Influence of metabolic substrate on rat heart function and metabolism at different coronary flows

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BURKHOFF, D., R. G. WEISS, S. P. SCHULMAN, R. KALIL-FILHO, T. WANNENBURG, AND G. GERSTENBLITH. *Influence of metabolic substrate on rat heart function and metabolism at different coronary flows.* Am. J. Physiol. 261 (Heart Circ. Physiol. 30): H741–H750, 1991.—The influence of metabolic substrate on contractile strength, myocardial oxygen consumption ($M\dot{V}O_2$), high- and low-energy phosphate levels, and intracellular pH were determined in isovolumically contracting isolated rat hearts perfused with solutions containing either glucose or hexanoate at both high and low coronary perfusion pressures (CPP). Contractile strength was not significantly influenced by substrate at a CPP of 80 mmHg. As coronary flow was decreased, developed pressure measured at a fixed left ventricular volume (LVV) was lower during hexanoate than glucose perfusion. The relationship between $M\dot{V}O_2$ and mechanical work determined at a CPP of 80 mmHg over a range of LVVs was shifted upward in a parallel manner when substrate was switched from glucose to hexanoate. The $M\dot{V}O_2$-work relationship measured at a fixed LVV but over a range of coronary flows (7–20 ml/min) was also parallel shifted upward on switching from glucose to hexanoate. Basal $M\dot{V}O_2$ was greater during hexanoate than glucose perfusion by an amount that accounted for two-thirds the total increase in $M\dot{V}O_2$ observed between the substrates under unloaded beating conditions. The remainder of the difference was attributed to increased energy requirements for excitation-contraction coupling. Inorganic phosphate concentrations increased more and phosphocreatine concentrations decreased more during low-flow conditions (3 ml/min) when hearts were perfused with hexanoate compared with glucose. Thus hexanoate decreases myocardial efficiency compared with glucose in large part by increasing non-work-related oxygen demands. This inefficiency impacts adversely on contractile strength and high-energy phosphate concentrations at low coronary flows.

free fatty acids; glucose; ischemia; myocardial efficiency; pressure-volume area

MYOCARDIAL METABOLISM provides the chemical fuel (i.e., ATP) needed to maintain contractile activity and to preserve cellular integrity during conditions of normal and decreased coronary flow. Although the heart is capable of utilizing a variety of substrates, the two most preferred are free fatty acids (FFA) and glucose. Whereas heart muscle will use predominantly FFA in preference to glucose under most conditions (15, 25, 29), results of several studies suggest that cardiac efficiency (the ratio between ventricular work and oxygen consumption) is greater with glucose utilization (3, 14, 21, 25, 30, 34, 35). Consequently, the choice of substrate may influence contractile performance during normal coronary flow and particularly during low coronary flow (14, 18, 16, 34).

There are several possible reasons why ventricular efficiency could be greater with glucose than with fatty acids (16). For example, glucose, but not fatty acids, supports anaerobic ATP generation via glycolysis. Also, fatty acids are thought to uncouple oxidative phosphorylation (1, 9, 19) and induce ATP wastage by mitochondria (26). The relative importance of such factors is not entirely clear.

The goal of the present study was to determine, in detail, how metabolic substrates influence the interrelationships between contractile strength, ventricular work, and myocardial oxygen consumption at high and low coronary flows. To further evaluate how substrates impact on myocardial energy demand, the influence of substrate on basal oxygen consumption was measured. Additionally, the impact of substrate-mediated alterations in efficiency on the ability of the heart to maintain intracellular high-energy phosphate stores during periods of low coronary flow were determined using phosphorus nuclear magnetic resonance (NMR) spectroscopy. Finally, the potential role of glycolysis in contributing to differences observed between substrates was assessed through measurement of lactate production at various flows. All studies were performed on isolated rat hearts that were supplied either glucose or hexanoate (a medium-chain FFA) as the exogenous metabolic substrate. The results indicate that FFA decrease myocardial efficiency compared with glucose by increasing non-work-related oxygen demands. The data further suggest that uncoupling of oxidative phosphorylation may play a role in mediating this increase in myocardial oxygen consumption ($M\dot{V}O_2$). An important consequence of the FFA-mediated inefficiency is that contractile strength and high-energy phosphates fall to a greater extent during low coronary flows when the heart is forced to utilize hexanoate instead of glucose.

METHODS

Surgical Preparation

Retired male exbreeder rats (Sprague-Dawley) weighing 500–700 g were heparinized (1,000 U ip) and then deeply anesthetized (pentobarbital sodium, 100–150 mg ip). After rapid excision of the heart, the aorta was

cannulated for retrograde perfusion with a flange-tipped piece of PE-190 tubing (3 mm OD) passed over a 16-gauge needle. A polyvinyl chloride balloon attached to PE-190 tubing was inserted into the left ventricle through the mitral valve and held in place by a suture tied around the left atrium. The other end of the tubing was connected to a Gould pressure transducer (model P23XL) for continuous measurement of left ventricular pressure. A second transducer was used to measure coronary perfusion pressure. Pacing was performed with a Grass SD-9 stimulator via electrodes attached to the right ventricular outflow tract and left ventricular apex. Hearts were paced at 180 beats/min.

Perfusate solutions contained (in mM) 144 sodium, 5 potassium, 1.5 calcium, 6 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 0.9 magnesium, and 152 chloride. Lidocaine (5 μg/ml) was also added for control of ventricular ectopy. This mixture was warmed to 37°C, the pH was adjusted to 7.40, and the mixture was then divided in two portions. Glucose (5 mM) was added to one portion and hexanoate (1 mM) was added to the other. When the perfusates were prepared in this manner, concentrations of all components were identical except for the metabolic substrate. Solutions were stored in separate containers, equilibrated with 100% O_2 before use, and bubbled continuously throughout the experiment. Perfusate was not recirculated. The flow rate of perfusate was regulated by an adjustable-speed rotary pump (Masterflex, Cole-Parmer, Barrlington, IL) with a digital flowmeter that was calibrated by timed volume collection before each experiment. The fluid passed through a condenser and bubble trap before reaching the heart. The condenser and storage containers were warmed by a constant temperature circulator (VWR 1130, Miles, IL) which maintained the temperature of the perfusate at ~37°C.

Two sets of preliminary studies were carried out to ensure that with a glucose concentration of only 5 mM the heart was principally using the exogenous glucose as opposed to endogenous triglycerides. First, in several experiments, the glucose concentration was increased to 15 mM and insulin (40 U/l) was added to the perfusate. No differences were detected in the performance or oxygen consumption between these two conditions over a range of loading and perfusion pressures. The lower glucose concentration was used so that the osmolalities of the glucose and hexanoate perfusates were nearly the same. Second, it was demonstrated, by observing the ^{13}C-^{13}C spin-spin splitting pattern of the [4-^{13}C]glutamate NMR resonance of heart extracts (20) obtained after perfusion with 5 mM [1-^{13}C]glucose that exogenous glucose accounted for 75–85% of the acetyl-CoA entering the tricarboxylic acid (TCA) cycle. Thus exogenous glucose was the principal substrate utilized with the 5 mM glucose concentration. It was demonstrated by the same techniques that, during hexanoate perfusion (1 mM), 85–90% of the acetyl-CoA entering the TCA cycle was accounted for by this exogenous substrate.

