Glucose and fatty acid metabolism in the isolated working mouse heart

Belke, Darrell D., Terje S. Larsen, Gary D. Lopaschuk, and David L. Severson. Glucose and fatty acid metabolism in the isolated working mouse heart. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1210–R1217, 1999.—Although isolated perfused mouse heart models have been developed to study mechanical function, energy substrate metabolism has not been examined despite the expectation that the metabolic rate for a heart from a small mammal should be increased. Consequently, glucose utilization (glycolysis, oxidation) and fatty acid oxidation were measured in isolated working mouse hearts perfused with radiolabeled substrates, 11 mM glucose, and either 0.4 or 1.2 mM palmitate. Heart rate, coronary flow, cardiac output, and cardiac power did not differ significantly between hearts perfused at 0.4 or 1.2 mM palmitate. Although the absolute values obtained for glycolysis and glucose oxidation and fatty acid oxidation are significantly higher than those reported for rat hearts, the pattern of substrate metabolism in mouse hearts is similar to that observed in hearts from larger mammals. The metabolism of mouse hearts can be altered by fatty acid concentration in a manner similar to that observed in larger animals; increasing palmitate concentration altered the balance of substrate metabolism to increase overall energy derived from fatty acids from 64 to 92%.

Within the last decade, the focus of cardiovascular research has shifted from traditional physiology to investigations at the cellular and molecular level to understand the pathophysiological basis of cardiac dysfunction. The ability to create transgenic animals has provided the opportunity to examine the actions of a particular gene on normal and abnormal heart function (9, 12, 18). Development of transgenic models has been paralleled by technological advances allowing measurements of cardiac function, both in vivo and ex vivo, in animals as small as a mouse (14–16). Although many studies have focused on functional parameters involving contractility and electrophysiology, little is known about energy substrate metabolism in the mouse heart despite the importance of cardiac metabolism in disease states such as diabetes and myocardial ischemia.

Due to technical limitations in working with small animals, the vast majority of studies measuring cardiac energy substrate metabolism have been conducted using animal models such as rats (~300 g) or larger. Studies that have been performed on smaller mammals have been mostly limited to microscopic analysis and characterization of the enzymes involved in energy metabolism (5, 23). Assumptions on metabolism in small mammals have often been made on the basis of scaling various physiological and biochemical parameters with body size (11, 13). One consideration when examining cardiac metabolism in mammals as small as a mouse (20–40 g) is that mice are much closer to the lower limitations governing body size (~2 g) in homeothermic mammals (11). As a result, mice have a high weight-specific metabolic rate, necessitating their need for a very high heart rate (500–600 beats/min). As mammals become smaller in body size, the volume that mitochondria occupy within the myocyte increases (3, 17), as does the surface area of the inner mitochondrial membrane within the mitochondria itself (23). Microscopic analysis of heart muscle morphology reveals an increased capillary density for substrate and O2 delivery to the myocardium (24), supporting the need for a higher capacity for oxidative metabolism. Supporting this, several studies have demonstrated an increase in the activity of enzymes involved in oxidative metabolism (citrate synthase, succinate dehydrogenase, etc.) in smaller mammalian species, with increased oxidative capacity scaling to smaller body size and increased heart rate (5, 13, 23). In contrast, enzymes involved in glycolysis are more uniform among mammalian species and do not scale with body weight (5, 13). This difference in the scaling of glycolytic and oxidative capacity raises the possibility that the capacity for glucose oxidation may be limited by glycolysis in smaller species. Such an effect could bias oxidative metabolism toward an increased reliance on fatty acid oxidation in the hearts of small mammals.

Despite inferences involving oxidative metabolism that can be derived from metabolic scaling, myocardial substrate metabolism in an animal as small as a mouse has not been measured, especially in regard to potential interactions between fatty acid and carbohydrate oxidation. The Randle cycle, which describes a reciprocal interaction between fatty acid and carbohydrate oxidation (25), forms the basis for the pathological effect of high plasma fatty acid concentrations on heart function (19, 28). The purpose of this study was to measure rates of glucose and fatty acid oxidation and glycolytic rate in mouse hearts perfused with 11 mM glucose and either 0.4 or 1.2 mM palmitate to determine whether the pattern of substrate metabolism in the perfused mouse heart is similar to that observed in...
larger mammals and whether carbohydrate metabolism is affected by changes in fatty acid concentration in a manner similar to that observed in hearts from larger mammals.

