Regulation of Energy Metabolism of the Heart during Acute Increase in Heart Work*

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We determined the contribution of all major energy substrates (glucose, glycogen, lactate, oleate, and triglycerides) during an acute increase in heart work (1 μM epinephrine, afterload increased by 40%) and the involvement of key regulatory enzymes, using isolated working rat hearts exhibiting physiologic values for contractile performance and oxygen consumption. We accounted for oxygen consumption quantitatively from the rates of substrate oxidation, measured on a minute-to-minute basis. Total β-oxidation (but not exogenous oleate oxidation) was increased by the work jump, consistent with a decrease in the level of malonyl-CoA. Glycogen and lactate were important buffers for carbon substrate when heart work was acutely increased. Three mechanisms contributed to high respiration from glycogen: 1) carbohydrate oxidation was increased selectively; 2) stimulation of glucose oxidation was delayed at glucose uptake; and 3) glycogen-derived pyruvate behaved differently from pyruvate derived from extracellular glucose. Despite delayed activation of pyruvate dehydrogenase relative to phosphorylase, glycogen-derived pyruvate was more tightly coupled to oxidation. Also, glycogen-derived lactate plus pyruvate contributed to an increase in the relative efflux of lactate versus pyruvate, thereby regulating the redox. Glycogen synthesis resulted from activation of glycogen synthase late in the protocol but was timed to minimize futile cycling, since phosphorylase became inhibited by high intracellular glucose.

Increased heart work, usually elicited by catecholamines, increases carbohydrate oxidation because of activation of the pyruvate dehydrogenase complex (PDC) by dephosphorylation (1); PDC phosphatase is stimulated by increased mitochondrial Ca²⁺ (2). Activation of PDC under conditions of high workload could be advantageous, tending to increase carbohydrate oxidation selectively. Under the assumption that the ATP yield per O₂ consumed is higher for oxidation of carbohydrate versus lipid, a selective increase in carbohydrate oxidation could provide increased ATP synthesis despite maximum oxygen extraction.

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1 The abbreviation used is: PDC, pyruvate dehydrogenase complex.

Whether increased substrate oxidation with workload is selective for carbohydrate remains unresolved. Studies by us (3) and by Collans-Nakai et al. (4), using isolated working rat hearts, indicated that increased substrate oxidation is selective for carbohydrate, with little or no increase in exogenous fatty acid oxidation. In contrast, early studies by Neely et al. (5, 6) and Crass et al. (7) suggested that fatty acid and carbohydrate oxidation increase in parallel. Hall et al. (8) showed that swine heart exhibit increased fatty acid uptake in vivo in response to dobutamine, presumably from stimulated β-oxidation, since levels of malonyl-CoA were reduced. Increased systemic non-esterified fatty acids resulting from peripheral lipolysis could also have promoted fatty acid uptake in that study. Awan and Saggerson (9) found reduced malonyl-CoA in isolated hearts and stimulated palmitate oxidation by isolated (nonworking) heart myocytes upon adrenergic stimulation. Therefore, the potential exists for direct adrenergic stimulation of total β-oxidation in the heart resulting from lowered malonyl-CoA, which is a potent inhibitor of a limiting enzyme for β-oxidation, carnitine palmitoyltransferase I.

The problem with existing studies is the failure to consider all relevant exogenous and endogenous substrates. The question of whether carbohydrate cannot be answered based on measures of glucose and exogenous fatty acid oxidation alone. Regulation of total β-oxidation should, ideally, consider both exogenous and endogenous lipids. Therefore, we extended existing studies in the following important ways. We examined oxidation of all carbohydrates (glucose, glycogen, and lactate) that together contribute virtually all PDC flux. We also considered total β-oxidation of exogenous and endogenous lipids. We used a pulse-chase technique to measure glycogen turnover continuously. Using this method, we find that the degree of coupling of glycogen utilization to oxidation varies depending on the relation between glycolysis and the capacity for carbohydrate oxidation (i.e. phosphorylase activation versus PDC activation). To explain the tight coupling of glycogen to subsequent oxidation, we examined the hypothesis that the burst of glycogenolysis is coordinated with activation of PDC. Surprisingly, it was not.

Lactate and pyruvate are released or consumed by tissues at different rates, since their systemic concentrations differ. We hypothesize that heart, which, at times, displays large fluxes for lactate or pyruvate across the plasma membrane, utilizes differential efflux of lactate versus pyruvate (a redox pair) as a supplemental mechanism for regulation of the cytosolic redox.

We previously found small amounts of simultaneous glycogen synthesis and degradation in heart, especially during glycogen depletion (10). Compared with total glycolytic flux, the contribution of flux through glycogen in the absence of cyclical changes in glycogen content ("glycogen cycling") (11) was minor. In the present study, we examined activities for synthase.
and phosphorylase and their allosteric effectors in relation to fluxes for glycolgen synthesis and glycogenolysis. We postulate that the well described reciprocal regulation of glycolgen synthase and phosphorylase, apparently designed to prevent futile cycling of glycolgen, is robust, preventing more than minor glycolgen cycling in heart.

**EXPERIMENTAL PROCEDURES**

**Materials**—Isotopes were from ICN (Costa Mesa, CA). Enzymes were from Boehringer Mannheim. Other chemicals were from Sigma. Fatty acid synthesis was purified from rat liver after inducing the enzyme; rats were fasted for 2 days and then fed bread (8% protein, no fat) for 3 days. Enzyme was purified from the cytosol of four rat livers (53 g) as described by Awan and Saggerson (9) except that chromatography was on DEAE-cellulose, as described by Linn (12). We obtained 68 units (1.1 unit/mg of protein), assayed by the method of Carey and Dils (13).

