Glucose metabolism in perfused mouse hearts overexpressing human GLUT-4 glucose transporter

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Glucose metabolism in perfused mouse hearts overexpressing human GLUT-4 glucose transporter. Am J Physiol Endocrinol Metab 280: E420–E427, 2001.—Glucose and fatty acid metabolism was assessed in isolated working hearts from control C57BL/KaJ-m+/+db mice and transgenic mice overexpressing the human GLUT-4 glucose transporter (db/+–hGLUT-4). Heart rate, coronary flow, cardiac output, and cardiac power did not differ between control hearts and hearts overexpressing GLUT-4. Hearts overexpressing GLUT-4 had significantly higher rates of glucose uptake and glycolysis and higher levels of glycogen after perfusion than control hearts, but rates of glucose and palmitate oxidation were not different. Insulin (1 μU/ml) significantly increased glycogen levels in both groups. Insulin increased glycolysis in control hearts but not in GLUT-4 hearts, whereas glucose oxidation was increased by insulin in both groups. Therefore, GLUT-4 overexpression increases glycolysis, but not glucose oxidation, in the heart. Although control hearts responded to insulin with increased rates of glycolysis, the enhanced entry of glucose in the GLUT-4 hearts was already sufficient to maximally activate glycolysis under basal conditions such that insulin could not further stimulate the glycolytic rate.

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The development of transgenic mice has provided the opportunity to examine the effects of genes involved in energy metabolism (15). Specifically, transgenic mice with glucose transporter isoform 4 (GLUT-4) knockout or with the overexpression of glucose transporters have been created to examine the effect of alterations in glucose transport on whole animal glucose homeostasis (2, 11, 14, 23). GLUT-4 serves as an insulin-recruitable glucose transporter expressed exclusively in insulin-sensitive tissues (skeletal muscle, adipose tissue, and heart), which is important in the regulation of blood glucose. Male mice in which GLUT-4 has been knocked out demonstrate moderate postprandial hyperglycemia, reduced adipose tissue mass, and cardiac hypertrophy (14). A heart-specific GLUT-4 knockout (1) also demonstrated modest cardiac hypertrophy with no change in contractile function. In contrast, overexpression of GLUT-4 in either adipose tissue (12) or skeletal and cardiac muscle (7, 23) leads to a reduction in blood glucose levels, especially during fasting, and increased tolerance to oral glucose loading.

In normal animals, insulin signaling is required for the translocation of GLUT-4 to the plasma membrane. However, in mice overexpressing GLUT-4, the amount of GLUT-4 associated with the plasma membrane is increased without the need for insulin signaling (7, 11, 13, 23). Thus GLUT-4 overexpression in skeletal muscle significantly increases glucose uptake in isolated muscle (13), leading to increased glycogen synthesis and storage and increased glycolysis and lactate formation (2, 13). Compared with skeletal muscle, overexpression of GLUT-4 in adipocytes also leads to a significant increase in glucose uptake and metabolism, including an increase in glycolysis and glucose incorporation into triacylglycerol (22). In addition, this latter study also demonstrated an increase in glucose oxidation in adipocytes overexpressing GLUT-4, suggesting a connection between increased glucose uptake, glycolysis, and glucose oxidation. It is not known whether GLUT-4 overexpression similarly increases glucose oxidation in skeletal muscle, or whether GLUT-4 overexpression has any effect on glucose metabolism in cardiac muscle. Furthermore, many of these previous studies have examined the metabolism of glucose as the sole oxidizable substrate, whereas fatty acids are an important substrate for myocardial metabolism that influence the utilization of glucose. The purpose of this study was to examine glycolysis and glucose oxidation, together with fatty acid oxidation, in isolated working hearts from mice overexpressing the human isoform of GLUT-4 (hGLUT-4) in cardiac muscle and to assess whether responsiveness to insulin was altered.
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METHODS