METHODS

Heart Isolation and Perfusion Conditions

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. Adult Swiss-Webster mice (average body weight 36 ± 1 g) were heparinized (100 U ip) 15 min before anesthesia with 10 mg ip pentobarbital sodium. Hearts (average weight 0.170 ± 0.006 g) were excised and placed in ice-cold Krebs-Henseleit bicarbonate (KHB) solution. Extraneous tissues (pericardium, lung, trachea, etc.) were removed, and the aorta was cannulated with an 18-gauge plastic cannula (1.5 cm length, 0.95 mm ID, 1.30 mm OD). After cannulating the aorta, the heart underwent a retrograde Langendorff perfusion (60 mmHg perfusion pressure) with KHB solution for ~20 min. During this period the left atrium was connected to the preload reservoir (oxygenator) by cannulating the pulmonary vein with a 16-gauge steel cannula (3 cm length, 1.14 mm ID, 1.52 mm OD). The preload line (and perfusate reservoir) was wrapped with a water jacket and heated to 38.5°C, resulting in a myocardial temperature very close to 37°C (36.4–36.7°C) when the heart was operating in the working mode. When the heart was switched from Langendorff to working mode, the left atrium was perfused at a preload pressure of 15 mmHg; the left ventricle worked against a hydrostatic column set at a height equivalent to a pressure of 50 mmHg. A schematic of the working heart apparatus is shown in Fig. 1.

Pressure development in the aortic (afterload) line was measured using a 2.5-Fr miniature pressure transducer (Millar Micro-Tip, Millar Instruments, Houston, TX). Before the experiment, the pressure transducer was calibrated using a mercury manometer. Pressure measurements were recorded online (10-s duration for each recording) at a sampling frequency of 500 Hz and processed using specialized software (CVSOFT 2.0, Odessa Computer Systems, Calgary, Alberta, Canada). Pressure measurements were recorded every 10 min. After data acquisition, the pressure signal was filtered at 75 Hz to remove noise, and subsequent traces were examined for peak systolic and minimum diastolic pressures to determine developed pressure for 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. To accomplish these flow measurements without disrupting metabolic measurements (e.g., the quantitative collection of 14CO2), graduated cylinders were sealed within the perfusion apparatus and flow was determined (at 10-min intervals) by measuring the time required to collect 2-ml samples. Cardiac output was calculated from the sum of the aortic and coronary flows. Cardiac power was calculated as the product of developed pressure and cardiac output; a conversion factor of 1.33 × 10−4 was used to convert cardiac power values from millimeters mercury per milliliter to joules (30).

Preparation of Perfusate Solution for the Working Heart

The KHB solution used for the initial Langendorff perfusion consisted of (in mM) 118.5 NaCl, 25 NaHCO3, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 2.5 CaCl2, 0.5 EDTA, and 11 glucose and was gassed with 95% O2-5% CO2 (pH 7.4). Theworking heart solution used in this experiment was a modified KHB solution containing palmitate (0.4 or 1.2 mM) bound to 3% BSA as a metabolic substrate in addition to 11 mM glucose. To bind palmitate to BSA, the palmitate was first dissolved in

Fig. 1. Perfusion apparatus for working mouse heart preparation. Preload and afterload pressures were set according to height above heart. Flow measurements were obtained by trapping effluent from afterload line (aortic flow) or solution dripping off heart (coronary flow) in graduated cylinders that could be filled at one end and drained through the other. AMP, amplifier; LV, left ventricle; LA, left atrium.
25 ml of a water:ethanol mixture (60:40 vol/vol) containing 0.5–0.6 g Na2CO3/g palmitate. The mixture was heated with constant stirring until the ethand was removed; this mixture was added to the modified KHB solution containing BSA (without glucose) while stirring rapidly to ensure adequate mixing. This solution was dialyzed overnight against 10 vol of KHB solution (without glucose) using 8–12,000 mol wt cut-off SPECTRAPOR dialysis tubing (Spectrum Medical Industries, Los Angeles, CA). Glucose (11 mM) was added to the perfusate solution the next day just before use.