**Heart Perfusion**—Hearts from chow-fed male Harlan Sprague-Dawley rats (349 ± 8 g, n = 25) were perfused using the working heart apparatus (14) in a gas-tight configuration described previously (10).

The sum of 14C-lactate plus 14C-pyruvate was determined in deproteinized perfusate by paper chromatography (16). Lactate and pyruvate migrated together in this system. The recovery of lactate from perfusate was initially 100 cm H2O, increased to 140 cm H2O at the time of adrenergic stimulation. The initial perfusate was Krebs-Henseleit buffer containing 1.4 mM free Ca2+ (1.5 mM CaCl2 plus 0.1 mM EDTA), equilibrated with 95% O2, 5% CO2. Hearts were perfused in the working mode using 200 ml of recirculated perfusate, lacking carbon substrate initially. The initial part of this protocol (Fig. 1) was designed to deplete and then resynthesize glycolgen, as we previously described (3). After 20 min, the perfusate was supplemented to 5 mM glucose, 40 microunits/ml regular insulin (Lilly), 5 mM sodium β-hydroxybutyrate, and 0.5 mM sodium t-lactate, and perfusion was continued for 25 min to allow glycolgen resynthesis while contractile function returned to base line. Hearts were then switched to a nonrecirculating mode as described previously (3). The perfusate during this period was Krebs-Henseleit buffer containing 5 mM glucose, 40 microunits/ml insulin, 0.5 mM sodium β-hydroxybutyrate, 3% (w/v) bovine serum albumin (fraction V, fatty acid-free; Intergen, Purchase, NY), and 1.4 mM free Ca2+ (the preparation was dialyzed against a large volume of albumin-free perfusate containing 1.5 mM CaCl2 and 0.1 mM EDTA). At 55 min, epinephrine bitartrate was added to 1 μM, and the height of the aortic overflow was raised to 140 cm above the heart. Hearts were freeze-clamped on their cannulae with aluminum tongs cooled in liquid N2.

We studied three treatment groups according to the time at which hearts were freeze-clamped: “unstimulated” (n = 5, clamped at 55 min of the protocol), “acute stimulation” (n = 5, clamped at 58 min at the peak of glycolgen oxidation, Fig. 3), and “prolonged stimulation” (n = 15, clamped at 75 min). Hearts subjected to prolonged stimulation were randomly assigned to one of three treatment groups according to which 14C labeled substrates (14CO2 from glucose, glycogen, or lactate) were employed. 14C-phosphate, phosphocreatine, Pi, pyruvate, and malonyl-CoA were measured with the acid molybdate reaction (18). Malonyl-CoA was measured following incubation for 30 min at 30 °C with 1 mM CaCl2 (25). The active form was measured directly, and the total activity of Pi measured with the acid molybdate reaction (18). Phosphorylase was measured in the presence of 3 mM AMP. Pyruvate dehydrogenase was measured by 14C2O2 production from 2 mM [14C]pyruvrate, as described by Harris et al. (25). The active form was measured directly, and the total activity was measured following incubation for 30 min at 30 °C with 1 mM CaCl2 plus 5 mM MgCl2, to allow dephosphorylation by endogenous PDC phosphatase. This procedure produced stable, maximal values for the total activity of PDC. We established that all of the enzyme assays were linear with respect to time and amount of tissue extract used in the assays.

Data are expressed as mean ± S.E. Statistical comparison was by analysis of variance with post hoc comparison by Newman-Keuls multiple comparison test. p < 0.05 was considered significant.

**RESULTS**

**Physiological Performance (Fig. 2 and Appendix Table IA)**—We performed five sets of matched perfusions, three of which were used to measure oxidation of three different 14C-labeled substrates ([14CO2 from glucose, glycogen, or lactate]). Physiologic performance of these three groups is shown in Fig. 2. Fig. 2A shows contractile activity (hydraulic power, watts), measured from panel shows oxygen consumption (MVO2). Two other treatment groups (unstimulated, and acute stimulation) were omitted from the figure for clarity, because the data points overlapped the values presented. These two groups were freeze-clamped at earlier times of the protocol, for tissue analysis. Various measures of performance for all the groups are given in the Appendix. The five groups were well matched for...
all values of performance measured. Performance is comparable with values measured \textit{in vivo} for resting and exercising rats (26).

During the first 20 min of the protocol (Fig. 1), the absence of exogenous substrates resulted in diminishing contractile function. The protocol was designed to deplete endogenous substrates, evidenced by continued oxygen consumption. The addition of substrates at 20 min (glucose plus insulin, lactate, and D-β-hydroxybutyrate) restored performance (Fig. 2). We used D-β-hydroxybutyrate as a co-substrate to maintain incorporation of glucose into glycogen. Perusions were switched to a nonrecirculating mode starting at 45 min (beginning of the “chase” for glycogen labeling), and the only change in substrate availability at that time was the replacement of β-hydroxybutyrate for a physiologic long-chain fatty acid, oleate prebound to albumin. Following a 10-min equilibration period, hearts were stimulated with epinephrine (1 \(\mu\)M), and at the same time, we raised the afterload by 40%. There was an immediate 95% increase in contractile performance and 103% increase in MVO\textsubscript{2}. As occurs \textit{in vivo}, oxygen extraction was slightly increased by this intervention, and \(O_2\) was almost completely extracted, but most of the increase in MVO\textsubscript{2} resulted from an increase in coronary flow (see Appendix). Immediately after the work jump, there was no change in the supply/demand ratio for oxygen (MVO\textsubscript{2}/power), suggesting that hearts did not experience acute demand ischemia (this issue is examined further below). There was a 14% reduction in the ratio MVO\textsubscript{2}/power after prolonged stimulation, consistent with the well known reduction in cardiac efficiency during adrenergic stimulation.