Animals. All experiments were approved by the University of Calgary Health Sciences Animal Welfare Committee and followed the guidelines of the Canadian Council on Animal Care. The mouse strain used in this study, C57BL/Ks-Jm+/+db (7), is an inbred mutant mouse strain with a recessive leptin receptor (lepr-/leprab) mutation. Transgenic animals (7, 11) with hGLUT-4 overexpression [db/+hGLUT-4, line 8 (hGLUT-4)] were used to test whether cardiac glucose metabolism was altered. Control nontransgenic mice (db/db) were used to test whether cardiac glucose enhancement in the ability to dispose of an oral glucose challenge in transgenic hGLUT-4-overexpressing mice showed a marked enhancement with the endogenous murine GLUT-4 gene in a variety of tissues (i.e., skeletal muscle, adipose tissue, heart). Transgenic hGLUT-4-overexpressing mice showed a marked enhancement in the ability to dispose of an oral glucose challenge, consistent with elevations in cell surface hGLUT-4 content (7).

The extent of GLUT-4 overexpression in hearts from control and hGLUT-4 mice was assessed by immunoblotting. Postnuclear membrane fractions were prepared from frozen hearts from male mice (8–9 wk of age), as described previously (20). Samples were subjected to electrophoresis and blotted using the NuPAGE electrophoresis system (Invitrogen, Carlsbad, CA). Samples were solubilized in sample buffer containing lithium dodecyl sulfate. Reduced proteins (5) overnight at 4°C. Immunoreactive bands were separated on 10% Bis-Tris z protein) were then separated on 10% Bis-Tris HCl buffered polyacrylamide gels and subsequently transferred to nitrocellulose membrane. The membranes were incubated with affinity-purified rabbit anti-GLUT-4 polyclonal antibodies (5) overnight at 4°C. Immunoreactive bands were visualized using chemiluminescent system (Lumi-LightPLUS, Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Images were captured using a Lumi-Imager F1 (Boehringer Mannheim, Mannheim, Germany) and quantitated using LumiAnalyst 3.1 software (Roche Diagnostics).

Heart perfusion conditions. Mice were heparinized with 100 U heparin (ip) 15 min before anesthesia with 10 mg of pentobarbital sodium (ip). The heart was excised and placed in ice-cold Krebs-Henselit bicarbonate (KHB) buffer. Extraneous tissues (pericardium, lung, trachea, etc.) were removed, and the aorta was cannulated with an 18-gauge plastic cannula. After the aorta was cannulated, the heart underwent a Langendorff perfusion (60 mmHg perfusion pressure) with KHB buffer for ~20 min to wash blood out of the heart. During this time, the left atrium was cannulated with a 26-gauge steel cannula that was connected to the preload reservoir (4). When the heart was switched from Langendorff to working mode, the left atrium was perfused at a preload pressure of 15 mmHg (height of preload column), and the left ventricle worked against an afterload pressure of 50 mmHg (afterload column height).

Pressure development in the aortic (afterload) line was measured using a 2.5 F miniature pressure transducer (Millar Micro-Tip, Millar Instruments, Houston, TX). Pressure measurements were recorded on-line (10 s duration for each recording) at 500 samples/s and processed using specialized software (CVSOPH 2.0, Odessa Computer Systems, Calgary, Alberta, Canada). After data acquisition, the pressure signal was filtered at 75 Hz to remove noise, and the subsequent traces were examined for peak systolic and minimum diastolic pressures to determine pressure development for use in calculating cardiac power.

Heart rate was determined from the pressure traces by measuring the time interval between peak systolic values. In conjunction with pressure measurements, aortic and coronary flow measurements were obtained by collecting the flow from the afterload line and the effluent dripping off the heart, respectively (4). Cardiac output was measured as the sum of aortic and coronary flow. Cardiac power was calculated as the product of developed pressure and cardiac output. A conversion factor of 1.33 × 10⁻⁹ was used to convert cardiac power values from millimeters of mercury per milliliter to Joules (21). The left ventricle wet weights of hearts from control and hGLUT-4 mice were 0.154 ± 0.005 and 0.159 ± 0.009 g, respectively.