Measurement of Oxygen Consumption

In experiments where oxygen consumption was to be measured, the inferior and superior venae cavae were ligated before excision of the heart so that the right atrium and ventricle were air tight. A short piece of Teflon tubing (3 mm OD, 1.75 mm ID) was placed into the right ventricle via the main pulmonary artery for collection of all coronary sinus and right ventricular thebesian flow. Insertion of this cannula did not affect left ventricular pressure generation.

Oxygen consumption was calculated by multiplying the coronary flow by the difference in oxygen content between arterial and venous perfusate. The coronary flow used in these calculations was the flow set on the perfusion pump. To exclude the possibility of a significant amount of aortic regurgitation, coronary sinus effluent flow was measured by timed collections through the pulmonary cannula at several flow rates in each experiment. This always accounted for >85% of the flow set on the pump, and this proportion did not vary in any experiment. To estimate oxygen content, oxygen tension (Po_2) was measured by a commercially available platinum oxygen electrode flow-through system (Instech Laboratories, Plymouth Meeting, PA), which allowed for on-line measurements of Po_2. A tubing system was designed to divert either all of the arterial flow or effluent from the heart to the electrode. The linearity of the system was confirmed at several flow rates by comparing the output of the system to direct measurements of oxygen content by a Lex-O_2-Con machine. Sodium dithionate (J. T. Baker Chemical, Phillipsburg, NJ), a compound that extracts oxygen from solution, was used to electrically zero the electrode before each experiment. The gain of the system was determined in each experiment by measuring the Po_2 (Corning pH/blood gas analyzer model 178, Corning, NY) of the oxygenated perfusate as it passed through the electrode sampling chamber and by assuming an oxygen solubility of 2.39 vol%/760 mmHg. The zero of the system was found to be independent of the flow rate. However, the gain of this electrode system was dependent on the rate of coronary flow. It was therefore necessary to measure both arterial and venous values provided by the electrode system at each level of coronary flow in order to determine the difference in Po_2 values at each flow.

Myocardial oxygen extraction ratio (ER_m) was also calculated from these measurements. ER_m was defined as (arterial Po_2 − venous Po_2)/arterial Po_2.

NMR Spectroscopy

For experiments in which ^{31}P-NMR spectra were to be measured, the hearts were positioned within a 20-mm probe of a Bruker AM 360-WB NMR spectrometer (field strength 8.5 T). Hearts were paced using a KCl wick electrode that was placed into the right ventricle. Magnetic field homogeneity was optimized during observation of the water proton signal using the decoupler coil. Proton-decoupled, minimally saturated ^{31}P spectra were obtained with a 2.1-s delay between pulses of 22-μs duration (approximate flip angle of 60°) using a 2K data table. Free induction decays were convoluted with an exponential function that introduced a line broadening of 15 Hz; this caused negligible distortion of the line shape. Concentrations of phosphorus-containing com-

pounds were determined by integrating the area under individual peaks by hand digitization (model 2210 digitizing board, Jandel Scientific, Corte Madera, CA) using commercially available software (SigmaScan, Jandel Scientific). The area under each peak was corrected for the degree of saturation and nuclear Overhauser effect (NOE), with scaling factors determined by comparison of spectra determined with the 2.1-s delay to fully relaxed spectra (delay of 10 s) with minimal NOE. At the beginning of each experiment a series of spectra were obtained during graded increments of intraventricular balloon volume with solution containing phenylphosphonic acid (either 100 or 50 mM, pH 7.4). The areas of this peak from these spectra provided a calibration curve for absolute metabolite quantification (37). Thus all values are presented as moles per gram of wet weight. Intracellular pH was indexed by the chemical shift of the inorganic phosphate (P_i) peak relative to the phosphocreatine (PCr) peak (23).

Protocols

Experiments were carried out on five groups of hearts. All hearts were allowed to equilibrate for 30 min before the start of the experiment. In each protocol, measurements were made with the hearts alternately perfused with solutions containing either glucose or hexanoate; the glucose substrate was tested first in half the hearts and hexanoate was tested first in the other half. In no case did the results depend on the order in which the substrates were tested, and no distinction will be made below. Enough time was allowed to pass after a switch in substrate for performance and oxygen consumption to reach stable states, and this was typically between 10 and 15 min. All measurements were made under steady-state conditions.

Substrate dependence of ventricular contractile strength and efficiency at normal perfusion pressure. The purpose of the first protocol was to determine the substrate dependence of ventricular contractile strength and efficiency at a normal perfusion pressure. Contractile strength was assessed by measurement of the end-systolic pressure-volume relation (ESPVR) (28). Ventricular efficiency was assessed by measurement of the relationships between $M\dot{V}O_2$ and two indexes of mechanical work: the rate-pressure product and the pressure-volume area (defined below). Coronary flow was adjusted to provide a perfusion pressure of 80 mmHg at the beginning of the experiment and was fixed at this value throughout the experiment. Coronary flows averaged 18 ± 4 ml/min (11.6 ± 1.7 $ml \cdot g^{-1} \cdot min^{-1}$). The basic protocol, performed in 10 hearts, consisted of measuring P_{O_2} in the coronary venous effluent, end-diastolic pressure, and end-systolic pressure at each of several balloon volumes during glucose or hexanoate perfusion. Ventricular volume was varied over an average range of 0.35 ml (from 0.2 to 0.55) in increments of 0.05–0.10 ml. The order in which volume was changed was varied randomly from one experiment to the next. Arterial P_{O_2} was measured at the beginning and at the end of a run, and this served to test the stability of the electrode system during the data-acquisition period as well as to provide the upper limit calibration signal for the electrode. Stability of ventricular performance was checked by making measurements at different times but at the same volume settings over the experimental period. Measurements were made at as many left ventricular volume settings as possible (range 4–10) over an ~20-min time period with each substrate.

Substrate dependence of ventricular efficiency at reduced coronary flows. In the second protocol ($n = 8$) the interrelations among coronary flow, $M\dot{V}O_2$, developed pressure, and rate-pressure product were determined during glucose and hexanoate perfusion in each heart. End-diastolic pressure, end-systolic pressure, and both arterial and venous P_{O_2} were determined at the control coronary flow rate (chosen to be 15 ml/min). Flow was then set at one of four test levels (7, 9, 12, or 20 ml/min), and the same measurements taken under steady-state conditions. The perfusate was then changed, and the measurements were repeated. Finally, the measurements were repeated after flow was restored to the control level with the second substrate. This procedure was repeated for each of the test flows. The order in which the test flows were used was randomized in each experiment.