**Rates for Substrate Oxidation**—Fig. 3 shows rates of substrate oxidation during the chase period for glycogen. Rates were measured by the Fick principle (\(V - A\) difference times coronary flow, but the \(A\) side was fresh perfusate or endogenous substrate), based on \(^{14}\text{CO}_2\) production for carbohydrate oxidation, or \(^3\text{H}_2\text{O}\) production from exogenous [9,10-\(^3\text{H}\)]oleate. The results are similar to those we reported previously (3), determined in the absence of insulin and lactate and without increasing the perfusion pressure. The new finding is that, like glycogen, lactate oxidation is rapidly increased, but unlike glycogen, increased lactate oxidation is sustained.

**Glycogen Content and Enrichment**—Table I gives values for the content of total glycogen, \(^{14}\text{C}\)-glycogen, and enrichment. In hearts clamped at 55 min (unstimulated), the glycogen enrichment achieved by the labeling protocol (pulse portion of the pulse-chase) was 55 ± 10%. With acute adrenergic stimulation, the decrease in glycogen content was not large, since the duration of stimulation was short (3 min). Following prolonged stimulation, the total glycogen content decreased by half, as did the content for \(^{14}\text{C}\)-glycogen. The enrichment of residual glycogen remained the same (49 ± 9\%, \(^{14}\text{C}\)-glycogen group in Table I). A similar value for enrichment (49\%) was calculated for the glycogen that was broken down, correcting for the small amount of \textit{de novo} synthesis (\(^{14}\text{C}\)glucose group in Table I). These findings indicate that glycogen was degraded randomly, without discretion for new \(^{14}\text{C}\)glycosyl residues and preexisting \(^{12}\text{C}\)glycogen. As expected, there was no glycogen synthesis from lactate (Table I, last line).

We next considered the possibility that the initial glycogen degraded was enriched to a greater extent than the rest (i.e. a hybrid of random and ordered degradation). The distinction
Table I

Glycogen content and enrichment

| Treatment group       | [Total glycogen] | [14C](Glycogen) (enrichment) |
|-----------------------|------------------|-------------------------------|
| Unstimulated (n = 5)  | 107 ± 6          | 59 ± 10 (55 ± 10%)            |
| Acute stimulation     | 80 ± 2           | ND*                           |
| Prolonged stimulation |                  |                               |
| (n = 15)              | 55 ± 5           | NA^b                          |
| [14C]Glucose group    | 57 ± 8           | 30 ± 9 (49 ± 9%)              |
| (n = 5)               | 46 ± 8           | 4.0 ± 0.7 (8.0 ± 0.5%)        |
| [14C]Lactate group    | 62 ± 11          | 0                             |

* Not determined.
^b Not applicable.

(purely random versus hybrid model) is important for interpretation of the present data, as well as data from 13C NMR spectroscopy studies. To do this, we examined the time course of glycogen enrichment by taking advantage of oxygen consumption measurements, but it was first necessary to account for oxygen consumption from all the substrates.

Triglyceride Turnover and Pyruvate Release—Total triglyceride content of the hearts increased slightly (not significantly) during the 20 min of adrenergic stimulation (from 56.9 ± 6.2 (n = 5) to 59.3 ± 6.3 (n = 15) μmol of acyl/g, dry weight for unstimulated and prolonged stimulation treatment groups, respectively). During the same interval, de novo synthesis, based on incorporation of [9,10-3H]oleate into triglycerides, increased (p < 0.05) from 1.38 ± 0.34 (n = 3) to 6.38 ± 1.40 (n = 15) μmol of acyl/g, dry weight. Therefore, the total triglyceride pool size expanded by 2.4 μmol acyl/g, dry weight, and de novo synthesis was 5.0 μmol of acyl/g, dry weight. Using the relation degradation = synthesis – change in pool size, the value for degradation is 5.0–2.4 = 2.6 μmol/g, dry weight, and the corresponding rate over 20 min is 0.13 μmol/min/g, dry weight (8% of exogenous oleate oxidation). To calculate oxygen consumption, we assumed that triglycerides were oxidized using a value of 25.5 mol of O2/μmol for the average acyl group (i.e. like oleate).

Release of pyruvate contributes slightly to oxygen consumption (0.5 mol of O2/μmol of pyruvate release) because pyruvate is more oxidized than its precursors (glucose, glycogen, or lactate). Rates of pyruvate release are given in Appendix Table II.

Oxygen Consumption from Total Substrate Oxidation and the Time Course of Glycogen Enrichment (Figs. 4 and 5)—Fig. 4 shows predicted and measured rates of oxygen consumption. Predicted rates are based on the sum for the measured rates of oxidation of every major substrate (glucose, glycogen, lactate, oleate, triglycerides, and release of pyruvate). To calculate rates of oxygen consumption resulting from glycogen oxidation, we assumed that there is uniform isotopic dilution (i.e. that a purely random pattern of synthesis and degradation applies) and used the values given in Fig. 3. Two conclusions can be drawn from the close agreement between measured and predicted MVO2. First, we accounted for every major oxidizable substrate for the heart quantitatively. Second, the assumption of uniform isotopic dilution is valid. The second conclusion is shown more clearly in Fig. 5, which depicts oxygen consumption resulting from glycogen oxidation over time. The upper curve is oxygen consumption from oxidation of all glycogen, based on the difference between MVO2 and oxygen consumption from every substrate except glycogen. The lower curve is oxygen consumption from oxidation of that portion of glycogen (55% of the total) that was labeled with 14C (mean ± S.E., n = 5).

