Accelerated rates of glycolysis in the hypertrophied heart: are they a methodological artifact?

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Leong, Hon Sing, Mark Grist, Hannah Parsons, Richard B. Wambolt, Gary D. Lopaschuk, Roger Brownsey, and Michael F. Allard. Accelerated rates of glycolysis in the hypertrophied heart: are they a methodological artifact? Am J Physiol Endocrinol Metab 282: E1039–E1045, 2002. First published January 29, 2002; 10.1152/ajpendo.00507.2001.—Glycolysis, measured by 3H2O production from [5-3H]glucose, is accelerated in isolated working hypertrophied rat hearts. However, nonglycolytic detritiation of [5-3H]glucose via the nonoxidative pentose phosphate pathway (PPP) could potentially lead to an overestimation of true glycolytic rates, especially in hypertrophied hearts where the PPP may be upregulated. To address this concern, we measured glycolysis using [5-3H]glucose and a second, independent method in isolated working hearts from halothane-anesthetized, sham-operated and aortic-constricted rats. Glycolysis was accelerated in hypertrophied hearts compared with control hearts regardless of the method used. There was also excellent concordance in glycolytic rates between the different methods. Moreover, activity of glucose-6-phosphate dehydrogenase and expression of transaldolase, enzymes controlling key steps in the oxidative and nonoxidative PPP, respectively, were not different between control and hypertrophied hearts. Thus nonglycolytic detritiation of [5-3H]glucose in the PPP is insignificant, and 3H2O production from [5-3H]glucose is an accurate means to measure glycolysis in isolated working normal and hypertrophied rat hearts. Furthermore, the PPP does not appear to be increased in cardiac hypertrophy induced by abdominal aortic constriction.

COMPARED WITH NONHYPERTROPHIED HEARTS, rates of glycolysis have been reported as being accelerated in isolated working hypertrophied rat hearts (2, 14). In these experiments, glycolysis was measured by quantitatively collecting 3H2O released from [5-3H]glucose in the perfusate, a well-established means of measuring glycolysis in isolated heart preparations (10, 22, 26). [5-3H]glucose, during its catabolism in the glycolytic pathway, is assumed to be completely detritiated by the triose phosphate isomerase and enolase reactions (26).

Despite the initial validation and widespread use of the [5-3H]glucose detritiation method, it has recently been suggested that rates of glycolysis obtained by quantitation of 3H2O production from [5-3H]glucose overestimate true rates of glycolysis in isolated working normal rat hearts (15). The authors of this recent report propose that nonglycolytic detritiation of [5-3H]glucose occurs in the nonoxidative portion of the pentose phosphate pathway by means of the transaldolase reaction in the heart. According to the authors, this detritiation of [5-3H]glucose in the pentose phosphate pathway takes place in the absence of net glycolytic flux, which thereby leads to an overestimation of true rates of glycolysis.

The main function of the pentose phosphate pathway is to produce ribose 5-phosphate and NADPH2 (39–41). The ribose 5-phosphate is used to synthesize nucleic acids, whereas NADPH2 is primarily used to protect the heart against oxidative stress by maintaining glutathione in a reduced state. The pentose phosphate pathway is comprised of two branches, the oxidative and the nonoxidative branches (39–41). The oxidative branch of the pentose phosphate pathway, which produces NADPH2 and ribulose 5-phosphate, is essentially irreversible, being controlled primarily by glucose-6-phosphate dehydrogenase (G6PDH) activity and, hence, by the NADPH2-to-NADP+ ratio. In fact, the NADP+ pool is normally sufficiently reduced (notably via NADP-dependent isocitrate dehydrogenase) that G6PDH is substantially inhibited, and flux through the oxidative span of the pentose phosphate pathway is extremely low in the heart (4, 13, 34, 43).

The nonoxidative branch is reversible and is responsible for the interconversion of glycolytic intermediates with pentose phosphates. The potential cyclic nature of the nonoxidative branch involving glycolytic inter-
mediates with repetitive passage through the triose phosphate isomerase reaction could hypothetically lead to significant nonglycolytic loss of $^3$H from [5-3H]glucose, particularly if flux through this branch is significant. Upregulation of the pentose phosphate pathway has been reported in a variety of models of cardiac hypertrophy, including aortic constriction (16, 25, 46, 47). As a consequence, and if substantial nonglycolytic detritiation of [5-3H]glucose occurs in myocardium, as suggested, it is conceivable that the acceleration of glycolysis reported in isolated working hypertrophied rat hearts is an artifact.