Substrate dependence of basal metabolism. To assess the degree to which changes in $M\dot{V}O_2$ detected in the first series resulted from substrate dependence of basal metabolism, $M\dot{V}O_2$ was measured before and during hyperkalemic cardiac arrest in six mechanically unloaded hearts. To obtain mechanically unloaded contractions, no balloon was placed in the ventricles and a thin vent was placed into the chamber through the mitral valve so that no external pressure could be generated by the ventricle. The solutions used to arrest the hearts contained 20 mM KCl and NaCl decreased to 125 mM (so as to maintain osmotic pressure the same as control) and either glucose or hexanoate; the other constituents were identical to those of the standard solutions. $M\dot{V}O_2$ was measured under four conditions: during unloaded, beating conditions with the first substrate; during arrest with the first substrate; during arrest with the second substrate; and during beating conditions with the second substrate.

Impact of substrate on phosphate metabolite concentrations at low coronary flow. In this protocol ($n = 7$) ^{31}P-NMR spectra were measured at one high flow (20 ml/min) and one low flow (3 ml/min) with both glucose and hexanoate perfusion. This low flow was chosen because results of five preliminary experiments indicated that changes in pH, [P_i], [PCr], and [ATP] were relatively small with less severe reductions in coronary flow during hexanoate and especially during glucose perfusion. End-diastolic and end-systolic pressures and data for determination of a ^{31}P-NMR spectrum were measured after a 30-min stabilization period at a flow of 20 ml/min. Ventricular pressure was allowed to stabilize for ~2.5 min at the low flow rate and a 144-pulse spectrum (measured over a 5-min period) was obtained. Flow was then restored to the control level, 5 min were given to allow ventricular pressure to stabilize, and a 144-pulse spectrum was obtained. The perfusate was then switched, a 15-min stabilization period was provided, and the entire procedure was repeated.

Influence of substrate on lactate production at various coronary flows. In the final protocol (n = 6) 3 ml of coronary effluent were collected from the hearts for analysis of lactate concentration at various coronary flows. Collections were made under steady-state conditions during either glucose or hexanoate perfusion at a control flow of 20 ml/min, then at flows of 12, 7, and 3 ml/min (set in a random manner), and finally during a recontrol period at 20 ml/min. After these collections, perfusate was switched to that containing the other substrate and the procedure repeated. Samples were stored frozen and lactate concentrations were subsequently determined in duplicate.

Data Analysis

Ventricular contractile state was quantified in the first protocol by the slope (E_{es}) and volume axis intercept (V_0) of the ESPVR. End-systolic pressure (P_{es}) was plotted as a function of ventricular volume (V) and linear regression analysis was applied

$$P_{es} = E_{es} (V - V_0) \quad (1)$$

The values of E_{es} and V_0, when considered together, provide a means of indexing left ventricular contractility, which is independent of changes in preload (12, 28). For purposes of comparing data from different experiments, E_{es} was normalized to a left ventricular mass of 1 g. There is no accepted method of normalizing V_0 for different heart sizes.

Mechanical energy generation by the ventricle under isovolumic conditions was assessed by two indexes. The first was the traditional rate-pressure product (RPP), which was obtained by multiplying developed pressure by heart rate. The second index of work was the pressure-volume area (PVA), which is defined as the area on the pressure-volume plane circumscribed by the ESPVR, the end-diastolic pressure-volume relation (EDPVR), and the systolic portion of the ventricular pressure-volume trajectory (31–33). This index, which has the units of work ($mmHg \cdot ml \cdot g^{-1}$), has been proposed as a measure of the total mechanical energy liberated during a contraction and is relatively independent of the afterload conditions on the ventricle (31). For the special case of isovolumic contractions where the pressure-volume trajectory is a vertical line on the pressure volume plane, PVA is equal to the area of a triangle with base ($V - V_0$) and height P_{es} minus the area beneath the EDPVR between V_0 and the actual ventricular volume ($A_{d,V}$)

$$PVA = 0.5\ P_{es} (V - V_0) - A_{d,V} \quad (2)$$

For the purpose of quantifying $A_{d,V}$, the EDPVR was described by a second-order polynomial.

The influence of substrate on ventricular efficiency was assessed from plots of $M\dot{V}O_2$ vs. RPP and PVA. For the case of the $M\dot{V}O_2$-PVA relation, $M\dot{V}O_2$ was expressed in units of milliliters of oxygen per beat per gram, whereas for the $M\dot{V}O_2$-RPP relation it was expressed in milliliters of oxygen per minute per gram. For both relations, linear regression analysis was performed to quantify the relations

$$M\dot{V}O_2 = a\text{PVA} + b \quad (3a)$$

$$M\dot{V}O_2 = c\text{RPP} + d \quad (3b)$$

where a and c are the slopes and b and d the intercepts.

Statistics

The substrate dependence of the ESPVRs, $M\dot{V}O_2$-PVA relations, and the $M\dot{V}O_2$-RPP relations were tested using multiple linear regression analysis with dummy variables coding for the presence of either glucose or hexanoate. Other data were pooled according to the level of coronary flow, and the statistical significance of differences between glucose and hexanoate perfusate was determined by the paired t test. Bonferroni correction was used in cases when multiple comparisons were made (NMR studies). The impact of flow and substrate on lactate production was tested by two-way analysis of variance. All statistical calculations were performed using a commercially available statistical package (SYSTAT, Evanston, IL).

RESULTS

Effect of Substrate on Ventricular Performance at Normal Coronary Perfusion Pressure

Results from a representative experiment in which a heart was alternately perfused at normal perfusion pressure with glucose and hexanoate are presented in Fig. 1. ESPVR (Fig. 1, *top*), $M\dot{V}O_2$-RPP (Fig. 1, *middle*), and $M\dot{V}O_2$-PVA (Fig. 1, *bottom*) relations were all described reasonably well by linear regression. EDPVR (also shown in Fig. 1, *top*) was described well by a second-order polynomial. Neither ESPVR nor EDPVR were influenced significantly by substrate. However, there was an upward shift of $M\dot{V}O_2$-RPP and $M\dot{V}O_2$-PVA relations observed with hexanoate compared with that obtained with glucose, indicating that for a given amount of mechanical work by the ventricle, it consumed more oxygen when perfused with hexanoate.

The mean (±SD) results from 10 hearts studied in this series are summarized in Table 1 along with the results of multiple linear regression analysis. Overall, there was no statistically significant influence of substrate on the slope of either $M\dot{V}O_2$-RPP or $M\dot{V}O_2$-PVA relations, but there was a statistically significant upward shift (higher intercepts) for both of these relations by $\sim 0.14 \times 10^{-3}$ ml $O_2 \cdot beat^{-1} \cdot g^{-1}$ (2.5×10^{-2} ml $O_2 \cdot min^{-1} \cdot g^{-1}$).