Fig. 4. Measured and predicted values for oxygen consumption. Oxygen consumption during the chase period of the protocol is depicted. Open symbols, values measured directly from the A – V difference for oxygen (MVO2, mean ± S.E., n = 15). Closed symbols, predicted values calculated from the sum for the measured rates of oxidation of glucose, glycogen, lactate, oleate, triglycerides, and release of pyruvate, using values depicted in Fig. 3 and Appendix Table IIA.

Fig. 5. Oxygen consumption resulting from total glycogen oxidation compared with [14C]glycogen oxidation. Oxygen consumption from glycogen oxidation during the chase is depicted. Open symbols, oxygen consumption resulting from oxidation of all glycogen, calculated by taking the difference between MVO2 (shown in Fig. 4) and oxygen consumption resulting from oxidation of every substrate except glycogen. Closed symbols, oxygen consumption resulting from oxidation of that portion of glycogen (55% of the total) that was labeled with 14C (mean ± S.E., n = 5).
stimulation. There was turnover following prolonged stimulation, since, as described above, there was robust glycogen oxidation (Fig. 3), and de novo synthesis occurred during the same period (Table I, [14C]glucose treatment group). Table II shows activity states for glycogen synthase at different times of the protocol, along with the content of glucose 6-phosphate (activator of the glucose 6-phosphate-independent form), which accounts for the observed changes in de novo synthesis. The activity state of synthase was increased by 65% following prolonged stimulation. However, the enzyme was not activated acutely. The content of glucose 6-phosphate was not significantly changed during the protocol. These data indicate that a small amount of glycogen synthesis occurred late in the protocol, since synthase did not become activated until after prolonged stimulation. The potential for futile cycling is therefore limited, since most of glycogenolysis occurred early (Fig. 3).

Lactate/Pyruvate Ratios, High Energy Phosphates, and Regulation of the Redox (Appendix Tables II A and III A)—We measured high energy phosphates and the intracellular ratio of lactate/pyruvate to determine if hearts experienced demand ischemia upon adrenergic stimulation, which would exaggerate glycogenolysis. The ratio of lactate/pyruvate, which rises dramatically during ischemia, reflects the cytosolic redox potential (NADH/NAD+), assuming lactate dehydrogenase is near equilibrium. We did not observe an acute increase in this ratio (Appendix Table IIA). Therefore, hearts were not ischemic during acute stimulation, when glycogenolysis occurred. The largest change in high energy phosphates was a 21% decrease in phosphocreatine during acute stimulation, in exchange with Pi for the most part (Appendix Table IIIA). The ATP content was not decreased during acute stimulation. That acute changes in high energy phosphates were small or absent further supports the conclusion of adequate oxygen supply.

Prior to adrenergic stimulation, there was small net lactate extraction resulting from a small lactate gradient across the plasma membrane (0.5 mM extracellular versus 0.45 mM in the cytosol). With adrenergic stimulation, extraction switched to net release, and there was a large transient burst of release (Appendix Table IIA). Subsequently, lactate release quickly decreased to a new steady state, which was maintained for the remainder of the protocol. There was simultaneous oxidation of exogenous lactate during net release (Fig. 3). This phenomenon has been observed previously (27, and could result from mixing of intracellular and extracellular lactate pools or from cellular heterogeneity. Not unexpectedly, a pyruvate gradient (roughly equal to the intracellular pyruvate concentration, Appendix Table IIA) produced pyruvate efflux. Although pyruvate release followed its concentration gradient throughout the protocol, the burst of lactate release immediately after adrenergic stimulation (derived primarily from glycogen) was out of proportion to the lactate gradient (0.5 mM extracellular versus 0.87 mM in the cytosol). The resulting large increase in the ratio for lactate efflux relative to pyruvate efflux (from −0.49 ± 0.62 to +4.42 ± 1.14, p < 0.05) prevented an acute increase in the lactate/pyruvate ratio (Appendix Table IIA). The ratio of lactate/pyruvate did eventually increase (after glycogenolysis had ceased) despite persistent increase in the ratio for lactate efflux relative to pyruvate efflux (3.85 ± 1.53, p < 0.05 versus the unstimulated state). We explain the result by a switch from glycogen to glucose as the glycolytic substrate and tighter coupling of glycogen to oxidation (see below), since 70% of released lactate plus pyruvate was derived from glycogen during acute stimulation, but it was entirely from exogenous glucose later in the protocol.

**Coupling of Glycolysis to Pyruvate Oxidation**—Fig. 6 shows cumulative values for metabolism of glycogen (A) or glucose (B) by glycolysis, oxidation, and the percentage of glycogen that was subsequently oxidized. We expressed the results as cumulative values (the slope is equal to the rate), because different

| Table II | Glycogen synthase activity and the content of glucose 6-phosphate |
|----------|---------------------------------------------------------------|
| Values are the mean ± S.E. (n = 5) for the glucose 6-phosphate-independent form. Values are the mean ± S.E. (n = 5) for the glucose 6-phosphate-independent form. The activity state for the glucose 6-phosphate-independent form, which accounts for the observed changes in de novo synthesis. The activity state of synthase is increased by 65% following prolonged stimulation. However, the enzyme was not activated acutely. The content of glucose 6-phosphate was not significantly changed during the protocol. These data indicate that a small amount of glycogen synthesis occurred late in the protocol, since synthase did not become activated until after prolonged stimulation. The potential for futile cycling is therefore limited, since most of glycogenolysis occurred early (Fig. 3).