Experimental Protocol

Once the hearts were fully cannulated for the working heart mode, the Langendorff line was closed and the preload and aortic lines were opened. The working heart apparatus was made air-tight by sealing the heart within the apparatus (Fig. 1); this allowed for the quantitative collection of 14CO2 arising from glucose oxidation (see Measurement of Substrate Metabolism). Any 14CO2 evolving from the KHB solution as the result of solution oxygenation was collected by bubbling the oxygen outflow through 15 ml of hyamine hydroxide (ICN, Irvine, CA). Hearts were perfused in working mode for 60 min. Hearts were allowed to beat unpaced at their natural rate because pacing electrodes could not be attached easily to the right atrium in this closed apparatus. This would also prevent the hearts from being forced to work at a rate that would exceed their ability to supply energy. The total volume of working heart solution used in each experiment was 35 ml. At 20-min intervals (starting at time = 0 min), a 2.5-ml sample of perfusate was withdrawn through an injection port mounted between the perfusate reservoir and the oxygenator (Fig. 1). The perfusate samples were stored in scintillation vials under a 2-ml layer of paraffin oil to prevent the loss of 14CO2. 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 (wet wt), and then dried to remove all water (dry wt). The ratio of this sample (dry/wet wt) was used to calculate the total dry mass of the heart. Metabolic rates were calculated using the total dry mass of the heart to correct for variations in heart size.

Measurement of Substrate Metabolism

Metabolism of radiolabeled glucose and palmitate was measured according to the methods outlined by Saddik and Lopaschuk (26). All determinations of substrate metabolism at each time point (0, 20, 40, 60 min) were made in duplicate. Rates of glycolysis and glucose oxidation were measured simultaneously in one set of hearts (n = 6), whereas palmitate oxidation was measured in a separate set of hearts (n = 6). Steady-state metabolic rates were calculated as the mean values from the three time periods (0–20, 20–40, 40–60 min) when perfusate samples were removed from the working heart apparatus. Values obtained for the various metabolic pathways were normalized for heart mass (dry wt) or the degree of cardiac power output from perfused mouse hearts.

Glycolysis. Glycolytic flux was determined by measuring the amount of H2O released from the metabolism of 5-[3H]glucose (specific activity = 400 M bq/mol) by the triosephosphate isomerase and enolase steps of the glycolytic pathway. To separate H2O from 5-[3H]glucose and [U-14C]glucose, perfusate samples were filtered through anion exchange resin (200–400 mesh Dowex 1-X4) pretreated with 0.4 M potassium borate. Dowex columns were extensively washed with distilled H2O before use. A 100-µl sample was loaded on the column and eluted into scintillation vials with 0.8 ml of H2O. After the addition of Ecolite scintillant (ICN), the sample was counted for 3H and 14C. Although the Dowex resin retains ~99% of glucose, a small amount of 5-[3H]glucose that passes through the resin contributes to the 3H signal. To correct for this, the assay takes advantage of measuring the amount of [U-14C]glucose that also passes through the resin as an internal standard to calculate the amount of contaminating 5-[3H]glucose in the sample. As a result, the contribution of 5-[3H]glucose to the H2O signal can be factored out.

Glycolysis oxidation. Glucose oxidation was determined by trapping and measuring 14CO2 released by the metabolism of [U-14C]glucose (specific activity = 400 M bq/mol). The 14CO2 released during glucose oxidation (pyruvate dehydrogenase and tricarboxylic acid cycle) was trapped using 1 M hyamine hydroxide (Fig. 1) as described by Saddik and Lopaschuk (26). Quantitative collection of 14CO2 was performed by continuously bubbling the outflow air from the perfusion apparatus through 15 ml of hyamine hydroxide and then sampling the hyamine hydroxide (300 µl) every 20 min, starting at 0 min. At the same time, the 14CO2 remaining in the perfusion medium (in the form of bicarbonate anion) was released by injecting 0.75 ml of the KHB solution sample into 0.75 ml of 9 N H2SO4 inside a sealed metabolic flask. The flasks were shaken overnight, and 14CO2 released from the solution was subsequently trapped in a center well containing 300 µl of hyamine hydroxide. The hyamine hydroxide samples were counted using CytoScint scintillation cocktail (ICN). Quantitative 14CO2 production was measured by adding together the values for 14CO2 obtained from the outflow air and solution.