Analysis of the pooled ESPVR data did reveal a statistically significant upward shift in this relation measured during hexanoate perfusion compared with glucose perfusion. However, the magnitude of this effect, as quantified by the change in V_0 values, averaged only 0.02 ml. Thus, although this shift was statistically significant, it was not large in magnitude. There was no detectable influence of substrate on the EDPVR.

Substrate Dependence of Developed Pressure and $M\dot{V}O_2$ During Graded Reductions in Coronary Flow

Results from a representative heart studied in the third series of experiments are summarized in Fig. 2. At high

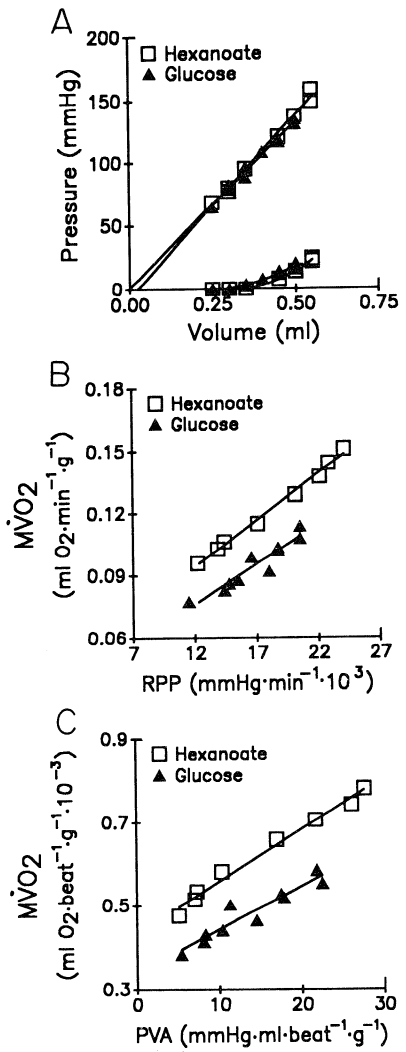

FIG. 1. Results from a representative heart showing influence of metabolic substrate on end-systolic and end-diastolic pressure-volume relations (*A*), myocardial oxygen consumption ($M\dot{V}O_2$)-rate-pressure product (RPP) relation (*B*), and $M\dot{V}O_2$-pressure-volume area (PVA) relation (*C*).

coronary flows, there was relatively little difference in developed pressure (DP) between glucose and hexanoate perfusion. However, at reduced coronary flow rates, DP was significantly greater when the hearts were perfused with glucose. Analysis of pooled data from the eight hearts studied, summarized in Table 2, indicated that, at coronary flows of <15 ml/min, the difference in DP during glucose and hexanoate perfusion was statistically significant.

At a flow of 20 ml/min, where DP was similar with glucose and hexanoate perfusion in the representative example (104 vs. 101 mmHg), $M\dot{V}O_2$ was significantly lower with glucose as substrate (0.107 vs. 0.121 ml $O_2 \cdot min^{-1} \cdot g^{-1}$). However, as coronary flow was decreased, the difference in $M\dot{V}O_2$ between the substrates became less. Statistical analysis of the results from all eight hearts, however, showed that $M\dot{V}O_2$ was greater during hexanoate perfusion at every level of coronary flow tested, except at a flow of 7 ml/min (Table 2).

The relations between $M\dot{V}O_2$ and RPP obtained with glucose and hexanoate perfusion for the representative heart are presented in Fig. 2 (*bottom*). At any RPP, $M\dot{V}O_2$ was significantly higher with hexanoate perfusion than with glucose perfusion. The mean (±SD) results for all hearts studied in this series are summarized in Table 3. There was no statistically significant influence of substrate on the slope of the $M\dot{V}O_2$-RPP relation, but there was a statistically significant upward shift of the relation with hexanoate compared with glucose perfusion (multiple linear regression analysis).

At a flow of 20 ml/min, ER_m averaged 58 ± 12% during glucose perfusion and 64 ± 10% during hexanoate perfusion (see Table 2). As flow decreased, ER_m increased for both substrates, but the differences in ER_m noted between substrates became much smaller. At the lowest flows tested, 7 ml/min, ER_m was 83 ± 7% for glucose and 85 ± 6% for hexanoate (P = NS), indicating substantial oxygen extraction at these low flows.

Influence of Substrate on Basal Metabolic $M\dot{V}O_2$

Mean (±SD) results from six unloaded hearts in which the influence of substrate on $M\dot{V}O_2$ before and during KCl arrest were measured are shown in Fig. 3. Actual mean values of $M\dot{V}O_2$ are shown in Fig. 3 (*top*), whereas differences in $M\dot{V}O_2$ measured with hexanoate and glucose perfusion under the two conditions are shown in Fig. 3 (*bottom*). Note that a derived parameter, labeled $M\dot{V}O_2$ for excitation-contraction coupling, has been included and was defined as the difference between $M\dot{V}O_2$ measured under unloaded beating conditions and $M\dot{V}O_2$ measured during KCl arrest.

Hearts perfused with glucose consumed less oxygen under both unloaded beating conditions and during KCl arrest; in addition, the $M\dot{V}O_2$ attributed to excitation-

TABLE 1. *Summary of results from rat hearts alternately perfused with hexanoate and glucose*

Substrate	ESPVR			$M\dot{V}O_2$-PVA			$M\dot{V}O_2$-RPP		
	E_{es}	V_o	r^2	a	b	r^2	c	d	r^2
Hexanoate	337.6±128.7	0.01±0.11	0.983±0.010	1.32±0.26	0.77±0.17	0.941±0.057	5.46±0.76	0.095±0.030	0.943±0.060
Glucose	333.0±108.9	0.03±0.11	0.988±0.008	1.46±0.42	0.63±0.18	0.941±0.066	6.95±1.94	0.070±0.027	0.935±0.070
Analysis of covariance	NS	$P < 0.001$		NS	$P < 0.001$		NS	$P < 0.001$	

Values are means ± SD; n = 10. Mean right and left ventricular masses are 0.29 ± 0.07 and 1.24 ± 0.20 g, respectively. ESPVR, end-systolic pressure-volume relation; $M\dot{V}O_2$, myocardial oxygen consumption; PVA, pressure-volume area; RPP, rate-pressure product. Parameters of linear regressions, as detailed in *Eqs. 1, 3a,* and *3b*: E_{es}, mmHg·g·ml^{-1}; V_o, ml; a, ml $O_2 \cdot mmHg^{-1} \cdot ml^{-1} \times 10^{-5}$; b, ml $O_2 \cdot beat^{-1} \cdot g^{-1} \times 10^{-3}$; c, ml $O_2 \cdot mmHg^{-1} \cdot g^{-1} \times 10^{-6}$; d, ml $O_2 \cdot min^{-1} \cdot g^{-1}$.