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TABLE III
Activity state of PDC and of phosphorylase and the content of effectors of phosphorylase, AMP, and glucose

| Parameter measured | Treatment group | | | |
|--------------------|-----------------|---|---|---|
| | Unstimulated (n = 5) | Acute stimulation (n = 5) | Prolonged stimulation (n = 15) | |
| PDC activity state | 17.8 ± 6.3 | 38.0 ± 2.5 | 62.6 ± 4.9 | |
| Phosphorylase activity state | 20.9 ± 3.4 | 50.0 ± 6.3 | 33.0 ± 1.1 | |
| AMP (µmol/g, dry wt) | 1.49 ± 0.33 | 2.35 ± 0.16 | 1.44 ± 0.15 | |
| Intracellular glucose (µmol/ml) | 0.10 ± 0.60 | 2.58 ± 1.54 | 2.15 ± 0.27 | *p < 0.05 versus unstimulated.

*Values are the mean ± S.E. The activity state of PDC is the active form/total activity × 100%. The activity state of phosphorylase is phosphorylase/total phosphorylase × 100%. The total activity of PDC did not differ between the groups and was 21.3 ± 1.4 µmol/min/g, dry weight (n = 25). Total phosphorylase activity did not differ between the groups and was 221 ± 6 µmol/min/g, dry weight (n = 25). Intracellular glucose was calculated using the formula given in Appendix Table IIA.

Temporal Dissociation of Activation of Glycogen Phosphorylase from PDC and Regulation of Phosphorylase (Table III)—The burst of release of glycogen-derived lactate plus pyruvate immediately after adrenergic stimulation arose, first, because PDC was slow to become activated relative to phosphorylase (Table III) and, second, because of competition between extracellular lactate with glycogen as sources of pyruvate for PDC (Fig. 3).

Adrenergic stimulation predictably converted a larger portion of phosphorylase to the α form (Table III), since phosphorylase kinase is stimulated by cAMP and Ca2+. However, because intracellular glucose was acutely increased, augmented phosphorylase flux must have resulted largely from elevated AMP, a potent allosteric activator of phosphorylase b. Increased F1, (derived mostly from phosphocreatine; see Appendix Table IIIA), being a substrate for phosphorylase, probably also contributed to increased phosphorylase flux. Because of accumulation of intracellular glucose, which inhibits phosphorylase a allosterically, phosphorylation of phosphorylase to the α form may have paradoxically inhibited flux through the enzyme. Indeed, the mechanism for cessation of phosphorylase flux, despite 50% residual glucogen, appears to be the return of AMP to control levels, since phosphorylase a and intracellular glucose remained elevated. We conclude that the transient burst of phosphorylase flux reflects the status of high energy phosphates and not the activation state of phosphorylase kinase.

Coordination of Glycogenolysis from Glucose Versus Glycogen—During the first minutes of adrenergic stimulation, there was a gradual switch from glycogen to glucose as the glycolytic substrate (Fig. 3). The coordination between glucose and glycogen during the switch resulted from inhibition of glucose transport by accumulation of intracellular glucose during rapid glycogen break-down (Table III), partially derived from glycogen itself (i.e. the 8% or so released as glucose instead of glucose-1-phosphate). The majority of glycolytic flux from glycogen bypasses hexokinase. Therefore, one would expect that if a bottleneck were at phosphofructo-1-kinase, then glucose-6-phosphate would increase during a burst of glycogenolysis, but this was not the case (Table II). We conclude that coordination between glucose and glycogen occurs at glucose transport.

Rates of Fatty Acid Oxidation and Regulation by Malonyl-CoA—Table IV gives values for exogenous oleate oxidation and total β-oxidation (exogenous plus endogenous lipids). We measured exogenous fatty acid oxidation directly ([1-3H]oleate). Total β-oxidation was measured indirectly by a completely independent method based on oxygen consumption not resulting from carbohydrate oxidation. To check the validity of this approach, we calculated total β-oxidation based on the sum for oleate oxidation plus the value for triglyceride oxidation measured during adrenergic stimulation (0.13 µmol/min/g, dry weight) that was described above (see “Triglyceride Turnover and Pyruvate Release”). Values for total β-oxidation calculated from oleate plus triglycerides during acute stimulation (1.71 µmol/min/g, dry weight) and prolonged stimulation (1.74 µmol/min/g, dry weight) are slightly less (not significant) than values for total β-oxidation based on oxygen consumption measurements (Table IV). This is the expected result, since most of endogenous lipid oxidation is from oxidation of triglycerides. Prior to adrenergic stimulation, total β-oxidation was in excellent agreement with oleate oxidation (Table IV). This result is not surprising, because we expect to find diminished rates of triglyceride oxidation in the unstimulated state. We used the values for total β-oxidation based on oxygen consumption measurements as the best estimate of total β-oxidation. With adrenergic stimulation, the increase in oleate oxidation (20%) was small and did not reach statistical significance. The increase in total β-oxidation (40%) was larger and was significant. The level of malonyl-CoA was decreased by 33% following prolonged stimulation (Table IV), which is consistent with the increase in total β-oxidation.

**DISCUSSION**

The present study provides a comprehensive analysis of fuel selection during acute transition from low to high workload of isolated working rat heart. We confirm preliminary studies (3) by showing that glycogen and, to a lesser extent, lactate are important energy substrates for aerobic heart when the workload is acutely increased (Fig. 3). Although exogenous fatty acid oxidation was not significantly changed, total β-oxidation was increased by 40%, and the increase was associated with a decrease in the level of malonyl-CoA. This is expected based on the regulation of total β-oxidation by malonyl-CoA inhibition of carnitine palmitoyltransferase I (28). The increase in total β-oxidation was not in proportion to energy demand, however, so increased carbohydrate oxidation, facilitated by activation of PDC, became relatively more important. The pattern of substrate oxidation that developed with more prolonged adrenergic stimulation (Fig. 3) is in keeping with the observation in vivo that nonesterified fatty acids and lactate are major respiratory substrates for the heart in the exercising state (29, 30).
Triglyceride oxidation resulted from turnover of the triglyceride pool. Our result agrees with the previous estimate that triglyceride turnover contributes about 10% to total β-oxidation in the steady state (31). The capacity for mobilization of triglycerides, reflecting maximum triglyceride lipase activity, is unknown. However, lipolytic capacity is probably less than the tremendous capacity for glycogen mobilization in heart, since triglycerides were not an important endogenous energy substrate compared with glycogen in the short term. Glycogen may serve to prevent transient supply-demand mismatch for carbon substrate during acute increase in heart work, which would impair contractile function in the short term.