Thus we set out to determine whether detritiation of [5-3H]glucose overestimates true rates of glycolysis in hypertrophied rat hearts. To this end, we used two independent methods to determine rates of glycolysis in isolated working hypertrophied and nonhypertrophied hearts. The first involved measurement of $^3$H2O released from [5-3H]glucose, whereas the second involved quantitative determination of pyruvate and lactate production coupled with glucose (pyruvate) oxidation. To assess the possibility that both oxidative and nonoxidative portions of the pentose phosphate pathway were increased in the hypertrophied heart, we also measured the activity or expression of two key enzymes, glucose-6-phosphate dehydrogenase and transaldolase, of the oxidative and nonoxidative portions of the pentose phosphate pathway, respectively.

**EXPERIMENTAL METHODS**

**Animal Model**

Pressure-overload left ventricular hypertrophy was produced in 3-wk-old male Sprague-Dawley rats (50–75 g) by constriction of the suprarenal abdominal aorta with a metallic clip (0.4 mm diameter) (2). In sham-operated control rats, the aorta was isolated but not constricted. Experiments were performed 8 wk after surgery. Rats, housed in a temperature-controlled (22 ± 1°C) and light-controlled (12:12-h light-dark cycle) room, had free, unlimited access to feed and water. Care of the animals was performed in accordance with guidelines set out by the Canadian Council on Animal Care.

**Isolated Heart Preparation and Perfusion Protocol**

Hearts from halothane (2–3%)-anesthetized, sham-operated, and aortic-constricted rats were perfused for 30 min with Krebs-Henseleit (KH) solution in the working heart mode at a preload of 11.5 mmHg and an afterload of 80 mmHg, as previously described (1–3, 21, 38). The KH solution contained 1.2 mM palmitate prebound to 3% fatty acid-free albumin, 5.5 mM [5-3H/U-14C]glucose, 0.5 mM lactate, 2.5 mM calcium, and 100 μU/l of insulin and was continuously circulated through the closed perfusion system. A high concentration of palmitate was used to minimize differences in fatty acid oxidation, evident at 0.4 mM palmitate, between hypertrophied and control hearts (2, 32). To ensure that glucose uptake was not limiting, a high physiological concentration of insulin was utilized. The solution was oxygenated with 95% O2-5% CO2 and maintained at 37°C throughout the perfusion.

A pressure transducer (Viggo-Spectramed, Oxnard, CA) inserted in the afterload line was used to measure heart rate and peak systolic pressure. Cardiac output and aortic flow were measured via external flow probes (Transonic Systems, Ithaca, NY) on the preload and afterload lines, respectively. Coronary flow was calculated as the difference between cardiac output and aortic flow. Rate-pressure product, the product of heart rate and peak systolic pressure, and hydraulic work, the product of cardiac output and peak systolic pressure, were used to measure external work performed by the heart (1–3, 21, 38). These measures of heart function were assessed every 10 min throughout the working heart perfusion.

At the end of 30 min, hearts were quickly frozen using aluminum tongs cooled to the temperature of liquid nitrogen. Frozen heart tissue was powdered using a mortar and pestle and then stored in cryovials at −70°C until use.