Perfusate solution for the working heart. The KHB buffer used for the initial Langendorff perfusion (pH 7.4) consisted of (in mM): NaCl 118.5, NaHCO₃ 25, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, CaCl₂ 2.5, EDTA 0.5, and glucose 11 and was gassed with a 95% O₂-5% CO₂ mixture. The working heart buffer used in these experiments was a modified KHB buffer containing 0.4 mM palmitate bound to 3% BSA as a substrate in addition to the 11 mM glucose (4). Insulin (1 μU/ml) was added to some working heart buffers to study its effect on glucose metabolism.

Experimental protocol. Once the hearts were cannulated for the working heart mode, the Langendorff line was closed, and the preload and aortic lines were opened. Hearts were allowed to beat un-paced at their natural rhythm. The working heart apparatus was made air tight by sealing the heart within the apparatus to allow for the collection of ¹⁴CO₂ as a result of glucose oxidation (4). The hearts were perfused in working mode for 60 min. The volume of working-heart buffer used in each experiment was 35 ml. Pressure and flow measurements were obtained every 10 min. At 20-min intervals (starting at t = 0 min), a 2.5-ml sample of buffer was withdrawn through an injection port mounted between the buffer reservoir and the oxygenator for determinations of metabolite content (see next section). At the end of the experiment, the hearts were quickly frozen between metal blocks cooled to ~80°C, weighed, and stored at ~80°C. A sample of heart tissue (~20 mg) was cut from the heart, weighed (wt), and dried to constant weight (dry wt). The ratio of this sample (dry wt:wet wt) was used to calculate the total dry mass of the heart. The remainder of the heart was used to determine glycogen levels. Metabolic rates were calculated using the total dry mass of the heart to correct for variations in heart size.

Measurement of substrate metabolism. The metabolism of glucose and palmitate was measured according to the methods outlined in detail by Belke et al. (4). All determinations of substrate metabolism for each time point were made in duplicate. Glycolysis and glucose oxidation were measured simultaneously in one set of hearts, whereas palmitate oxidation was measured in a separate set of hearts. Glycolytic flux was determined by measuring the amount of ³H₂O released from the metabolism of [⁵-³H]glucose (specific activity = 400 MBq/mol) by the triosephosphate isomerase and enolase steps of the glycolytic pathway with the use of anion exchange chromatography to separate ³H₂O from unmetabolized [⁵-³H]glucose and [U-¹⁴C]glucose.

Glucose oxidation was determined by trapping and measuring ¹⁴CO₂ released by the metabolism of [U-¹⁴C]glucose (specific activity = 400 MBq/mol). The ¹⁴CO₂ released during glucose oxidation (pyruvate dehydrogenase and TCA cycle)
was cut into 20- to 30-mg blocks, taking care to prevent the tissue from shawing. The frozen tissue was placed in test tubes and dissolved in 0.3 ml of 30% KOH (wt/vol) by immersion in boiling water for 1 h. Glycogen was precipitated by the addition of 0.2 ml of 2% (wt/vol) Na₂SO₄ and 2 ml of ice-cold absolute ethanol, and the samples were stored overnight at −20°C. The next day, samples were centrifuged at 3,500 rpm for 10 min, and the supernatant was discarded. The resulting pellet was washed once with 2 ml of ice-cold 66% ethanol. Glycogen was converted into monosaccharides by boiling the pellet in 1 ml of 3.79 M H₂SO₄ for 3 h. After this, 100 μl of 0.33 M MOPS was added to each tube, and the solution was neutralized with 10 M KOH to pH 7. The final volume of the solution was measured, and glucose concentration was determined by use of a glucose determination kit (Sigma, St. Louis, MO). Glycogen content of the heart is expressed as milligrams of glucose per gram wet weight of heart tissue.