Palmitate oxidation. Palmitate oxidation was determined by measuring the amount of H2O released from 9,10-3H]palmitate (specific activity = 18.5 GBq/mol) as outlined by Saddik and Lopaschuk (26). 3H2O was separated from 9,10-3H]palmitate by mixing 0.5 ml of KHB solution with a 1.88 ml of chloroform:methanol (1:2 vol/vol). This was followed by the addition of 0.625 ml of chloroform and mixing; then 0.625 ml of 1.1 M KCl dissolved in 0.9 M HCl was added and the samples were mixed. Samples were allowed to separate into their polar and nonpolar phases (~1 h), and the polar phase was pipetted into another tube. The polar phase was mixed sequentially with 1 ml chloroform, 1 ml methanol, and 0.9 ml of the KCl:HCl mixture. The samples were again allowed to settle into their polar and nonpolar phases (~1 h), and the polar phase was pipetted into another tube. The polar phase was mixed sequentially with 1 ml chloroform, 1 ml methanol, and 0.9 ml of the KCl:HCl mixture. The samples were again allowed to settle into their polar and nonpolar phases, and a 0.5-ml aliquot from the polar phase was taken and counted for 3H. Palmitate oxidation rates were calculated from 3H2O production, taking into account the dilution factor incurred from the process of separating 3H2O from 9,10-3H]palmitate. ATP production. The yield of ATP from glucose and palmitate metabolism was calculated using a stoichiometric ratio of 2 mol ATP/mol glucose passing through glycolysis, 30 mol ATP/mol glucose being oxidized, and 105 mol ATP/mol palmitate being oxidized (as outlined by Opie in Ref. 22). This calculation assumes 100% coupling of mitochondrial oxidative phosphorylation.

Statistics

Differences in cardiac function and substrate metabolism between hearts perfused with 0.4 and 1.2 mM palmitate were determined using an unpaired t-test or a nonparametric Mann-Whitney U test when variances were sufficiently different to preclude using a t-test. Repeated-measures analysis of variance was used to determine the significance of individual time points shown in Fig. 3. Differences between means were regarded as statistically significant when P values were <0.05.
RESULTS

Functional Parameters

Cardiac power output obtained from hearts perfused with either 0.4 or 1.2 mM palmitate over the 60-min perfusion period is shown in Fig. 2. In both groups, cardiac power increased during the first 20 min of perfusion after the switch-over from Langendorff to working mode; subsequent cardiac power output measurements were relatively stable. Power output for the 1.2 mM palmitate group was not significantly different from the 0.4 mM palmitate group. Average values for heart rate, coronary flow, cardiac output, and cardiac power over the 60-min perfusion period are shown in Table 1. Increasing perfusate palmitate concentration from 0.4 to 1.2 mM did not significantly alter any of the parameters of mechanical function. The values shown in Table 1 are similar to those reported previously for isolated working mouse hearts using a hydrostatic column of 50 mmHg to achieve afterload (9, 14).

Glucose and Palmitate Metabolism

Rates of glycolysis, glucose oxidation, and palmitate oxidation in mouse hearts perfused in working mode were measured during the 60-min perfusion (Fig. 3). Glycolysis was not altered in mouse hearts perfused with 0.4 mM palmitate compared with hearts perfused with 1.2 mM palmitate (Fig. 3A). In contrast, large differences were observed between the groups for glucose and palmitate oxidation (Fig. 3, B and C). Glucose oxidation was markedly higher in hearts perfused with 0.4 mM palmitate, whereas the opposite was observed for palmitate oxidation. Although the time course of glucose and palmitate oxidation was linear over the

Table 1. Functional parameters

<table>
<thead>
<tr>
<th>Palmitate Concn.</th>
<th>Heart Rate, beats/min</th>
<th>Coronary Flow, ml/min</th>
<th>Cardiac Output, ml/min</th>
<th>Cardiac Power, mJ/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4 mM</td>
<td>311 ± 20</td>
<td>3.2 ± 0.2</td>
<td>11.6 ± 0.9</td>
<td>111.6 ± 10.6</td>
</tr>
<tr>
<td>1.2 mM</td>
<td>330 ± 17</td>
<td>3.0 ± 0.1</td>
<td>12.3 ± 0.6</td>
<td>129.1 ± 8.6</td>
</tr>
</tbody>
</table>

Values represent means ± SE of 12 animals in each group. Each value represents average of data collected between 10- and 60-min time points.
60-min perfusion time course for most groups, glucose oxidation in the hearts perfused with 1.2 mM palmitate lagged over the first 20 min (Fig. 3B). Similarly, glycolysis (Fig. 3A) was slower over the first 20 min than the last 40 min of the perfusion period. This may reflect the increase in cardiac power observed over the first 20 min in working mode (Fig. 2).

