose uptake occurred despite impaired GLUT translocation in the hearts exposed to ischemia and adrenergic blockade.

3.5. Cardiac Workload, Cardiomyocyte Contraction and Glucose Transport

The mechanisms regulating the translocation of GLUT4 in skeletal muscle in response to contraction are believed to involve activation of AMPK. The distinction between skeletal muscle and cardiac muscle is the fact that *in vivo*, the heart is constantly contracting, rendering it more difficult to discern if contraction mediated translocation of GLUT4 plays a role in mediating basal cardiac glucose uptake. In mice with cardiomyocyte ablation of the insulin receptor, a 2-fold increase in GLUT4 protein abundance is observed. This is associated with increased cardiac glucose uptake in isolated working hearts and in vivo, despite significant reductions in cardiac GLUT1 content (17). In contrast, basal glucose uptake is markedly reduced in non-contracting, isolated cardiomyocytes from these mice, suggesting that in the quiescent myocytes, most of the GLUT4 is in an intracellular location. Also, transgenic mice that overexpress GLUT4 (using its endogenous promoter) have increased rates of glycolysis in isolated working hearts (122). These data suggest that GLUT4 may contribute to basal glucose uptake in the contracting heart. Very few studies have addressed the mechanism for contraction mediated GLUT4 translocation in isolated cardiomyocytes. Field stimulation of rat cardiomyocytes @ 1Hz has no effect on basal or insulin-mediated glucose uptake, but 5Hz stimulation is associated with increased plasma membrane GLUT4 content and a 2-fold increase in glucose uptake, that does not increase further with the addition of insulin (123). Intriguingly, this group also reported that the increase in GLUT4 translocation in field-stimulated cardiomyocytes could be inhibited by wortmannin, and that a 200kDa protein could be co-immunoprecipitated with the p85-?lpha subunit of PI-3 Kinase, but the complex was not associated with any members of the proximal insulin signaling machinery (124). These findings are in contrast to the wortmannin insensitivity of GLUT4 translocation in response to contraction in skeletal muscle (125).

Increasing workload in the intact contracting heart is also associated with increased glucose transport and

Table 1. Intracellular mediators/modulators of cardiac glucose transport

Mediator	Effect on glucose transport
Cyclic GMP	Impairs basal and insulin or hypoxia-mediated glucose uptake, due in part to impaired GLUT4 redistribution from the intracellular pool to the cell surface. This effect is antagonized by cyclic AMP (142).
Alpha -Lipoic Acid	Products of the lipo-oxygenase pathway are involved in insulin-stimulated actin reorganization. Specific blockade of this pathway inhibits actin reorganization and insulin-mediated GLUT4 translocation in cardiomyocytes (143). The stimulation of glucose transport in cardiomyocytes by ? lpha-lipoic acid is mediated via PI-3 Kinase and is not additive to that of insulin (144).
Serotonin	Serotonin causes translocation of GLUT4 (1.5 fold) and GLUT1 (1.8 fold) and a 3.5 fold increase in glucose transport in rat cardiomyocytes. This requires the generation of H_2O_2 by monoamine oxidase (145). Tyramine mediates glucose uptake by similar mechanisms (146).
Bradykinin	Perfusion of isolated hearts with bradykinin increases GLUT4 and GLUT1 translocation (147). Bradykinin is the likely mediator of the increase in glucose transport by angiotensin converting enzyme-inhibitors (148).
Endothelin-1	Endothelin-1 acting via the ET (A) receptor causes GLUT4 translocation and increases glucose uptake, via mechanisms that are independent of the activation of PI3-Kinase, PKC and PKA, but is associated with increased ERK phosphorylation (149).
Lactate	Lactate is an important cardiac substrate. It promotes translocation of GLUT4 and GLUT1 (as determined by immunoblotting) in isolated perfused heart via PI3-Kinase independent mechanisms. Despite GLUT translocation, there is no increase in glucose transport suggesting that an activation step might have been inhibited (150).
Intracellular pH	Cytosolic alkalinization by insulin is important in the final stages of docking, fusion and activation of GLUT4 transporters in cardiomyocytes (151).

increased translocation of glucose transporters (86, 106, 126). The mechanism for increased glucose uptake and GLUT4 translocation in response to increased cardiac work are incompletely understood. However, a recent study demonstrated that a graded exercise protocol was associated a progressive increase in cardiac AMPK activity, and increased sarcolemmal GLUT4 (127) implicating AMPK as the mediator of this effect. Additional mechanisms for the increased glucose uptake in response to increased workload likely exist and may include a PI3 Kinase dependent increase in PFK-2 activity that occurs independently of any change in AMPK activity (128).