Glucose uptake. To measure glucose uptake, control and transgenic hGLUT-4 hearts were perfused with the usual working heart buffer (11 mM glucose, 0.4 mM palmitate) supplemented with 0.2 μCi/ml 2-deoxy-1-[1,2-³H]glucose (ICN) and 0.05 μCi/ml inulin-[14C]carboxylic acid (Amersham Pharmacie Biotech, Baie d’Urfé, QC, Canada), for 30 min (without insulin). Hearts were perfused next for 10 min with KHB buffer to remove the radioactive perfusate and were then removed from the perfusion apparatus. After removal of atria, ventricles were cut open and rinsed in 20 ml KHB buffer and finally frozen as described above. Frozen heart tissue samples of ~70 mg were homogenized in 2 ml double-distilled H₂O using a polytron (60 s at one-half power), and radioactivity in duplicate 100-μl aliquots of the homogenate was measured by dual-label (¹⁴C/³H) liquid scintillation spectrometry. The content of 2-deoxy-[³H]glucose was corrected for extracellular space from [¹⁴C]inulin radioactivity; glucose uptake is expressed as disintegrations per minute per milligram of tissue.

Statistics. Differences in function and substrate metabolism between control and hGLUT-4 hearts were determined using an unpaired t-test or a Mann-Whitney rank sum test when groups had unequal variance. Differences between means were regarded statistically significant when the P values were <0.05.

RESULTS

hGLUT-4 expression and glucose uptake. The total content of GLUT-4 in hearts from control and transgenic hGLUT-4 mice, assessed by immunoblotting, is shown in Fig. 1. GLUT-4 immunoreactivity in hearts from hGLUT-4 mice was 1.4-fold greater than in control hearts (P = 0.051, two-tailed t-test).

To assess the functional consequences of this modest degree of cardiac overexpression of GLUT-4, glucose uptake by perfused mouse hearts was measured under basal conditions. The uptake of 2-deoxy-[³H]glucose was 242 and 526 dpm/mg tissue for control and hGLUT-4 hearts, respectively (mean of 2 experiments), a 2.2-fold increase in hGLUT-4 hearts. Therefore, a substantial fraction of total cardiac hGLUT-4 must be located on the cell surface to mediate the enhanced glucose uptake.

Functional parameters. For both control and hGLUT-4 hearts perfused under working conditions, cardiac power output was stable during 60 min of perfusion and was not significantly different between control and hGLUT-4 hearts (Fig. 2). Average values for heart rate, coronary flow, cardiac output, and cardiac power between the 10-min and the 60-min time points for hearts perfused in the presence or absence of 1 mU/ml insulin are shown in Table 1. None of these parameters was significantly different between control and hGLUT-4 hearts perfused in the presence or absence of insulin.

Glucose and palmitate metabolism. We also examined rates of glycolysis, glucose oxidation, and palmitate oxidation in working hearts over the course of 60 min of perfusion. The time course for glycolysis, glucose oxidation, and palmitate oxidation in control and hGLUT-4 working hearts is shown in Fig. 3. Glycolysis was higher in hearts overexpressing hGLUT-4 than in...
In contrast, neither glucose oxidation or palmitate oxidation was different between the two groups (Fig. 3, B and C). The average rates of glycolysis, glucose oxidation, and palmitate oxidation obtained from these graphs are shown in Table 2. The rate of glycolysis obtained for hGLUT-4 hearts (4,810 nmol/min g dry wt) was significantly higher (55% increase) than the corresponding rate obtained with control hearts (3,110 nmol/min g dry wt). Despite this higher rate of glycolysis in hGLUT-4 hearts, there was not a parallel increase in glucose oxidation (385 vs. 451 nmol/min g dry wt in control hearts). To eliminate the possibility that fatty acid oxidation might be depressed in hGLUT-4 hearts (leading to a greater reliance on glucose metabolism), palmitate oxidation was also measured in these hearts. As seen in Table 2, palmitate oxidation was also not affected by hGLUT-4 overexpression (353 and 387 nmol/min g dry wt for control and hGLUT-4 hearts, respectively).

The addition of 1 mU/ml of insulin to the perfusion medium (Table 2) significantly increased the rate of glycolysis in control hearts (from 3,110 to 5,050 nmol/min g dry wt) but not in hGLUT-4 hearts (4,810 and 5,460 nmol/min g dry wt for hearts perfused in the absence and presence of insulin, respectively). As a result, the rate of glycolysis was no longer significantly different between control and hGLUT-4 hearts (5,050 vs. 5,460 nmol/min g dry wt, respectively) perfused with insulin. Insulin also led to a significant increase in glucose oxidation in both groups.