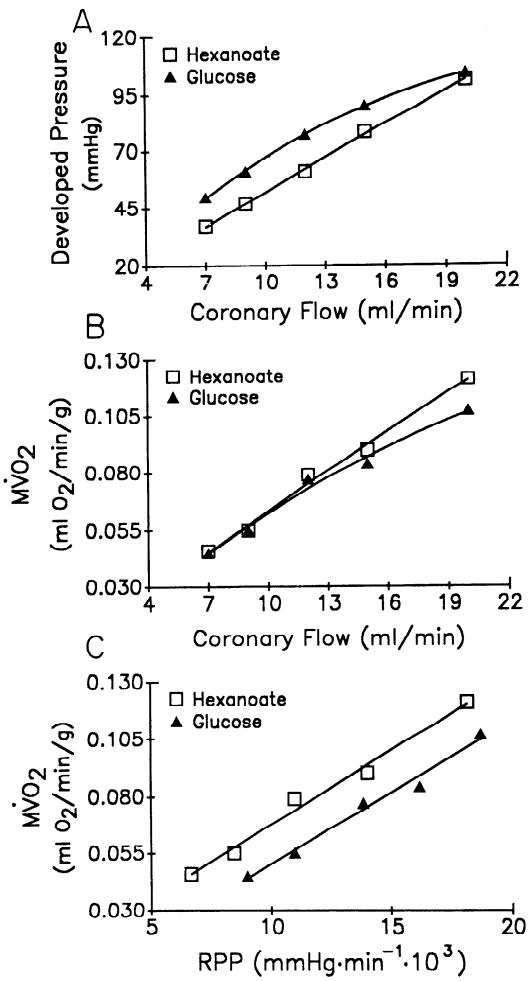

FIG. 2. Influence of coronary flow on left ventricular developed pressure (*A*) and $M\dot{V}O_2$ (*B*) and their modulation by metabolic substrate from a representative heart. *C*: relation between RPP and $M\dot{V}O_2$. For every level of work performed heart consumes more oxygen when perfused with hexanoate.

contraction coupling was also less with glucose than with hexanoate. The mean difference in unloaded $M\dot{V}O_2$ between glucose and hexanoate perfusion was 0.018 ml $O_2 \cdot min^{-1} \cdot g^{-1}$. The difference in $M\dot{V}O_2$ measured during KCl arrest between glucose and hexanoate perfusion was 0.012 ml $O_2 \cdot min^{-1} \cdot g^{-1}$, so that basal $M\dot{V}O_2$ decreased by 30% with glucose. The $M\dot{V}O_2$ attributed to excitation-contraction coupling was 0.006 ml $O_2 \cdot min^{-1} \cdot g^{-1}$ less with glucose than with hexanoate, so that $M\dot{V}O_2$ for excitation-contraction coupling decreased by only 9% with glucose. Thus two-thirds of the difference between unloaded $M\dot{V}O_2$ measured with the two substrates was accounted for by changes in basal $M\dot{V}O_2$, and only one-third was attributed to excitation-contraction coupling mechanisms.

Influence of Substrate on Intracellular pH, P_i, and High-Energy Phosphates

Representative ^{31}P-NMR spectra obtained from one heart during glucose and hexanoate perfusion at control (20 ml/min) and low (3 ml/min) flow rates are shown in Fig. 4. On moving from high to low flow, P_i increased and PCr decreased significantly during both hexanoate and glucose perfusion. However, the degree to which the concentrations of these compounds (i.e., areas under the respective peaks) changed was much greater when the heart was perfused with hexanoate. Results from the seven hearts studied in this series are summarized in Fig. 5, which shows the mean (±SD) value of each of the parameters measured at the control flow, then at the reduced flow, and after reaching a steady state following reflow during perfusion with each substrate. As for the previous series of hearts, DP was similar at the control flow but less at the low flow with hexanoate perfusion ($P < 0.05$). DPs measured under control and reflow conditions were not statistically different from each other. There were no statistically significant differences in end-diastolic pressure between the substrates or between different flow rates. [P_i], [PCr], and [ATP] were comparable during the initial and recontrol periods. During low flow, [P_i] averaged 5.06 ± 1.59 mol/g wet wt during hexanoate perfusion and only 3.03 ± 1.23 mol/g wet wt during glucose perfusion ($P = 0.001$). Changes in [PCr] complemented those observed in [P_i] in that [PCr] was significantly greater at low flow during glucose than during hexanoate perfusion (5.7 ± 1.83 vs. 4.7 ± 1.55 mol/g wet wt, respectively, $P = 0.001$). Concentrations of ATP, quantified by the calibrated area under the (β-P)ATP peak, were not statistically different between control, low-flow, or reflow conditions, nor were there differences introduced by substrate. Intracellular pH decreased during low flow from an average of 7.15 ± 0.05 under control conditions to 7.05 ± 0.07 with hexanoate and to 7.01 ± 0.06 with glucose perfusion (P = NS between glucose and hexanoate).

Impact of Substrate and Coronary Flow on Lactate Production.

At a coronary flow of 20 ml/min, lactate production averaged 0.37 ± 0.20 $\mu mol \cdot min^{-1} \cdot g$ wet wt^{-1} during

TABLE 2. *DP, $M\dot{V}O_2$, and ER_m measured in rat hearts alternately perfused with hexanoate and glucose at various coronary flows*

Coronary Flow, ml/min	DP, mmHg		$M\dot{V}O_2$, ml $O_2 \cdot min^{-1} \cdot g^{-1}$		ER_m, %	
	Hexanoate	Glucose	Hexanoate	Glucose	Hexanoate	Glucose
7	33.0±8.1	47.7±8.9*	0.056±0.007	0.055±0.006	85±6	83±7
9	47.5±10.2	61.6±12.0*	0.068±0.011	0.065±0.011*	79±6	76±8*
12	66.5±10.6	80.4±13.2*	0.090±0.014	0.084±0.014*	74±7	69±9*
15	87.7±14.8	94.0±16.2	0.111±0.017	0.105±0.020*	73±8	69±10*
20	105.6±14.1	105.9±13.3	0.149±0.031	0.133±0.029*	64±10	58±12*

Values are means ± SD; $n = 8$. DP, developed pressure; $M\dot{V}O_2$, myocardial oxygen consumption; ER_m, myocardial oxygen extraction ratio.
* $P < 0.05$ by paired t test compared with hexanoate.

TABLE 3. *Influence of metabolic substrate on relationship between rate-pressure product and myocardial oxygen consumption*

Substrate	Slope, ml $O_2 \cdot mmHg^{-1} \cdot g^{-1} \times 10^{-6}$	Intercept, ml $O_2 \cdot min^{-1} \cdot g^{-1}$	r^2
Hexanoate	7.32±1.23	0.0052±0.0185	0.978±0.020
Glucose	7.95±1.88	−0.0246±0.0329	0.944±0.040
Analysis of covariance	NS	$P < 0.001$	

Average right ventricular plus left ventricular weight was 1.57 ± 0.22 g.