Steady-state rates of glycolysis, glucose oxidation, and palmitate oxidation are shown in Table 2. Increasing the palmitate concentration from 0.4 to 1.2 mM did not significantly change the rates of glycolysis (4,100 and 3,150 nmol·min⁻¹·g dry wt⁻¹, respectively). In contrast, glucose oxidation decreased more than twofold from 715 to 259 nmol·min⁻¹·g dry wt⁻¹, whereas palmitate oxidation increased threefold from 494 to 1,560 nmol·min⁻¹·g dry wt⁻¹. A similar pattern was observed when oxidative metabolism was normalized for cardiac power output, because oxidatively derived energy contributes to muscle contraction. Glucose oxidation normalized for cardiac work was significantly lower in the 1.2 mM palmitate group, whereas palmitate oxidation was significantly higher. Under these conditions, glycolysis normalized for cardiac power output was also significantly lower in the 1.2 mM palmitate group, despite the fact that glycolysis was not significantly different between the groups when the rate was expressed per gram dry weight. This result is due to the additive effect of the lower glycolytic rate and the higher level of cardiac power observed in the 1.2 mM palmitate group (neither of which were significantly different from the 0.4 mM palmitate group alone). However, although it is reasonable to normalize oxidative metabolism to cardiac power output (owing to the contribution of oxidative metabolism to muscle contraction), glycolytic rate is determined by a number of factors that may not be related to muscle contraction. As a result, normalizing glycolysis for cardiac power output may create an artificial bias between the 0.4 and the 1.2 mM palmitate groups.

The capacity of the pathway for glycolysis and glucose oxidation was determined in one mouse heart perfused in the presence of 11 mM glucose and 0.4 mM palmitate by adding 100 µU/ml of insulin to the perfusion medium. Under these conditions, glycolysis increased to 8,776 nmol·min⁻¹·g dry wt⁻¹, whereas glucose oxidation increased to 1,182 nmol·min⁻¹·g dry wt⁻¹, indicating that neither metabolic pathway had reached its maximal potential under our basal assay conditions.

When steady-state rates for substrate metabolism are converted into ATP production (see METHODS), fatty acid oxidation accounted for ~64% of total ATP production in hearts perfused with 0.4 mM palmitate (Fig. 4), whereas glucose oxidation and glycolysis accounted for 26 and 10%, respectively. Thus, under these conditions, the perfused working mouse heart obtains approximately two-thirds of its energy from fatty acid oxidation. When the palmitate concentration was raised to 1.2 mM, the increase in fatty acid oxidation and the decrease in glucose oxidation increased the contribution of fatty acid oxidation to 92% and decreased glucose oxidation and glycolysis to 4.4 and 3.5%, respectively. Thus increasing the palmitate concentration in the perfusate shifted the balance of substrate metabolism markedly toward fatty acid oxidation, with glucose oxidation being severely depressed.

**DISCUSSION**

Creation of transgenic mouse strains has necessitated measurements of physiological parameters in hearts from much smaller animals (9) than used previously for myocardial substrate metabolism. Although predictions based on metabolic scaling suggest a high oxidative capacity in hearts from small mammals (3, 5, 23), direct comparative measurements of energy metabolism have not previously been made. Nuclear magnetic resonance has been used to study the high-energy phosphate profile in Langendorff perfused mouse hearts (27); however, the metabolism of individual substrates under physiological conditions of energy demand (i.e., the working heart) has not been determined. Our study provides the first measurements of glycolysis, glucose oxidation, and palmitate oxidation in the isolated working perfused mouse heart. The working mouse heart models developed to date have used only carbohydrates as a fuel source (9, 10, 14, 16), despite numerous studies showing that fatty acids are the preferred energy substrate for the mammalian myocardium (22, 26).