3.6. Substrate Utilization and the Regulation of Cardiac Glucose Transport

The heart possesses enormous metabolic flexibility. Because of its continuous need to generate ATP in order to maintain contractile function, the heart is able to utilize almost any substrate with which it is presented. Thus substrate utilization and metabolic flux rates are coordinately regulated so as to maintain a tight balance between substrate availability and ATP generation. Although the most widely studied regulation relates to the inverse relationship between mitochondrial fatty acid oxidation and glucose oxidation (Randle's cycle), important substrate derived regulation also takes place at the level of glucose transport. The presence of alternative substrates reduces glucose transport and the abundance of GLUT4 transporters in the sarcolemma of sub-maximally insulin-stimulated or phenylephrine-stimulated cardiomyocytes. There is an inverse correlation between citrate and malate levels and glucose transport. Thus, activation of the TCA cycle by substrates that increase the concentration of TCA cycle intermediates, negatively regulate glucose transport in the heart, independently of changes in PDH (129). In isolated perfused hearts, the presence of alternative oxidizable substrates reduces glucose utilization. One mechanism for this may involve reductions in cyclic AMP (cAMP) because maintaining high levels of cAMP blocks the inhibition of glucose utilization by alternative substrates by increasing glucose transport, increasing glycolysis (2^o to increased phosphofructokinase activity), increasing PDH and increasing glycogen phosphorylase (leading to increased glycogenolysis) (130). Long chain fatty acids such as palmitate, leads to increased intracellular retention of GLUT4 and reduced abundance of GLUT4 and GLUT1 on sarcolemmal membranes (126). Inhibition of fatty acid metabolism increase both the rates of glucose oxidation and glucose transport in isolated rat myocytes (131, 132) and the increase in glucose transport may be related to increased intrinsic activity of GLUT4 (133). In isolated perfused hearts, lipids and amino acids acutely induce a state of insulin resistant glucose uptake (134) and prolonged exposure of cardiomyocytes to ketone bodies induces insulin resistance in cardiomyocytes and impairs glucose uptake in response to various stimuli such as insulin, vanadate and PMA (phorbol 12-myristate 13-acetate) (135).

3.7. Intracellular Mediators and Other Modulators of Glucose Transport

Various other regulators of glucose transport in cardiomyocytes or perfused hearts have been described. These are summarized in table 1.

4. GLUCOSE TRANSPORTER KNOCKOUT AND TRANSGENIC MICE

4.1. Knockout Mice

Germ-line disruption of the GLUT4 gene resulted in striking cardiac hypertrophy, and impaired cardiac function. These mice were hyperinsulinemic and had profound changes in cardiac substrate delivery, thus it was uncertain if the cardiac hypertrophy was a primary or secondary consequence of GLUT4 ablation (136).

Cardiomyocyte restricted ablation of GLUT4 also resulted in cardiac hypertrophy although the degree of cardiac hypertrophy was more modest (137). The mechanisms linking GLUT4 deletion to cardiac hypertrophy are not known. Deletion of GLUT4 in hearts leads to unique metabolic adaptations. In mice with germ-line (G4-/-) as well as cardiomyocyte restricted GLUT4 deletion (G4H-/-), levels of cardiac creatine and creatine phosphate (pCr) (a storage pool for high energy phosphates in the heart), are increased (75, 138). There is also increased glucose utilization that may be facilitated in part by the compensatory upregulation of GLUT1 (75, 138). These adaptive mechanisms are maintained only in hearts from random fed mice; because after an overnight fast, levels of pCr fall as do the increased rates of glucose utilization. The energetic deficit in fasted G4H-/- contributes to impaired regeneration of ATP and severe functional impairment following low flow ischemia (75). Preliminary studies in G4H-/- hearts reveal marked elevation in rates of fatty acid utilization, the mechanisms of which remain to be elucidated (139). Taken together, these studies imply that GLUT4 is an important regulator of cardiac size and substrate utilization in the heart. Mice that are null for the insulin-regulated aminopeptidase (IRAP), have decreased expression of GLUT4 (140). The cardiac phenotype of this mouse has not yet been evaluated.