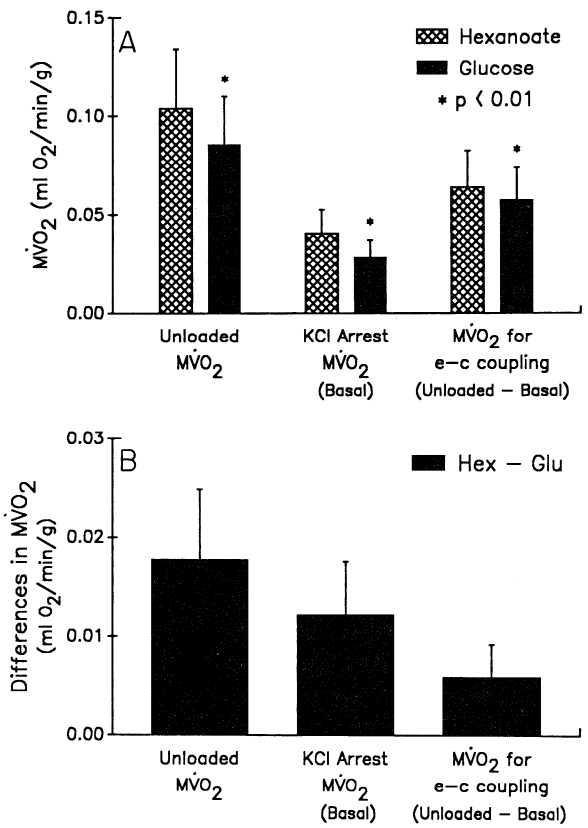

FIG. 3. Influence of substrate on $M\dot{V}O_2$ measured at normal coronary perfusion pressure with the hearts unloaded but beating at a rate of 180 beats/min and on basal metabolic $M\dot{V}O_2$ measured during KCl arrest. Also included is derived parameter, $M\dot{V}O_2$ for excitation-contraction (e-c) coupling, which was assumed to be the difference between the unloaded $M\dot{V}O_2$ and the basal $M\dot{V}O_2$. *A*: average ± SD; *B*: differences between hexanoate (Hex) and glucose (Glu) perfusion under each condition.

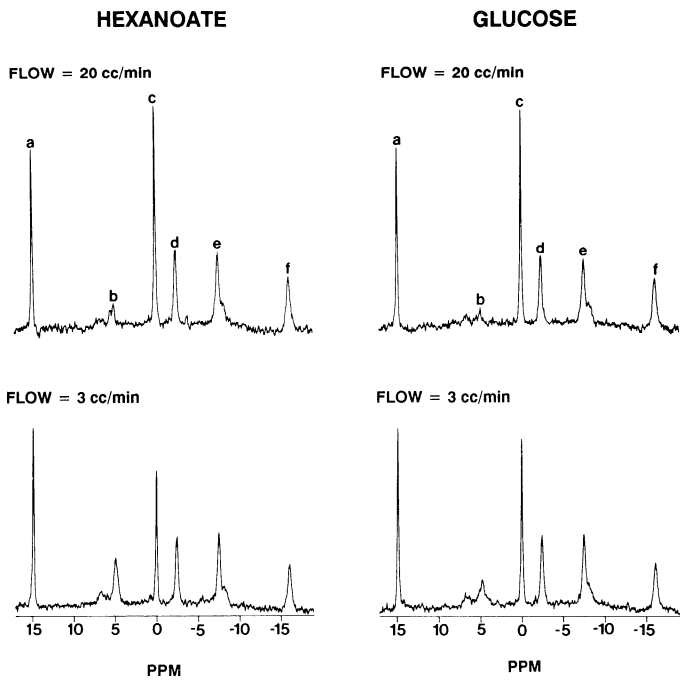

FIG. 4. Typical ^{31}P-NMR spectra obtained under control flow (20 ml/min) and low-flow (3 ml/min) conditions measured in same heart with glucose perfusate and hexanoate perfusate. These are 144 pulse spectra. Peaks are as follows: a, phenylphosphonic acid (100 mM concentration within intraventricular balloon); b, inorganic phosphate; c, phosphocreatine; d, (γ-P)ATP; e, (α-P)ATP; f, (β-P)ATP.

hexanoate perfusion and 0.70 ± 0.29 $\mu mol \cdot min^{-1} \cdot g^{-1}$ during glucose perfusion ($P < 0.001$). Lactate production did not change significantly as flow was decreased to 12 or 7 ml/min with either substrate. There was a trend for lactate production to increase during glucose perfusion at a flow of 3 ml/min (to 1.06 ± 0.31 $\mu mol \cdot min^{-1} \cdot g^{-1}$, $P = 0.04$ by paired t test compared with control).

DISCUSSION

The impact of metabolic substrate on contractile strength and the relation between ventricular work and oxygen consumption were assessed at varied coronary flows. There was no significant impact of substrate on contractile strength at a normal coronary perfusion pressure. However, at lower flows, contractile strength was depressed by hexanoate compared with glucose perfusion. The relation between $M\dot{V}O_2$ and mechanical work (indexed by either RPP or PVA) shifted upward in a parallel manner on switching from glucose to hexanoate at both high and low flows. Thus FFA perfusion imposed an increase in $M\dot{V}O_2$ that was independent of the amount of mechanical energy generation by the ventricle. This was accompanied by a work load-independent reduction in lactate production, suggesting a decreased contribution of anaerobic (glycolytic) ATP generation during hexanoate perfusion. Comparison of oxygen consumption measurements made during unloaded contractions and during KCl arrest indicated that two-thirds of the increase in $M\dot{V}O_2$ can be attributed to an increase in basal metabolic requirements and one-third to an increase in the oxygen consumed by excitation-contraction coupling mechanisms. This obligate increase in energy expenditure, not related to useful mechanical work, indicates a decrease in overall ventricular efficiency (defined in terms of the ratio between mechanical work and oxygen consumption) during medium-chain FFA perfusion. The consequences of this inefficiency were that at low coronary flows developed pressures were less, [PCr] could not be maintained as well, and there was more P_i

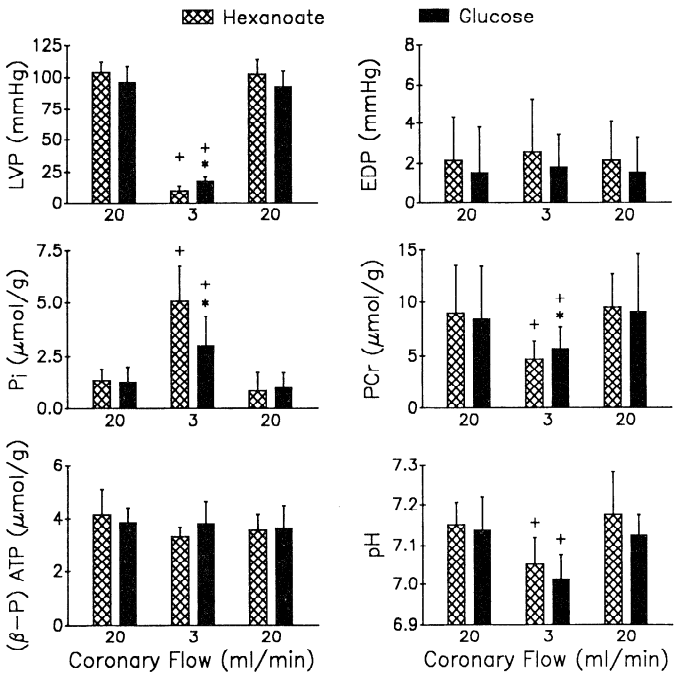

FIG. 5. Summary of experiments performed on 7 hearts (mean right ventricular plus left ventricular weight 1.52 ± 0.29 g) in which NMR spectra were measured. Measurements of each parameter were made under control conditions (20 ml/min), with reduced coronary flow rates (3 ml/min), and after restoration of flow to control level. LVP, developed left ventricular pressure; EDP, end-diastolic pressure; P_i, inorganic phosphate; PCr, phosphocreatinine. All results expressed as means ± SD from all hearts.

accumulation when hearts were perfused with hexanoate than when perfused with glucose.