**Measurement of Glycolysis and Glucose Oxidation**

Rates of glycolysis were determined simultaneously in the same hearts by two different and independent methods. Glycolysis was calculated as the sum of the rate of lactate and pyruvate accumulation in the perfusate and the rate of glucose oxidation (15). For this calculation, accumulation of total lactate and pyruvate or [14C]lactate and [14C]pyruvate in the perfusate was determined. Total lactate and pyruvate were measured enzymatically using a commercial kit (Sigma, St. Louis, MO). Accumulation of radiolabeled lactate and pyruvate was determined by means of a two-step assay described by Lehoux et al. (20). Briefly, the [14C]lactate in the sample was enzymatically converted to [14C]pyruvate in the first step. All of the [14C]pyruvate in the sample, including that originating from [14C]lactate, was then decarboxylated enzymatically to $^{14}$CO2 in the second step. The $^{14}$CO2 produced as a gas and from [14C]bicarbonate, released after addition of H2SO4, was collected into hyamine hydroxide-soaked filter paper, suspended in a center well in the reaction vial, and subsequently counted by standard techniques. Recovery rates with the use of this method, tested by spiking KH solution with known quantities of [14C]lactate, ranged from 85 to 90%. Rates of accumulation of lactate and pyruvate (total or 14C labeled) were calculated by taking account of the volume of the perfusate, the perfusion time, the dry heart weight, and the specific activity of perfusate [14C]glucose, where appropriate. Production of lactate and pyruvate is expressed as glucosyl units.

Glycolysis was also determined by measuring the rate of $^3$H2O production from [5-3H]glucose (1–3, 21, 38). Glucose oxidation rates were measured by quantitative collection of $^{14}$CO2 from [U-14C]glucose released as a gas and dissolved in the perfusate as [14C]bicarbonate, as previously described (1–3, 21, 38). Perfusate and gaseous samples were taken every 10 min of perfusion. Samples for determination of glycolysis and glucose oxidation were ultimately placed in vials containing scintillation cocktail and counted by standard techniques.

**Immunoblot Analysis of Transaldolase**

Expression of transaldolase protein in myocardium was determined by a previously described method (3). Briefly, samples of frozen ventricular tissue homogenate (containing 40–50 μg total protein) were solubilized by boiling in reducing sample buffer, separated by electrophoresis on 10% SDS-polyacrylamide gels, and transferred by electroblotting to a nitrocellulose membrane. After nonspecific blocking, the blots were probed overnight with a primary rabbit anti-rat transaldolase antibody (kindly donated by Dr. A. Perl, SUNY, Syracuse, NY). After incubation with anti-rabbit secondary antibody, the signal was detected by an enhanced
chemiluminescence-based detection system. Bands were quantified by densitometry. Equivalence of protein loading was confirmed by detection of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Measurement of Glycogen and G6PDH Activity

Myocardial glycogen content was determined after extraction of frozen, powdered ventricular tissue with 30% KOH, ethanol precipitation, and acid hydrolysis of glycogen (1, 21). G6PDH activity in myocardium was measured by a standard spectrophotometric technique (11).

Data Analysis

Data are expressed as means ± SE. Individual group means were compared using t-tests. The Bonferroni procedure was applied to all tests to correct for multiple tests and comparisons. A corrected value of $P < 0.05$ was considered significant.

RESULTS

Heart and Body Weight Data

Heart weight of aortic-constricted rats ($2.17 ± 0.05$ g, $n = 7$) was ~22% greater than that of the control rats ($1.78 ± 0.04$ g, $n = 7$, $P < 0.05$), consistent with our previous studies (1–3, 38) showing that this model produces a mild cardiac hypertrophy. Body weight was not significantly different between aortic-constricted (464 ± 12 g) and control (472 ± 10 g) rats.

Heart Function

Mechanical function was stable throughout the perfusion in both groups (data not shown). Heart rate, peak systolic pressure, and rate-pressure product were similar in control and hypertrophied hearts. Other parameters of heart function were lower in hypertrophied hearts than in control hearts, with statistically significant differences in cardiac output, hydraulic work, and coronary flow (Table 1).

Rates of Glycolysis

Determined from release of lactate plus pyruvate and glucose oxidation. Over the duration of the perfusion, accumulation of lactate and pyruvate was linear in both hypertrophied and control hearts (Fig. 1). Lactate and pyruvate were both released into the perfusate at significantly higher rates by hypertrophied hearts than by control hearts, which led to a greater overall accumulation of lactate and pyruvate in hypertrophied hearts (Table 2). Correspondingly, rates of accumulation of lactate (hypertrophy, 2,948 ± 375 vs. control, 1,095 ± 288 nmol glucose equivalents·min$^{-1}$·g dry wt$^{-1}$, $P < 0.05$) and pyruvate (hypertrophy, 647 ± 99 vs. control, 423 ± 54 nmol glucose equivalents·min$^{-1}$·g dry wt$^{-1}$, $P < 0.05$) were significantly greater in hypertrophied hearts than in control hearts. In contrast,