Table 1. Functional parameters of cardiac function in perfused working hearts from control and hGLUT-4 mice

<table>
<thead>
<tr>
<th></th>
<th>Heart Rate, beats/min</th>
<th>Coronary Flow, ml/min</th>
<th>Cardiac Output, ml/min</th>
<th>Cardiac Power, mJoule/min</th>
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<tr>
<td></td>
<td>-Ins</td>
<td>+Ins</td>
<td>-Ins</td>
<td>+Ins</td>
</tr>
<tr>
<td>Control</td>
<td>290 ± 17</td>
<td>298 ± 20</td>
<td>2.3 ± 0.3</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>hGLUT-4</td>
<td>310 ± 18</td>
<td>308 ± 13</td>
<td>2.0 ± 0.2</td>
<td>2.4 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12 control hearts and n = 11 hGLUT-4 hearts under basal conditions (-Ins) and n = 6 each for control and hGLUT-4 hearts perfused with 1 mU/ml insulin (+Ins). Control, nontransgenic (db/+) mice; hGLUT-4, transgenic mice overexpressing human GLUT-4 (hGLUT-4; db/+ -hGLUT-4).
GLUCOSE METABOLISM IN HEARTS OF TRANSGENIC hGLUT-4 MICE

Table 2. Energy substrate metabolism in perfused control and hGLUT-4 mouse hearts

<table>
<thead>
<tr>
<th></th>
<th>Glycolysis, nmol·min⁻¹·g⁻¹</th>
<th>Glucose Oxidation, nmol·min⁻¹·g⁻¹</th>
<th>Palmitate Oxidation, nmol·min⁻¹·g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−Ins</td>
<td>+Ins</td>
<td>−Ins</td>
</tr>
<tr>
<td>Control</td>
<td>3110 ± 340</td>
<td>5050 ± 413*</td>
<td>451 ± 78</td>
</tr>
<tr>
<td>hGLUT-4</td>
<td>4810 ± 340*</td>
<td>5460 ± 460</td>
<td>385 ± 73</td>
</tr>
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</table>

Values are means ± SE; n = 6 animals in each group, except for the hGLUT-4 group for palmitate oxidation, where n = 5. Control and hGLUT-4 mouse hearts were perfused in the absence (−Ins) and in the presence of 1 mU/ml insulin (+Ins). ND, values not determined. *P < 0.05 relative to control hearts; †P < 0.05 relative to hearts perfused without insulin.

(~80%); the rate of glucose oxidation did not differ between control and hGLUT-4 hearts perfused with insulin, as noted previously for basal perfusions without insulin.

Glycogen levels. The level of glycogen in hearts from both groups frozen at the end of the 60-min perfusion period is shown in Table 3. In perfusions without insulin, the level of glycogen was significantly higher in hGLUT-4 hearts than in control hearts (3.92 vs. 2.75 mg glucose/g wet wt). The addition of insulin to the perfusion medium led to a significant increase in glycogen levels in both groups; however, in the presence of insulin, the level of glycogen at the end of 60 min of perfusion was no longer different between control and hGLUT-4 hearts.

DISCUSSION

The GLUT-4 glucose transporter serves as an insulin-recruitable glucose transporter in muscle and adipose tissue. In whole animal studies, overexpression of GLUT-4 leads to lower blood glucose during fasting (12, 23), the result of increased GLUT-4 levels in the plasma membrane (even in the absence of insulin) leading to increased glucose uptake into cells (7, 23). Studies on the fate of this glucose have revealed an increase in glycolysis and glycogen synthesis in skeletal muscle (2, 13) and an increase in glucose incorporation into triacylglycerol, glycogenolysis, and glucose oxidation in adipocytes (22). The present study provides the first examination of the effects of GLUT-4 overexpression on function and metabolism in the heart.