Metabolic Substrate and Ventricular Efficiency

The influence of metabolic substrate on myocardial oxygen consumption at both normal and low coronary perfusion pressures has been investigated in several earlier studies, many of which have been reviewed in detail previously (4, 5, 34). A majority of studies do indicate that $M\dot{V}O_2$ increases with FFA perfusion at normal coronary perfusion pressures (3, 13, 14, 18, 21, 25, 30, 35), although results of a few studies have failed to reproduce this finding (5, 7, 8, 24, 27). In most of the previous studies, comparisons of $M\dot{V}O_2$ measured during glucose and FFA perfusion have been made at a single hemodynamic loading condition in each heart and efficiency indexed by a ratio between work and $M\dot{V}O_2$. It is not possible to determine from such a limited evaluation the mechanisms by which myocardial efficiency is altered and whether the observed changes in $M\dot{V}O_2$ result from increased energy demands for mechanical activity, basal metabolism, or other processes. In contrast, such information may be inferred when the energetics of ventricular contraction are evaluated, as in the present study, within the context of a relation between mechanical work generation and oxygen consumption determined over a range of loading conditions. Such an approach has been proposed by Suga and colleagues (31–33) with regard to interpretation of changes in the $M\dot{V}O_2$-PVA relation. The slope of the relation has been proposed to reflect the metabolic efficiency of pressure generation by the ventricle. The absence of an influence on slope by metabolic substrate therefore suggests that the predominant energy-wasting effect of FFA does not involve inefficiency of contraction-dependent oxygen utilization. The $M\dot{V}O_2$ axis intercept of the relation has been proposed to reflect the sum of energy required for basal metabolism plus excitation-contraction coupling. The influence of substrate on both of these were evaluated (Fig. 3). The increase in basal $M\dot{V}O_2$ accounted for two-thirds of the total increase observed during hexanoate perfusion, whereas the remainder was attributed to energy for excitation-contraction coupling.

Although excess concentrations of FFA have been shown to depress contractile strength with little impact on $M\dot{V}O_2$ at low coronary flows (14, 18, 21), the effect of substrate on the relation between $M\dot{V}O_2$ and work as coronary flows is changed has never been evaluated prior to this study. Hexanoate caused a parallel upward shift of this relation (Fig. 2). This was a manifestation of an increase in $M\dot{V}O_2$ with constant contractile state at high flows and a decrease in contractile state with constant $M\dot{V}O_2$ at low flows. Both of these reflect a decrease in efficiency during hexanoate perfusion.

Mechanisms by Which FFA Decrease Myocardial Efficiency

FFA can impact on myocardial metabolism in a number of ways that can cause increased $M\dot{V}O_2$ over that measured during glucose perfusion. First, on theoretical grounds, the ratio between the rate of ATP production and the rate of oxygen consumption (i.e., stoichiometric ratio of P/O) is 2.7 for FFA and 3.0 for glucose. If this were the sole explanation for the effects of FFA, then a 10% increase in $M\dot{V}O_2$ would be expected at any level of pressure generation upon switching from glucose to hexanoate perfusion. Furthermore, via this mechanism, the oxygen consumption attributed to various processes (e.g., contraction, basal metabolism, and excitation-contraction coupling) would be expected to increase by the same proportion. However, the overall increase in $M\dot{V}O_2$ was greater than the expected 10%, and although $M\dot{V}O_2$ attributed to excitation-contraction coupling did increase by ~10%, $M\dot{V}O_2$ for contraction was not changed appreciably and basal $M\dot{V}O_2$ increased by nearly 30%. Thus the changes in P/O expected from the stoichiometry of the metabolic pathways could account for only a portion of the increase in $M\dot{V}O_2$ during FFA perfusion.

ATP production by glycolysis accounts for only a small proportion of the total ATP pool under aerobic conditions (15). Lactate production was shown to be enhanced during glucose compared with hexanoate perfusion. Thus a portion of the decreased $M\dot{V}O_2$ observed during glucose perfusion may be accounted for by enhanced glycolysis. Assuming the theoretical P/O of 2.7 for hexanoate, it is estimated that enhanced glycolysis accounts for 20% of the $M\dot{V}O_2$ difference between glucose and FFA perfusion. Although this estimate is subject to a number of assumptions, it is clear that enhanced glycolysis cannot account for the entire reduction of $M\dot{V}O_2$ observed during glucose perfusion.

Another effect of FFA, identified originally in isolated

mitochondrial preparations, is uncoupling of oxidative phosphorylation (1, 9, 19). By this effect, FFA cause oxygen to be consumed that is not linked to generation of ATP. FFA are also believed to stimulate latent adenosinetriphosphatase activity (26). By this mechanism, FFA cause ATP to be consumed, but this consumption is not linked to useful work within the cell (e.g., not used for contraction, basal metabolism, excitation-contraction coupling). It has also been proposed that FFA act as ionophores (36), which could increase energy demands to maintain ionic gradients. Any of these mechanisms could cause a work load-independent increase in oxygen consumption and thus explain a parallel upward shift of the $M\dot{V}O_2$-work relation and account for the increase in basal metabolism observed in this study. Recently, Kingsley-Hickman et al. (13) showed in isolated rat hearts that even though $M\dot{V}O_2$ increased upon switching exogenous substrate from pyruvate to FFA (octanoate), the rate of ATP generation did not change, as measured by magnetization transfer techniques. This result suggests that mitochondrial uncoupling may be a more important mechanism by which FFA impact on metabolism than stimulation of ATP wastage. However, Kinsley-Hickman et al. studied the effects of octanoate, not hexanoate, and it is possible that their results would not apply to the results of the present study.