Table 2. Energy substrate metabolism in mouse heart

<table>
<thead>
<tr>
<th>Palmitate Conc.</th>
<th>Glycolysis</th>
<th>Glucose Oxidation</th>
<th>Palmitate Oxidation</th>
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<tbody>
<tr>
<td></td>
<td>Steady-state, nmol·min⁻¹·g dry wt⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4 mM</td>
<td>4100 ± 404</td>
<td>715 ± 119</td>
<td>494 ± 60</td>
</tr>
<tr>
<td>1.2 mM</td>
<td>3150 ± 341</td>
<td>259 ± 59*</td>
<td>1560 ± 140*</td>
</tr>
<tr>
<td>Rate normalized for cardiac power, nmol·mJ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4 mM</td>
<td>1.78 ± 0.33</td>
<td>0.23 ± 0.04</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>1.2 mM</td>
<td>0.72 ± 0.04*</td>
<td>0.06 ± 0.01*</td>
<td>0.40 ± 0.05*</td>
</tr>
</tbody>
</table>

Values represent means ± SE for 6 animals in each group. *Significantly different from hearts perfused with 0.4 mM palmitate.
Although the inclusion of fatty acids in the perfusion medium does not directly affect the mechanics of contraction under normal aerobic conditions (i.e., dP/dt, left ventricular developed pressure), fatty acids can influence heart function under pathological conditions such as ischemia-reperfusion and diabetes (19). Therefore, an understanding of both fatty acid and glucose metabolism will be essential to assess myocardial function in mouse models of cardiovascular disease.

In the present study, mouse hearts were perfused with 11 mM glucose and 0.4 mM (or 1.2 mM) palmitate. Although the concentrations of glucose and free fatty acid in mouse plasma are ~8 and 0.7 mM, respectively (unpublished observations), we chose concentrations to facilitate comparison with previous studies using perfused rat hearts. Rates of glycogenolysis (4.100 nmol·min⁻¹·g dry wt⁻¹), glucose oxidation (715 nmol·min⁻¹·g dry wt⁻¹), and palmitate oxidation (494 nmol·min⁻¹·g dry wt⁻¹) are all higher than rates measured in rat hearts (26) under similar conditions (glycolysis = 2,480, glucose oxidation = 357, palmitate oxidation = 363 nmol·min⁻¹·g dry wt⁻¹). Similar results were obtained when mouse hearts were perfused with 1.2 mM palmitate compared with perfused rat hearts (26): glycolysis, 3,150 vs. 1,680; glucose oxidation, 259 vs. 124; palmitate oxidation, 1,560 vs. 633 nmol·min⁻¹·g dry wt⁻¹. Palmitate oxidation in perfused mouse hearts was also higher than values observed in dog hearts in vivo (494 in mice vs. 54 nmol·min⁻¹·g dry wt⁻¹ in dogs; calculated from Ref. 29 assuming a dry-to-wet weight ratio of 0.2). Thus it appears that the rate of myocardial substrate oxidation (glucose, palmitate) increases with decreasing body mass.

More important than absolute rates of substrate metabolism is the balance between fatty acid and carbohydrate metabolism in the heart. Under basal conditions (11 mM glucose, 0.4 mM palmitate), fatty acid oxidation accounted for 64% of ATP production, whereas glucose oxidation accounted for 26% and glycogenolysis for 10% (Fig. 4). These values are similar to results reported previously (1, 26) for carbohydrate and fatty acid metabolism in rat hearts (fatty acid oxidation ~70%, carbohydrate oxidation ~20–25%). The contribution of fatty acid oxidation to energy production in dog heart (0.4 mM plasma free fatty acids) was reported to be 60% (21). Similarly, fatty acid oxidation in the human heart has been estimated at 60% (2). As a result, although the absolute rates of substrate oxidation are higher in mouse hearts in comparison with larger animals, the pattern of substrate utilization remains similar. Previous studies suggested that although the capacity for oxidative metabolism increases in hearts from small animals, the capacity for glycolysis does not (5, 13), potentially creating an imbalance between the supply and oxidation of pyruvate, but this was not observed in perfused mouse hearts. The previous studies (5, 13) were based on in vitro measurements of enzymes involved in glycolytic and oxidative metabolism under optimal conditions. In the intact heart, the supply of pyruvate from glycolysis was greater than its rate of oxidation, suggesting that even in the absence of scaling with body size the activity of the glycolytic pathway is greater than the rate of oxidation that can be achieved even under working conditions (high oxidative demand).