To answer the question posed in the Introduction, when all relevant exogenous and endogenous substrates are examined, the increase in carbohydrate oxidation upon adrenergic stimulation is, in fact, selective. Carbohydrate oxidation is increased selectively because total β-oxidation, regulated mostly by malonyl-CoA levels in the cytosol, is independent of the activity state of PDC in the mitochondria (compare PDC activities in Table III to malonyl-CoA levels in Table IV). Our data do not support the suggestion that PDC activation, by providing substrate for the synthesis of malonyl-CoA in the cytosol (by acetyl-CoA carboxylase), is a mechanism for reciprocal regulation of carbohydrate and fatty acid oxidation (32), at least in the setting of adrenergic induced activation of PDC.

In very broad terms, we confirmed early studies of Neely et al. (5, 6) and Crass et al. (7) by showing that stimulation of heart work increases both carbohydrate and fatty acid oxidation. However, these early studies examined only exogenous substrate oxidation. Presently, we did not find an increase in exogenous fatty acid oxidation (there was a 20% increase, but this was not statistically significant). This result agrees with our previous report (3) and a similar study by Collins-Nakai et al. (4). Further, the small increase in total β-oxidation that we did find (40%) was not in step with carbohydrate oxidation or the workloads, contrary to Neely et al. (6). We also confirmed the report by Awan and Saggerson that epinephrine decreases the level of malonyl-CoA in isolated hearts (9). We extend the results to include the relation between malonyl-CoA and total β-oxidation, which is consistent with stimulation of β-oxidation at carnitine palmitoyltransferase I and inhibition of carnitine palmitoyltransferase I by malonyl-CoA (28). Since the concentration of nonesterified fatty acids was fixed in our perfusions, increased β-oxidation must have resulted directly from adrenergic stimulation and/or increased heart work and is not secondary to changes in delivery of nonesterified fatty acids. Although Hall et al. (8) found increased exogenous fatty acid uptake by dobutamine in pig heart in vivo, the result does not contradict our findings, since, as we will show elsewhere, 2 the sensitivity to workload of β-oxidation and triglyceride turnover becomes enhanced under metabolic conditions (high lactate and nonesterified fat) that develop in vivo during exercise or adrenergic stimulation.

The Strategy for Substrate Selection Based on ATP Yield—We can now consider why the heart adopted the strategy of preferential increase in carbohydrate oxidation to deal with increased energy demand, at least under idealized conditions of fixed substrate availability. Using a revised estimate for the stoichiometry of oxidative phosphorylation (33), the ATP/O2 ratio calculated for complete oxidation of glucose (5.2) is only 4% higher than for oleate (5.0), and the value for complete oxidation of lactate (4.8) is actually less than oleate. Based on these calculations, the advantage of selective increase in carbohydrate oxidation during high oxygen extraction seems marginal, or it is a slight disadvantage in the case of lactate. The analysis is flawed, because carbohydrates are not completely oxidized. The actual value for ATP/O2 is higher for glucose or glycogen relative to oleate, partially because carbohydrates carry more of their own oxygen but also because a portion of carbohydrates are metabolized anaerobically. Like skeletal muscle, during high workload states, a portion of glucose taken up by heart is diverted to the release of lactate plus pyruvate. This occurs irrespective of whether there is net release or extraction of lactate (Ref. 27; and compare Fig. 3 with net lactate fluxes given in Appendix Table IIA). Using values for the distribution between oxidation and release of lactate or pyruvate that were obtained during adrenergic stimulation in this study, we calculate that the effective value of ATP/O2 is 5.4 for glucose and 5.7 for glycogen. Therefore, the advantage of using glycogen during periods of high energy demand is pronounced.

Coordination of Glucose and Glycogen Utilization—Upon adrenergic stimulation, the increase in glucose oxidation was delayed relative to the burst of glycogenolysis (Fig. 3). We explain the delay by interaction between glucose and glycogen at the level of glucose transport. Therefore, phosphorylase effectively regulates glucose uptake in this setting. We also conclude that acute regulation of phosphorylase mostly reflects the status of high energy phosphates (particularly AMP) and not the activity state of phosphorylase kinase. It follows that glucose uptake, in the setting of adrenergic stimulation, is regulated indirectly by the status of high energy phosphates. Blocked glucose uptake upon acute reduction in energy charge, and the associated burst of glycogenolysis, obviates the investment of ATP to prime glucose for glycolysis until after glycogen has been exploited.

