

letters to the editor

The following is the abstract of the article discussed in the subsequent letter:

Leong, Hon Sing, Mark M. 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* 283: E1039–E1045, 2002. First published January 29, 2002; 10.1152/ajpendo.00507.2001.—Glycolysis, measured by $^3\text{H}_2\text{O}$ production from $[5\text{-}^3\text{H}]\text{glucose}$, is accelerated in isolated working hypertrophied rat hearts. However, nonglycolytic detritiation of $[5\text{-}^3\text{H}]\text{glucose}$ 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 $[5\text{-}^3\text{H}]\text{glucose}$ 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 $[5\text{-}^3\text{H}]\text{glucose}$ in the PPP is insignificant, and $^3\text{H}_2\text{O}$ production from $[5\text{-}^3\text{H}]\text{glucose}$ 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.

Overestimating glycolysis in rat heart

To the Editor: When considering the paper by Leong et al. (4), the reader may be left with the impression that an earlier paper on a similar subject (3) is irrelevant, if not erroneous. Here we offer an explanation for the discrepancy of the results.

The validity of isotopic methods for the measurement of metabolic fluxes rests on the assumption that the isotope is metabolized in the same manner as unlabeled glucose. Errors can be introduced at different levels, e.g., by inadequate models, substrate cycling, recycling of isotopes, or lack of specific methods to measure glucose radioactivity (2). When we noted inconsistencies in our results from experiments using two different methods to measure myocardial glucose metabolism, we considered the pentose phosphate pathway (PPP) by measuring $^3\text{H}_2\text{O}$ release from $[5\text{-}^3\text{H}]\text{glucose}$ and by a second independent method. We found that apparent glycolytic flux obtained by $^3\text{H}_2\text{O}$ production from $[5\text{-}^3\text{H}]\text{glucose}$ overestimated true glycolytic flux (3). Our conclusions were based on rate determinations obtained at 5-min intervals over a 30-min period of stable cardiac performance and a lack of

constancy of the rate of detritiation. Using the same preparation, but imposing a three times greater workload on the heart (which accounts for the higher rates of glucose oxidation), Leong et al. (4) now report that significant production of $^3\text{H}_2\text{O}$ from $[5\text{-}^3\text{H}]\text{glucose}$ by detritiation in the PPP is unlikely. The apparent discrepancy with our results is explained by the small number of observations ($n = 3$), by the small number of time points ($n = 3$), and by the way the results are analyzed and displayed (cumulative counts and not rates). In graphs of cumulative flux, any later data points carry over the variability of earlier time points. By selecting three of our experiments and plotting means of cumulative glycolytic flux at three time points, we also observe an apparent linearity (Fig. 1A). The same data plotted as individual experiments show a nonlinear increase in cumulative flux (Fig. 1B). In Fig. 1C, we have plotted rates of glycolytic flux for the same three experiments at 5-min intervals, and there is a significant increase over time ($P < 0.05$). In short, we are not certain whether the analysis of Leong et al. (4) had sufficient power and was performed in a manner to detect nonlinearity. A second point is that any

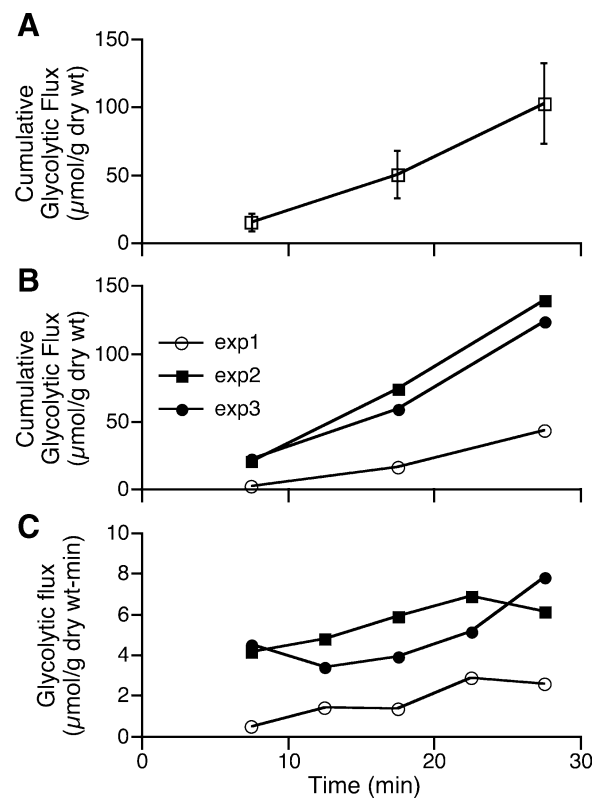


Fig. 1. Cumulative and noncumulative rates of glycolytic flux in rat heart ($n = 3$). Please see text for details.

error is a systematic error that should not affect the final interpretation of the results, unless nonoxidative PPP is different in the two groups. We made a similar systematic error in an earlier study (1), where we were also not aware of a difference between the different tracers. This earlier study cannot be directly compared with our later study (3), because it addressed an entirely different issue: ischemia and reperfusion, and the partitioning of glucose between different pathways (glycolysis and oxidation), with glucose as the only exogenous substrate supplied to the heart. Finally, we would like to suggest that there is no a priori reason to suggest that flux through the nonoxidative PPP should be affected by hypertrophy. The authors have shown this very clearly in their study.