The reciprocal regulation of fatty acid and carbohydrate oxidation (Randle cycle) plays a role in mediating the pathological effects of high fatty acid concentrations in disease states (19, 28). In our study, fatty acid oxidation increased and glucose oxidation decreased when palmitate concentration was elevated from 0.4 to 1.2 mM. Glycolysis was not depressed to the same extent as glucose oxidation (Table 2), consistent with previous observations using rat hearts (26). Oxidation of 1.2 mM palmitate accounted for over 92% of calculated ATP production, whereas the contribution of glycolysis and glucose oxidation was reduced to 3.5 and 4.4%, respectively. This pattern of substrate metabolism is similar to results with rat hearts under similar perfusion conditions (6), where palmitate oxidation accounted for 89%, glucose oxidation 5.4%, and glycolysis 5.5% of calculated ATP production. In human studies, raising plasma free fatty acids to 1.19 mM increased cardiac fatty acid utilization to 85% (2). Therefore, the observed shift in the pattern of substrate metabolism for mouse hearts at the higher concentration of palmitate indicates that the isolated working mouse heart model also has the capability of adapting substrate metabolism to changes in substrate availability.

Limitations of This Study

Although energy substrate metabolism from exogenous substrates can be measured in isolated working perfused mouse hearts, the contribution of endogenous stores of triglyceride and glycogen was not considered. Although the mobilization of endogenous triglyceride stores occurs in rat hearts perfused in the absence of fatty acids, mobilization decreases when fatty acid concentrations in the perfusate are increased (26). This reduces the likely contribution of triglyceride stores to energy production in our study. However, given the high metabolic rate of the mouse and the observation that myocardial triglyceride levels are typically higher in mouse than rat hearts (20), the possible contribution of endogenous triglyceride stores to energy production in perfused mouse hearts will have to be evaluated in future experiments.

Although glycogen mobilization can also contribute to energy production in the heart, it is unlikely to play a significant role in the present study. Increased glycogen mobilization could account for the depression in exogenous glucose oxidation in hearts perfused in the presence of 1.2 mM palmitate, but levels of glycogen were higher after 60 min of perfusion with 1.2 mM palmitate compared with hearts perfused with 0.4 mM palmitate (3.15 vs. 2.41 mg/g wet wt, respectively), suggesting that differences in glycogen mobilization could not account for our results. De Windt et al. (9) observed that cardiac glycogen content increased during mouse heart perfusions.
Calculations of ATP production (see Methods) are based on ideal rates of ATP production per mole of glucose or fatty acid metabolized (22). It is unlikely that such ideal conditions exist within the living cell, because the proton gradient generated by substrate oxidation in the mitochondria may be dissipated through pathways not involved in ATP synthesis (i.e., leak pathway, ion exchange pathways). However, because both glucose and fatty acid metabolism contribute acetyl-CoA to the tricarboxylic acid cycle, any inefficiency in ATP production arising as a result of non-ATP producing pathways using the proton gradient would affect energy production from both carbon sources equally. Therefore, calculations of the percent contribution of glucose and palmitate to ATP production is a reasonable indication of patterns for myocardial substrate utilization.

Finally, O2 consumption was not measured, so we cannot eliminate the possibility that the high level of glycolysis observed in mouse hearts is not partly due to insufficient O2 delivery. Brooks and Apstein (7) have raised the possibility that KHB solution may not be able to deliver sufficient O2 to the mouse heart under high work loads. However, in the present study hearts were unpaced to reduce the possibility of working the hearts beyond their capacity to supply energy. Furthermore, the normal response of glycolysis to fatty acids and insulin suggests that glycolysis was not abnormally increased in our working mouse heart model.

In conclusion, despite high rates of energy substrate metabolism observed in isolated working mouse hearts, the pattern of substrate metabolism was similar to that observed in hearts from larger mammals. Furthermore, the pattern of mouse heart substrate metabolism was affected by changes in perfusate fatty acid concentrations in a manner similar to that observed in hearts from larger mammals. Although scaling of enzyme activities for glycolytic and aerobic metabolism (11, 13) have suggested that glycolytic capacity might limit glucose oxidation, this does not appear to be the case for the working mouse heart.

Perspectives

The use of transgenic mouse models to explore cardiac physiology and pathology is increasing, requiring methods to assess changes in energy substrate metabolism. For example, transgenic mouse models with deficiencies in creatine kinase (27), acid α-glucosidase (Pompe disease) (4), and cytochrome-c oxidase (8) have been created, but it is not yet known how these metabolic abnormalities alter overall substrate metabolism. Our study demonstrates that although metabolic rates are high the working mouse heart, the metabolism of individual substrates appears to be regulated similarly by the same factors that influence metabolism in hearts from larger animals. Therefore, perfused working mouse hearts will be a useful experimental model to assess the regulation of cardiac metabolism under both physiological and pathological conditions.

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