Redox Regulation during Glycogenolysis and the Coupling to Oxidation—We confirmed our earlier observation (16) that glycogen-derived pyruvate is more tightly coupled to oxidation than is pyruvate derived from extracellular glucose (Fig. 6). We hypothesized that tighter coupling would reflect coordination between phosphorylase activation and PDC activation. Contrary to our hypothesis, PDC activation was delayed relative to the burst of glycogenolysis. We would have predicted pronounced activation of PDC resulting from the combined effect of Ca2⁺ stimulation of PDC phosphatase and feed-forward activation resulting from inhibition of PDC kinase by high levels of glycogen-derived pyruvate. Paradoxically, we found that glycogen-derived pyruvate was preferentially oxidized despite the fact that PDC activation was slow to develop. Further, the portion not oxidized was preferentially reduced to lactate and was exported as such. Since the relative efflux of lactate and pyruvate varied during the protocol (Appendix Table IIA), the process contributed to regulation of the cytosolic redox potential. During the burst of glycolytic flux from glycogen, differential efflux of lactate versus pyruvate prevented the occurrence of cytosolic reduction that results when glycolytic flux is stimulated in excess of the capacity for pyruvate oxidation. In so doing, inhibition of glyceraldehyde-3P dehydrogenase and phosphofructo-1-kinase from cytosolic reduction and H⁺ accumulation, which would otherwise have self-limited pathway flux, was obviated.

Activation of Synthase and Phosphorylase Were Timed to Minimize Glycogen Cycling—We postulated that there is robust reciprocal regulation of glycogen synthase and phosphorylase, preventing more than minor occurrence of glycogen futile cycling in heart. We did observe activation (dephosphorylation) of synthase late in the protocol (Table II), accounting for a small amount of de novo glycogen synthesis (Table I) during net glycogenolysis (Fig. 3). Synthase activation could serve to prime the system for glycogen repletion during recovery but was timed to minimize futile

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2 G. W. Goodwin and H. Taegtmeyer, manuscript in preparation.
Is Glycogenolysis a Physiologic Response to Epinephrine?—Myocardial glycogenolysis is a physiological response to exercise of the whole animal (35), mediated in large part by increased adrenergic tone and circulating catecholamines. However, the glycogenolytic response to epinephrine has not been observed consistently. There are at least three explanations for the inconsistency. First, hearts perfused with saline in vitro are very sensitive to epinephrine induced glycogenolysis (3, 36). This could be an artifact resulting from low reserve for O₂ delivery and from the induction of demand ischemia when no provision is made to increase O₂ delivery (37). In the present study, we did make provision for increased O₂ delivery at the onset of adrenergic stimulation (we increased the perfusion pressure at the same time), and we verified by various methods (intracellular ratio for lactate to pyruvate, content of high energy phosphates, oxygen consumption relative to contractile performance) that hearts did not experience demand ischemia. The increased AMP and small decrease in phosphocreatine that we observed during acute stimulation are similar to changes in vivo in response to increased pressure development and/or dobutamine infusion (37, 38) and therefore represent a physiologic response. Second, we find that myocardial glycogenolysis is blunted under conditions of high lactate and non-physiologic response. Second, we find that myocardial glycogenolysis is blunted under conditions of high lactate and non-physiologic response. Third, studies of glycogenolysis based on disappearance of the 13C NMR signal from glycogen will underestimate true glycogenolysis by an amount determined by the degree of isotopic dilution of [13C]glycogenolysis. By adopting the last on-first off model proposed by Brainard et al. (42), apparent glycogenolysis based on disappearance of the 13C NMR signal from glycogen will underestimate true glycogenolysis by an amount determined by the degree of isotopic dilution of [13C]glycogenolysis. The degree of 13C isotopic enrichment has not generally been specified, but it is probably considerably less than the value of 55% 14C enrichment achieved in the present study. For example, Laurent et al. (44) recently concluded that epinephrine does not stimulate glycogenolysis in skeletal muscle. If the degree of isotopic enrichment of glycogen in that study were as high as the value for extracellular glucose (13.9%), then glycogenolysis was underestimated by a factor of 7.

In conclusion, we characterized total substrate utilization during an acute low to high work transition of the heart, described the mechanism responsible for high respiratory rates from glycogen, and explained the inconsistency among reports of epinephrine-induced glycogenolysis. High glycogen respiration was one component of the overall pattern of selective increase in carbohydrate oxidation. The advantage of increased carbohydrate oxidation may relate to a higher effective value for the ratio of ATP synthesized/O₂ consumed for carbohydrate versus lipid, not to be confused with the ATP/O₂ ratio calculated for complete oxidation.

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APPENDIX

Tables IA–IIIA show physiological performance and lactate, pyruvate, and high energy phosphate content in the treatment groups.

### TABLE IA

| Parameter measured | Prolonged stimulation | Acute stimulation | Unstimulated |
|--------------------|-----------------------|------------------|--------------|
|                    | [14C]Glucose group    | [14C]Glycogen group | [14C]Lactate group |                  |
| Coronary flow (ml/min) | 16.9 ± 1.4 | 17.4 ± 1.6 | 15.7 ± 1.0 | 16.2 ± 2.8 | 18.4 ± 1.0 |
| Cardiac output (ml/min) | 47.7 ± 8.1 | 50.7 ± 2.1 | 46.5 ± 5.0 | 44.4 ± 1.7 | 50.0 ± 0.5 |
| Heart rate (min⁻¹) | 267 ± 33 | 247 ± 21 | 260 ± 13 | 252 ± 47 | 282 ± 25 |
| Peak systolic pressure (mm Hg) | 116 ± 6 | 120 ± 5 | 114 ± 7 | 112 ± 11 | 117 ± 9 |
| Developed pressure (mm Hg) | 60.7 ± 18.8 | 65.6 ± 5.1 | 58.8 ± 8.6 | 52.8 ± 12.0 | 61.1 ± 12.4 |
| Peak pressure × rate × 10⁻³ | 30.0 ± 2.7 | 31.7 ± 4.2 | 29.5 ± 1.6 | 28.2 ± 3.5 | 32.5 ± 1.4 |
| MVO₂ (µmol/min/g, dry wt) | 37.6 ± 3.9 | 40.9 ± 2.6 | 41.3 ± 3.3 | 36.8 ± 4.2 | 37.4 ± 2.7 |

Values are the mean ± S.E. n = 5 in each group.