In this study, we used the lean C57BL/KsJ-db/db heterozygote (7) as a model to study the effects of hGLUT-4 overexpression on cardiac metabolism. This characterization is required for comparative studies on the effect of hGLUT-4 overexpression on the metabolism and contractile function of genetically diabetic C57BL/KsJ-leprdb/db hearts with insulin resistance (3). Although db/+ animals carry a single mutant copy of the leptin receptor gene, they exhibit a lean phenotype and do not demonstrate any of the diabetic-obese characteristics observed in diabetic (db/db) mice carrying two mutant copies of the leptin receptor (11).

Surprisingly, the extent of hGLUT-4 overexpression in transgenic hGLUT-4 hearts was modest, only 1.4-fold. By comparison, total GLUT-4 expression in skeletal muscle (7) was increased approximately threefold in these same hGLUT-4 mice (line 8). This suggests that GLUT-4 expression may be different in skeletal muscle and heart, although both tissues showed the same enhancement in diabetic (db/db) + hGLUT-4 mice (11). Nevertheless, although the difference in total GLUT-4 content in control and hGLUT-4 hearts was modest, glucose uptake was increased 2.2-fold in hGLUT-4 hearts, indicating that a substantial fraction of hGLUT-4 had been translocated to the cell surface and was functional. Therefore, a detailed examination of both glucose and fatty acid metabolism by control and hGLUT-4-perfused hearts was undertaken.

Our study used an isolated working mouse heart model perfused with palmitate in addition to glucose as a source of energy. The use of palmitate as an oxidizable substrate in addition to glucose was chosen to reflect the physiological preference of the heart for fatty acids as an energy source (17) and to avoid artifacts in glucose metabolism arising from the use of glucose (or carbohydrates) as the sole energy source. For example, glucose oxidation is abnormally high in hearts perfused in the absence of fatty acids (19), which could mask any effect that GLUT-4 overexpression could have on glucose metabolism. Also, fatty acids inhibit glucose uptake and antagonize the translocation of GLUT-4 to the plasma membrane of perfused hearts (24); so the presence of fatty acids such as palmitate in the perfusion buffer is an important physiological factor that influences the partition of GLUT-4 between its functional site in the plasma membrane and intracellular locations. As a result, perfusion of hearts in the presence of glucose and fatty acids provides a more physiologically relevant condition than simple perfusion with glucose alone.

Table 3. Glycogen content in control and hGLUT-4 hearts

<table>
<thead>
<tr>
<th>Glycogen Content, mg/g wet wt</th>
<th>−Insulin</th>
<th>+Insulin</th>
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<tbody>
<tr>
<td>Control</td>
<td>2.75 ± 0.19</td>
<td>5.68 ± 0.57†</td>
</tr>
<tr>
<td>hGLUT-4</td>
<td>3.92 ± 0.44*</td>
<td>5.60 ± 0.39†</td>
</tr>
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</table>

Values are means ± SE; n = 6 in each group. Glycogen content was measured after 60-min perfusion in the absence (−Insulin) and in the presence (+Insulin) of 1 mU/ml insulin. *Significantly different from control (P = 0.035 by Mann-Whitney ranked sum test); †significantly different from hearts perfused without insulin (P = 0.001 for control hearts; P = 0.017 for hGLUT-4 hearts, by Mann-Whitney ranked sum test).
Overexpression of hGLUT-4 did not affect the mechanical performance of the isolated working heart relative to nontransgenic control animals (Table 2). As cardiac power output was similar in both groups over the course of the perfusion (Fig. 2), the metabolic demand by these hearts as the result of their respective levels of cardiac power output will be the same, making the comparison of the metabolic profiles between the groups easier. The increase in glycolysis (1.55-fold) and glycogen levels (1.43-fold) in perfused mouse hearts overexpressing hGLUT-4 (Tables 2 and 3) is consistent with the 1.4-fold increase in hGLUT-4 expression (Fig. 1), providing further evidence that the content of functional glucose transporters has increased in the hearts of hGLUT-4 mice. These results agree with previous observations showing enhanced glycolysis and glycogen levels in skeletal muscle overexpressing GLUT-4 (2, 13). An increase in myocardial glycogen has also been reported in unperfused hearts from mice overexpressing hGLUT-4 transporters (7, 23). Although hGLUT-4 overexpression increased glucose uptake and glycolytic flux, the rate of glucose oxidation was not increased (Table 2). This result differs from that obtained in adipocytes overexpressing GLUT-4 (22), where all aspects of glucose metabolism, including glucose oxidation, were enhanced as a result of GLUT-4 overexpression. The similarity of glucose oxidation rates between control and hGLUT-4 hearts observed in our study could simply reflect precise regulation of glucose oxidation through the pyruvate dehydrogenase step under basal conditions in a perfused heart with an unchanged energy demand (Table 1), as the addition of insulin to the perfusion medium significantly enhanced glucose oxidation in both groups (Table 2). Also, fatty acid oxidation rates were similar for both groups, consistent with no change in glucose oxidation rates. If glucose oxidation had increased in hGLUT-4 hearts, a corresponding reduction in palmitate oxidation would have been anticipated from operation of the Randle cycle (16, 18).