Table 1. Mechanical function in control and hypertrophied working rat hearts

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<th>Control</th>
<th>Hypertrophied</th>
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<tr>
<td>Heart rate, beats/min</td>
<td>265 ± 18</td>
<td>285 ± 16</td>
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<tr>
<td>Peak systolic pressure, mmHg</td>
<td>119 ± 6</td>
<td>113 ± 6</td>
</tr>
<tr>
<td>Rate pressure product, mmHg·min$^{-1}$·10$^{-3}$</td>
<td>31.1 ± 0.9</td>
<td>31.9 ± 0.6</td>
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<tr>
<td>Cardiac output, ml/min</td>
<td>77.7 ± 5.3</td>
<td>63.0 ± 1.2</td>
</tr>
<tr>
<td>Hydraulic work, mmHg·ml·min$^{-1}$·10$^{-2}$</td>
<td>91.5 ± 9.4</td>
<td>70.7 ± 3.5$^*$</td>
</tr>
<tr>
<td>Coronary flow, ml·min$^{-1}$·g wet wt$^{-1}$</td>
<td>37.0 ± 1.4</td>
<td>26.0 ± 3.0</td>
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</table>

Values represent means ± SE of data obtained at 30 min of perfusion; $n = 7$ per group. $^*$Significantly different from Control, $P < 0.05$.

Table 2. Perfusate lactate and pyruvate production in control and hypertrophied working rat hearts

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<thead>
<tr>
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<th>Control</th>
<th>Hypertrophied</th>
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<tbody>
<tr>
<td>Lactate</td>
<td>66.4 ± 17.3</td>
<td>198.2 ± 22.2$^*$</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>19.0 ± 1.4</td>
<td>31.5 ± 3.5$^*$</td>
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Values represent means ± SE; $n = 7$ per group. Values for lactate and pyruvate represent total accumulation in perfusate over the duration of 30-min perfusion. $^*$Significantly different from Control, $P < 0.05$.
GLYCOLYSIS IN CARDIAC HYPERTROPHY

Fig. 2. Rates of myocardial glycolysis in control (open bars) and hypertrophied (filled bars) hearts. Rates of glycolysis were determined from rates of lactate and pyruvate accumulation, measured enzymatically (Lactate + Pyruvate) or from 14C-labeled products ([14C]-Lactate + [14C]-Pyruvate) added to glucose oxidation, or from rates of 3H2O production from [5-3H]glucose. *Significantly different from Control, P < 0.05; n = 7 per group.

glycolysis, calculated by adding rates of lactate and pyruvate accumulation to rates of glucose oxidation, was, therefore, higher in hypertrophied hearts than in control hearts (Fig. 2). Similar results were obtained when glycolysis was calculated from [14C]lactate and [14C]pyruvate in the perfusate (Fig. 2).

Determined from detritiation of [5-3H]glucose. Cumulative rates of 3H2O production from [5-3H]glucose were linear in both control and hypertrophied hearts (Fig. 1). As with the other method, rates of glycolysis were also greater in hypertrophied hearts than in control hearts (Fig. 2). Glycolytic rates obtained in this way did not differ significantly from rates calculated on the basis of accumulation of total or [14C]lactate and [14C]pyruvate in either hypertrophied or nonhypertrophied hearts. A separate series of hearts perfused with 0.4 mM palmitate in the perfusate showed a similar concordance between rates of glycolysis determined by the two independent methods in both hypertrophied and control hearts (data not shown). Glycolysis that is determined from the detritiation of [5-3H]glucose is, therefore, a true measure of myocardial glycolysis, as obtained from lactate/pyruvate production and glucose oxidation.

Enzymes of the Pentose Phosphate Pathway and Glycogen Content

Expression of transaldolase [based on protein abundance, (Fig. 3)] and of G6PDH [based on enzyme activity (Table 3)] did not differ between hypertrophied and control hearts. Glycogen content at the end of the perfusion was also not significantly different between hypertrophied and control hearts (Table 3).