4.2. Transgenic Mice

Mice with overexpression of the human GLUT4 gene have increased rates of glucose uptake, glycolysis and glycogen. There were no differences in rates of glucose or palmitate oxidation. Insulin administration did not increase glycolysis further but the ability of insulin to increase glycogen content, increase glucose oxidation rates and to decrease palmitate oxidation rates were similar in transgenics and their controls (122). Db/Db mice have significant decrements in glucose oxidation and glycolysis, and increased rates of palmitate oxidation. Increasing GLUT4 expression in these hearts (by crossing with the human GLUT4 transgenics described above) is associated with a normalization of their metabolic profiles and restoration of normal cardiac function (61). These data suggest that increased glucose uptake into the heart could prevent the metabolic changes associated with diabetic cardiomyopathy. Cardiac restricted overexpression of GLUT1 produces a heart that has markedly increased glucose utilization that apparently protects the heart from developing heart failure that follows pressure overload hypertrophy (141).

5. CONCLUSIONS AND PERSPECTIVES

Glucose is an important metabolic substrate for the heart and assumes increased importance in the response of the heart to ischemia and in the adaptation of the heart to cardiac hypertrophy. Secondly, the heart demonstrates a unique ability to alter its substrate utilization on the basis of changes in substrate supply and cardiac work. Thus understanding the factors that regulate glucose entry into the heart will increase our understanding of cardiac physiology and pathophysiology. In this review, evidence for similarities in the subcellular location and trafficking of GLUT4 between cardiac muscle and skeletal muscle has been provided. However, there are important differences that exist such as the quantitatively important role of insulin-mediated glucose uptake in the regulation of skeletal muscle glucose utilization in contrast to the potentially more important role of cardiac contraction in the regulation of glucose entry into the heart. Thus obtaining a more comprehensive understanding of the molecular mechanisms responsible for translocation of glucose transporters in the contracting heart *in vivo* remains a key area of future investigation. It is also evident that the expression of cardiac glucose transporters and the regulation of cardiac glucose uptake are intimately linked to the availability of alternative substrates in the heart. Although, many groups have described this basic phenomenon, key molecular mechanisms still remain to be elucidated such as the mechanisms linking fatty acid utilization to changes both in glucose transporter expression as well as to changes in glucose transport. Alterations in glucose transporter expression and function occur in many pathophysiological states such as diabetes mellitus and in response to cardiac hypertrophy. Modifying glucose utilization could potentially be of therapeutic benefit. For example, increasing cardiac glucose utilization in hypertrophied hearts may delay the transition to congestive heart failure, and improve recovery in the ischemic heart. Finally, the possibility exists that glucose transporters may play important roles in the heart that might be distinct from their function as glucose transporters. In this regard, understanding the link between glucose transporter expression and the development of cardiac hypertrophy (a significant phenotype of GLUT4 knockout mouse models) is an important question that remains to be elucidated.

6. ACKNOWLEDGMENT

The work in my laboratory was supported by grants from the National Institutes of Health (U01-HL70525, HL 62886 and DK58073), the American Diabetes Association, the American Heart Association and the Ben and Iris Margolis Foundation.

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Abbreviations: ANF – atrial natriuretic factor, ET - endothelin, FFA - free fatty acids, IGT- impaired glucose tolerance, IR-insulin receptor, IRS-1 –insulin receptor substrate -1, IRAP – insulin regulated aminopeptidase, LVAD – left ventricle assist device, LVH -left ventricle hypertrophy, MCAD medium chain acyl CoA dehydrogenase, MHC –myosin heavy chain, MI-myocardial infarction, MSG –monosodium glutamate, MVO_2 –oxygen consumption, PDH –pyruvate dehydrogenase, PDK4- pyruvate dehydrogenase kinase, PGC-1 – PPAR gamma coactivator-1, PKC –protein kinase C, PKA –protein kinase A, PMA – phorbol-12-myristate 13-acetate, PPAR- peroxisome proliferator activated receptor, RVH –right ventricle hypertrophy, SERCA – sarcoplasmic endoplasmic reticulum Ca^{2+}/Mg^{2+} ATPAse, SHR – spontaneously hypertensive rat, TCA -tri-carboxylic acid cycle, UCP-2 –uncoupling protein -2, UCP-3-uncoupling protein -3, VMH- ventromedial hypothalamus

Key Words: Glucose Transporters, GLUT1, GLUT4, Heart, Cardiomyocytes, Review

Send correspondence to: E. Dale Abel MB.BS, D.Phil., Division of Endocrinology, Metabolism and Diabetes and Program in Human Molecular Biology and Genetics, University of Utah School of Medicine, 15 North 2030 East, Building 533, Room 3410B, Salt Lake City, Utah, 84112, USA. Tel: 801-585-0727, Fax: 801-585-0701; E-mail: dale.abel@hmbg.utah.edu