We also examined glucose metabolism in working mouse hearts treated with 1 mU/ml insulin. Glycolysis was significantly increased in control hearts perfused with insulin but not in hGLUT-4 hearts (Table 2); thus the addition of insulin abolished any differences in the pattern of glucose metabolism between control and hGLUT-4 hearts. It appears that glucose uptake was increased in both groups, because glycogen levels were increased significantly in insulin-treated control and hGLUT-4 hearts (Table 3). These results suggest that glycolysis was already maximal in hGLUT-4 hearts under the present perfusion conditions, whereas the increase in glucose entry stimulated by insulin in control hearts increased flux through the glycolytic pathway. This result differs from observations made in skeletal muscle isolated from GLUT-4 overexpressing mice (13); insulin resulted in an increase in glycolysis in both control and GLUT-4 overexpressors. This difference may be due to the fact that the muscle bed (cardiac) in the present study was perfused under working conditions to create a metabolic demand, whereas skeletal muscles were incubated (unperfused) in the study by Hansen et al. (13). In our study, perfusing the hearts with insulin did increase glucose oxidation and glycogen levels, proving that the intracellular pathways for insulin signaling are intact in the hearts from hGLUT-4 overexpressors. Anoxia and increasing workload, like insulin, increase glucose uptake in perfused hearts because of GLUT-4 translocation (24); therefore it will also be of considerable interest to examine the effect of these interventions on glucose metabolism in perfused hearts from db/+ hGLUT-4 mice.

It is important to acknowledge that hGLUT-4 mice have global hGLUT-4 expression in insulin-sensitive tissues such as skeletal muscle and adipose tissue in addition to heart. As a consequence, hGLUT-4 mice are hypoglycemic and hypoinsulinemic in the fed state, suggesting increased sensitivity to insulin (7). Indeed, oral glucose disposal was markedly enhanced in transgenic mice. In addition, fed hGLUT-4 mice had elevated plasma glucagon levels and reduced total plasma cholesterol (7). Therefore, these systemic effects could also contribute to the altered glucose metabolism observed in perfused hearts, together with the direct effect of increased cardiac hGLUT-4 expression. Investigations with cardiac-specific hGLUT-4 overexpression will be required to address this issue.

Limitations of study. The db/+ heterozygote (10–14 wk old) was utilized as a model to investigate the effects of hGLUT-4 overexpression on heart metabolism. Investigations with this line of mice allows comparative studies on the effect of diabetes on cardiac function and metabolism to be conducted with perfused hearts from diabetic db/db homozygotes and transgenic db/db-hGLUT-4 mice (3). This age was chosen because perfused hearts from diabetic db/db mice at 10–14 wk demonstrated considerable contractile dysfunction and altered metabolism (3).