DISCUSSION

Goodwin et al. (15) have recently reported that use of [5-3H]glucose to measure glycolysis leads to an overestimation of “true” rates of glycolysis in isolated working rat hearts because of nonglycolytic loss of tritium from [5-3H]glucose. This suggestion was based on their finding that rates of glycolysis determined by measuring 3H2O production from [5-3H]glucose were two to three times higher than rates of glycolysis determined as the sum of lactate and pyruvate released to the perfusate and glucose oxidation rates measured using [14C]glucose. These authors proposed that glucose detritiation occurred extensively via the nonoxidative pentose phosphate pathway, specifically, by the enzyme transaldolase.

In contrast to Goodwin et al., we found an excellent concordance between rates of glycolysis determined by different and independent methods (Fig. 2) and confirmed that glycolysis is accelerated in hypertrophied hearts. Our data indicate that the nonglycolytic loss of tritium from [5-3H]glucose is insignificant, relative to loss via the glycolytic pathway, in both hypertrophied and nonhypertrophied hearts under the conditions used in our experiments. The concordance of glycolytic rates determined by the different methods also indicates that glycogen did not contribute significantly to total lactate and pyruvate accumulation in the perfusate, in keeping with the finding that the glycogen content did not differ among the two groups.

We attribute the discrepancy between the two studies primarily to two unexpected observations by Goodwin et al. The first is the exceedingly low glucose oxidation rates reported by Goodwin et al. (~100 nmol·min⁻¹·g dry wt⁻¹), especially when considered in relation to rates of glycolysis (15). In their study, glycolytic rates were at least 26-fold higher than glucose oxidation rates. This observation contrasts dramatically with the findings of Kantor et al. (18), in which

Fig. 3. Representative immunoblots of transaldolase (TA) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in control (Control) and hypertrophied (Hypertrophy) hearts. Each lane represents protein isolated from a single heart.
isolated working hearts were perfused with KH solution containing a similarly low (i.e., 0.4 mM) concentration of fatty acid. Compared with values in the study by Goodwin et al., glucose oxidation rates in isolated normal rat hearts were found to be substantially higher (∼1,900 nmol·min⁻¹·g dry wt⁻¹) by Kantor et al. The relatively high rates of glucose oxidation observed in the latter study are to be expected in the presence of low concentrations of fatty acid because of the well-established reciprocal relationship between catabolism of fatty acids and glucose in the heart (27, 29, 35).

The explanation for the dramatically lower glucose oxidation rates obtained in the study by Goodwin et al. is unclear but might be explained by the modified working heart preparation in which the coronary flow was not recirculated (15), as it is in the more traditional preparation (22). Thus, rather than calculating rates of glucose oxidation from the accumulation of 14CO₂ over the duration of the perfusion, perfusate containing 14CO₂ released in a single pass through the myocardium was collected into open preweighed vials over short time periods (i.e., 5 min). An aliquot (10 ml) of this perfusate was subsequently used to measure the 14CO₂ produced.

The use of nonrecirculating perfusion does provide some potential advantages. For example, the [14C]lactate and [14C]pyruvate that accumulate in the recirculating working heart preparation could contribute to overall 14CO₂ production. It might therefore be argued that the low glucose oxidation rates reported with the use of the nonrecirculating perfusion are a better representation of true glucose oxidation rates. However, elegant isotopomer analysis in nonrecirculating isolated rat hearts perfused with 0.4 mM oleate shows that this is not the case (9, 37). In the isotopomer studies, glucose oxidation rates in normal hearts exceeded ∼1,000 nmol·min⁻¹·g dry wt⁻¹ (∼10-fold higher than the values reported by Goodwin et al.) and are comparable to values obtained in a recirculating working heart preparation studied under similar conditions (18).

The second unexpected observation by Goodwin et al. (15) is the lack of linearity of 3H₂O production over a 30-min period. At the outset of perfusion, glycolytic rates determined from rates of 3H₂O production from [5-3H]glucose were relatively similar to those determined from lactate and pyruvate production rates. However, with time, 3H₂O production rates increased, whereas lactate and pyruvate production rates did not. This increase, which is the primary basis for their conclusions with respect to detritiation of [5-3H]glucose by the pentose phosphate pathway, is inconsistent with the findings of others, including those in the present study and previous investigations originating in Goodwin’s laboratory (2, 6, 22, 26).