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REPLY

To the Editor: In attempting to explain discrepant findings between their earlier paper (3) and a more recent one from our laboratory (5), Drs. Taegtmeier and Cohen raise issues that warrant further discussion.

We remain puzzled by the nonlinear glycolytic flux from [5-³H]glucose reported by the authors (3), because linearity of ³H₂O production from [5-³H]glucose has been consistently observed by us and others (6). In response to their concerns about the power and manner of analysis in our recent study (5), we reviewed our previous work. The results of one extensive study (1) are summarized here, expressed as cumulative glycolysis (Fig 1A) or rates of glycolysis (Fig 1B). This representative study unequivocally demonstrates linearity of glycolysis. Apart from the one study cited above (3), we are not aware of any other studies in which

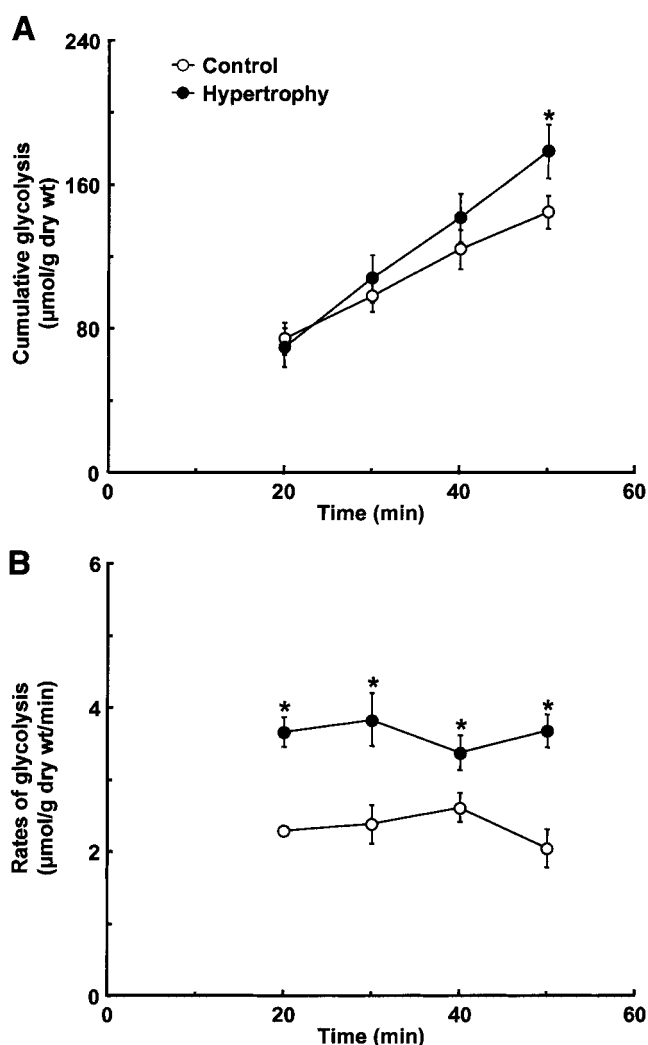


Fig. 1. Cumulative glycolysis (A) and rates of glycolysis (B) in isolated rat hearts ($n = 8$ per group). * $P < 0.05$ vs. Control.

nonlinearity of ³H₂O production from [5-³H]glucose has been reported.

In our view, Drs. Taegtmeier and Cohen fail to adequately address a second and crucial discrepancy between our two studies. Rates of glucose oxidation reported in their study are very low, despite the fact that the hearts were perfused with lower concentrations of fatty acid than in our study (0.4 vs. 1.2 mM). In the presence of low fatty acid concentrations, glucose oxidation rates ≥ 10 - to 20-fold higher than those reported by the authors are commonly observed by use of similar (4) or different methodologies (2). This issue is of critical importance, because glucose oxidation rates are used to calculate “true” rates of glycolysis (3). Significantly, discrepancies between “true” rates of glycolysis (estimated from the sum of rates of lactate-pyruvate production and glucose oxidation) and “apparent” glycolysis (from [5-³H]glucose) disappear if the higher glucose oxidation rates are used to calculate “true” glycolysis. With respect to workload as an explanation, careful review indicates that the relevant (i.e., pressure-

related) workload was at most 20% higher in our study and not threefold higher as suggested.

We agree that there was no a priori reason to suspect upregulation of the nonoxidative pentose phosphate pathway (PPP) in cardiac hypertrophy. However, we addressed this issue because Drs. Taegtmeyer and Cohen themselves had previously noted that the oxidative PPP is upregulated in hypertrophied hearts (3), a situation that would likely raise doubts about the utility of using [5-³H]glucose methodology in hypertrophied hearts if not specifically addressed.

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