The db/+ heterozygotes are lean and do not demonstrate any of the diabetic-obese characteristics observed in the diabetic db/db homozygotes with two mutant copies of the leptin receptor (7, 11). However, carrying one recessive copy of the mutant gene does appear to confer some advantage to db/+ heterozygotes in terms of conserving energy reserves when faced with a food shortage (9); db/+ mice are predisposed to develop a phenotype of increased fat mass relative to littermates homozygous for the normal leptin receptor (8). This raises some questions as to the validity of using these animals as a model to study the effects of GLUT-4 overexpression on cardiac metabolism. If the contribution of glycolysis, glucose oxidation, and palmitate oxidation to myocardial ATP production [under ideal conditions as described in Opie (17)] is calculated for db/+ mice and compared with results from Swiss-Webster mouse hearts (which have normal leptin receptors) perfused under identical conditions (4), values are very similar between the mouse strains (glycolysis, 11 vs. 10% of calculated ATP production; glucose oxidation 24 vs. 26%; palmitate oxidation 65 vs. 64%, for db/+ and Swiss-Webster mice, respectively).
This suggests that the overall pattern of cardiac metabolism in lean heterozygotes (db/+) does not differ substantially from animals that do not possess a defective gene for the leptin receptor. Brozinick et al. (7) also concluded that the phenotype of db/+ mice overexpressing hGLUT-4 was similar to results obtained with other strains of mice (23). However, until the effects of hGLUT-4 overexpression on cardiac metabolism can be examined in mice without a defective copy of the leptin receptor, caution should be used in extrapolating these findings to other mouse strains.

One problem associated with isolated working mouse heart perfusions is whether there is an adequate supply of O2 for oxidative metabolism. Brooks and Apstein (6) have raised concerns that a crystalloid KHB buffer may not be able to deliver sufficient O2 to the mouse heart under high work loads, leading to the development of an ischemic (or hypoxic) heart. Because ischemia stimulates the translocation of GLUT-4 to the plasma membrane (24), any degree of ischemia could complicate the interpretations of our results. Brooks and Apstein (6) added red blood cells to the perfusion medium to increase O2 delivery to perfused mouse hearts, but the glycolytic metabolism of glucose by red blood cells would prevent us from measuring cardiac rates of glycolysis. Furthermore, it is unlikely that ischemia, if present, could account for the differences observed between groups in the present study. Our perfused working hearts were unpaced so as not to force them to work beyond their capacity to generate ATP for contractile function. The hearts maintained a constant level of power output over the last 40 min of perfusion (Fig. 2). If these hearts were ischemic, power output would be expected to decline severely in this time period. Also, the addition of insulin to the perfusion medium increased O2 delivery to perfused mouse hearts, but the glycolytic metabolism of glucose by red blood cells would prevent us from measuring cardiac rates of glycolysis. Furthermore, it is unlikely that ischemia, if present, could account for the differences observed between groups in the present study. Our perfused working hearts were unpaced so as not to force them to work beyond their capacity to generate ATP for contractile function. The hearts maintained a constant level of power output over the last 40 min of perfusion (Fig. 2). If these hearts were ischemic, power output would be expected to decline severely in this time period. Also, the addition of insulin to the perfusion medium increased glycogen content in these hearts. An effect which would not be observed if ischemia were leading to glycogen mobilization rather than deposition. De Windt et al. (10) have also studied the metabolism in an isolated ex vivo working mouse heart model: functional and metabolic evaluation. Pflügers Arch 437: 182–190, 1999.

In summary, we provide the first study that examines the effect of hGLUT-4 overexpression on glucose metabolism in an isolated ex vivo working mouse heart. Although hGLUT-4 overexpression increased glycogen levels and glycolysis, rates of glucose or fatty acid oxidation were unchanged. The addition of insulin to the perfusion medium increased glycogen levels in both hearts but led to a significant increase only in glycolysis in control hearts. Under these conditions, insulin abolished any differences in glucose metabolism arising from the overexpression of hGLUT-4. These results showing tissue-specific effects of GLUT-4 overexpression on glucose metabolism in isolated perfused mouse hearts contribute to our understanding of how differences in GLUT-4 expression in both physiological and pathophysiological situations can influence in vivo glucose homeostasis.

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