As shown in Fig. 1, we do not see a time-dependent increase in 3H₂O production rates, a finding consistent with the published literature (2, 22, 26). Of additional importance is the fact that the recent conclusions by Goodwin et al. are inconsistent with data from a previous study (6), in which the same authors reported that detritiation of [5-3H]glucose does adequately trace glycolytic flux from exogenous glucose. In this previous study, it was found that 3H₂O production from [5-3H]glucose did not differ from 3H₂O production from [2-3H]glucose. Other studies have also shown that glycolytic rates measured using [5-3H]glucose and [2-3H]glucose are comparable (23, 24, 31). This is important, because detritiation of [2-3H]glucose, which occurs at the hexose 6-phosphate isomerase reaction, is not affected by the reactions in the pentose phosphate pathway implicated as being responsible for nonglycolytic loss of 3H. Therefore, significant production of 3H₂O from [5-3H]glucose by detritiation in the pentose phosphate pathway is unlikely. This view is consistent with evidence that the capacity of the oxidative pentose phosphate pathway in heart is very low compared with liver and other tissues and that maximal cardiac oxidative pentose phosphate pathway flux (generally <50 nmol·min⁻¹·g dry wt⁻¹) is far lower than that of glycolysis (7, 28, 33, 34, 40). Furthermore, nonoxidative pentose phosphate pathway flux between the hexose and ribose pools in the heart is likely even lower, probably not exceeding 2 nmol·min⁻¹·g dry wt⁻¹ (12, 30, 44).

Our finding that rates of glycolysis are accelerated in hypertrophied hearts, regardless of the method used, is in keeping with previous in vivo and in vitro observations that are based on a variety of independent parameters. For example, increased accumulation of 2-deoxyglucose 6-phosphate has been observed in hypertrophied dog (42) and rat (17) hearts in vivo. Furthermore, activity of a number of glycolytic enzymes is greater in hearts exposed to a pressure overload than in normal hearts (36), and isoenzymes of lactate dehydrogenase (5) and enolase (19) shift toward more anaerobic, fetal forms in hypertrophied hearts. The acceleration of glycolysis in hypertrophied hearts has been proposed as a compensatory response to low fatty acid oxidation rates in these hearts (2, 14).

With respect to the pentose phosphate pathway, we found no significant upregulation of key enzymes from either oxidative (G6PDH) or nonoxidative (transaldolase) branches in hypertrophied hearts (Table 3 and Fig. 3). Coupled with the fact that nonglycolytic loss of 3H from [5-3H]glucose is insignificant, these data suggest that the pentose phosphate pathway is not upregulated in the model of cardiac hypertrophy used in the current experiments. This conclusion is also consistent with previous studies of the pentose phosphate

Table 3. Biochemical parameters in control and hypertrophied working rat hearts

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<tr>
<th>Parameter</th>
<th>Control</th>
<th>Hypertrophied</th>
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<tbody>
<tr>
<td>Glycogen, μmol/g dry wt</td>
<td>113.9 ± 12.6</td>
<td>107.5 ± 12.4</td>
</tr>
<tr>
<td>G6PDH, 1 mU/mg protein</td>
<td>17.2 ± 0.9</td>
<td>18.0 ± 1.6</td>
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Values represent means ± SE; n = 7 per group. Measurements were made in hearts frozen at the end of the 30-min perfusion period.

1 Units of glucose-6-phosphate dehydrogenase (G6PDH) are μmol/min.
pathway in cardiac hypertrophy, in which changes in pentose phosphate pathway enzyme activities, when observed, were transient and reversed with time after aortic constriction (8, 25, 45).

The results of our study also have great general relevance to investigators in the field. Specifically, a very important corollary of the data obtained is that measurement of the rate of 3H2O production from [5-3H]glucose is an accurate means to determine rates of glycolysis in isolated working normal and pathological rat hearts. Moreover, the data obtained alleviate any doubts about conclusions from many studies over the years that have used this methodology to measure glycolysis in isolated hearts.

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