We thank Qiuying Han and Patrick H. Guthrie for technical assistance.

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TABLE IA—continued

| Parameter measured                                      | Treatment group | Prolonged stimulation | Acute stimulation | Unstimulated |
|--------------------------------------------------------|-----------------|-----------------------|-------------------|--------------|
|                                                        | [14C]Glucose group | [14C]Glycogen group | [14C]Lactate group |               |
| Coronary flow (ml/min)                                 | 28.2 ± 2.5      | 29.2 ± 1.8            | 25.2 ± 2.1        | 26.2 ± 1.8   | NA^           |
| Cardiac output (ml/min)                                | 68.5 ± 12.8     | 73.9 ± 2.7            | 64.0 ± 2.6        | 60.4 ± 2.0   | NA            |
| Heart rate (min⁻¹)                                     | 358 ± 11        | 331 ± 6               | 339 ± 8           | 325 ± 23     | NA            |
| Peak systolic pressure (mm Hg)                         | 145 ± 4         | 150 ± 1               | 139 ± 6           | 152 ± 6      | NA            |
| Developed pressure (mm Hg)                             | 65.0 ± 7.1      | 69.7 ± 3.5            | 58.5 ± 5.9        | 79.9 ± 12.9  | NA            |
| Peak pressure × rate × 10⁻³                            | 51.8 ± 2.4      | 49.7 ± 1.3            | 48.5 ± 1.4        | 49.1 ± 5.6   | NA            |
| MVO₂ (μmol/min/g, dry wt)                              | 75.5 ± 6.4      | 84.5 ± 3.3            | 78.8 ± 3.3        | 75.6 ± 4.5   | NA            |

During acute stimulation (56–58 min)

| Coronary flow (ml/min)                                 | 27.6 ± 2.5      | 28.6 ± 1.5            | 25.0 ± 2.2        | 25.0 ± 2.2   | NA            |
| Cardiac output (ml/min)                                | 58.9 ± 11.6     | 67.2 ± 3.0            | 53.0 ± 3.6        | 53.0 ± 3.6   | NA            |
| Heart rate (min⁻¹)                                     | 351 ± 15        | 346 ± 14              | 360 ± 11          | 360 ± 11     | NA            |
| Peak systolic pressure (mm Hg)                         | 145 ± 4         | 148 ± 2               | 140 ± 5           | 140 ± 5      | NA            |
| Developed pressure (mm Hg)                             | 62.2 ± 7.2      | 66.0 ± 3.2            | 54.4 ± 6.3        | 54.4 ± 6.3   | NA            |
| Peak pressure × rate × 10⁻³                            | 50.8 ± 0.7      | 51.0 ± 2.6            | 50.6 ± 2.5        | 50.6 ± 2.5   | NA            |
| MVO₂ (μmol/min/g, dry wt)                              | 75.5 ± 8.3      | 81.1 ± 3.9            | 79.5 ± 3.3        | 79.5 ± 3.3   | NA            |

After prolonged stimulation (71–75 min)

| Coronary flow (ml/min)                                 | 27.6 ± 2.5      | 28.6 ± 1.5            | 25.0 ± 2.2        | NA           |
| Cardiac output (ml/min)                                | 58.9 ± 11.6     | 67.2 ± 3.0            | 53.0 ± 3.6        | NA           |
| Heart rate (min⁻¹)                                     | 351 ± 15        | 346 ± 14              | 360 ± 11          | NA           |
| Peak systolic pressure (mm Hg)                         | 145 ± 4         | 148 ± 2               | 140 ± 5           | NA           |
| Developed pressure (mm Hg)                             | 62.2 ± 7.2      | 66.0 ± 3.2            | 54.4 ± 6.3        | NA           |
| Peak pressure × rate × 10⁻³                            | 50.8 ± 0.7      | 51.0 ± 2.6            | 50.6 ± 2.5        | NA           |
| MVO₂ (μmol/min/g, dry wt)                              | 75.5 ± 8.3      | 81.1 ± 3.9            | 79.5 ± 3.3        | NA           |

^* NA, not applicable.

TABLE IIA

| Parameter measured | Treatment group |
|--------------------|-----------------|
|                    | Unstimulated (n = 5) | Acute stimulation (n = 5) | Prolonged stimulation (n = 15) |
| Tissue lactate (μmol/g, dry wt) | 2.97 ± 0.68 | 4.87 ± 1.41 | 5.91 ± 0.40 |
| Tissue pyruvate (μmol/g, dry wt) | 1.19 ± 0.07 | 2.11 ± 0.04 | 1.04 ± 0.03 |
| Intracellular lactate (μmol/ml) | 0.45 ± 0.22 | 0.87 ± 0.39 | 1.27 ± 0.12 |
| Intracellular pyruvate (μmol/ml) | 0.42 ± 0.03 | 0.65 ± 0.03 | 0.32 ± 0.01 |
| Tissue lactate/pyruvate | 2.49 ± 0.50 | 2.31 ± 0.67 | 5.78 ± 0.37 |
| Intracellular lactate/pyruvate | 1.30 ± 0.59 | 1.36 ± 0.63 | 4.07 ± 0.36 |
| Lactate efflux (μmol/min/g, dry wt) | −0.74 ± 1.3 | 13.9 ± 1.7 | 7.2 ± 1.1 |
| Pyruvate efflux (μmol/min/g, dry wt) | 1.41 ± 0.23 | 3.08 ± 0.37 | 2.06 ± 0.48 |

^* p < 0.05 versus unstimulated.

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