Limitations

A limitation of the present study relates to the use of crystalloid solutions rather than blood to perfuse the myocardium. Because of markedly decreased oxygen-carrying capacity, coronary vascular resistance is much lower in crystalloid perfused hearts and the flow needed to maintain coronary perfusion pressure within a physiological range is much greater than with blood perfusion. This could impact not only on tissue oxygenation but may also alter the normal mechanical coupling between coronary vasculature and myocardial performance. Despite this limitation, the data of the present study are in striking accordance with data obtained in blood-perfused hearts demonstrating the effects of excess FFA on performance and $M\dot{V}O_2$ in high- and low-flow conditions (14, 18).

Many previous studies of fatty acid metabolism have employed long-chain fatty acids. Our choice of hexanoate was based on the fact that this medium-chain fatty acid does not require transport into mitochondria. Thus we can exclude the possibility that the observations made at low coronary flows were caused by the impact of these conditions on fatty acid transport that might have limited substrate availability had long-chain fatty acids been used.

Implications

Myocellular integrity and viability can be ensured during periods of low coronary flow only if the balance between energy supply and demand is maintained (11). Maintenance of this balance is reflected by preservations of high-energy phosphate concentrations within the cell. The results of the present study suggest that the proportion of total chemical energy available to support contraction and excitation-contraction coupling is decreased during perfusion with fatty acids alone compared with that available during perfusion with glucose alone. This had no significant metabolic effect at normal perfusion pressures; but when flow was reduced, high-energy phosphate concentrations could not be maintained as well during fatty acid perfusion compared with glucose perfusion. Thus the degree to which coronary flow can be decreased and still maintain the energy supply-demand balance is reduced by fatty acids. Therefore the results of the present study suggest that strategies that would both enhance glucose utilization and inhibit fatty acid utilization may limit myocardial damage during periods of low coronary flow. Previous clinical attempts to manipulate metabolism during ischemic episodes have been predominantly limited to enhancing glucose utilization, and results of these studies have not demonstrated a consistent benefit (2). Interestingly, results of several animal studies have suggested that myocardial efficiency can be improved by the administration of agents that inhibit fatty acid utilization (10, 22). However, clinical testing of such strategies must await the availability of fatty acid inhibitors that can be used in patients.

Conclusion

The results of this study indicate that oxygen consumption is increased during hexanoate compared with glucose perfusion when work load is the same. Approximately two-thirds of the added oxygen requirements are attributable to an increase in basal metabolism and about one-third to an increase in energy for excitation-contraction coupling. Whereas at normal perfusion pressures fatty acids increase oxygen consumption without influencing contractile strength, it has been hypothesized previously that at decreased flow fatty acids would decrease function with no impact on oxygen consumption (14). This important hypothesis is confirmed for the first time by the results of the present study. Finally, both high-energy phosphates and ventricular performance are preserved better when substrate is switched from fatty acid to glucose at low coronary flow. This observation illustrates an important consequence of the decrease in metabolic efficiency caused by fatty acids.

The authors are grateful for help provided by Dr. V. P. Chacko during several of the NMR experiments and to Koenraad Vandegaer for performing lactate assays.

Support for this study was provided by the National Heart, Lung, and Blood Institute Specialized Center of Research Grant HL-17655-15. D. Burkhoff was supported by a fellowship grant from the American Heart Association, Maryland Affiliate.

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Received 5 December 1990; accepted in final form 10 May 1991.

REFERENCES

1. BORST, P., J. A. LOOS, E. J. CHRIST, AND E. C. SLATER. Uncoupling activity of long-chain fatty acids. *Biochim. Biophys. Acta* 62: 509–518, 1962.
2. BRACHFELD, N. The glucose-insulin-potassium (GIK) regimen in

the treatment of myocardial ischemia. *Circulation* 48: 459–462, 1973.
3. CHALLONER, D. R., AND D. STEINBERG. Effect of free fatty acid on the oxygen consumption of perfused rat heart. *Am. J. Physiol.* 219: 280–286, 1966.
4. DRAKE, A. J. Substrate utilization in the myocardium. *Basic Res. Cardiol.* 77: 1–11, 1982.
5. DRAKE-HOLLAND, A. J., G. ELZINGA, M. I. M. NOBLE, H. E. D. J. TER KEURS, AND F. N. WEMPE. The effect of palmitate and lactate on mechanical performance and metabolism of cat and rat myocardium. *J. Physiol. Lond.* 339: 1–15, 1983.
6. FOX, K. A., H. NOMURA, B. E. SOBEL, AND S. R. BERGMANN. Consistent substrate utilization despite reduced flow in hearts with maintained work. *Am. J. Physiol.* 244 (*Heart Circ. Physiol.* 13): H799–H806, 1983.
7. GMEINER, R., C. S. APSTEIN, AND N. BRACHFELD. Effect of palmitate on hypoxic cardiac performance. *J. Mol. Cell. Cardiol.* 70: 227–235, 1975.
8. HENDERSON, A. H., A. S. MOST, W. W. PARMLEY, R. GORLIN, AND E. H. SONNENBLICK. Depression of myocardial contractility in rats by free fatty acids during hypoxia. *Circ. Res.* 26: 439–449, 1970.
9. HULSMANN, W. C., W. B. ELLIOTT, AND E. C. SLATER. The nature and mechanism of action of uncoupling agents present in mitochrome preparations. *Biochim. Biophys. Acta* 39: 267–276, 1960.
10. HUTTER, J. F., C. SCHWEICKHARDT, H. M. PIPER, AND P. G. SPIECKERMANN. Inhibition of fatty acid oxidation and decrease of oxygen consumption of working rat heart by 4-bromochrotonic acid. *J. Mol. Cell. Cardiol.* 16: 105–108, 1984.
11. JENNINGS, R. B. Relationship of acute ischemia to functional defects and irreversibility. *Circulation* 53: 26–29, 1976.
12. KASS, D. A., AND W. L. MAUGHAN. From 'E_{max}' to pressure-volume relations: a broader view. *Circulation* 77: 1203–1212, 1988.
13. KINGSLEY-HICKMAN, P. B., E. Y. SAKO, K. UGURBIL, A. H. L. FROM, AND J. E. FOKER. ^{31}P NMR measurement of mitochondrial uncoupling in isolated rat hearts. *J. Biol. Chem.* 265: 1545–1550, 1990.
14. KJEKSHUS, J. L., AND O. D. MJØS. Effect of free fatty acids on myocardial function and metabolism in the ischemic dog heart. *J. Clin. Invest.* 51: 1767–1776, 1972.
15. KOBAYASHI, K., AND J. R. NEELY. Control of maximum rates of glycolysis in rat cardiac muscle. *Circ. Res.* 44: 166–175, 1979.
16. LIEDTKE, A. J. Alterations of carbohydrate and lipid metabolism in the acutely ischemic heart. *Prog. Cardiovasc. Dis.* 23: 321–336, 1981.
17. LIEDTKE, A. J., S. H. NELLIS, AND O. D. MJØS. Effects of reducing fatty acid metabolism on mechanical function in regionally ischemic hearts. *Am. J. Physiol.* 247 (*Heart Circ. Physiol.* 16): H387–H394, 1984.
18. LIEDTKE, A. J., S. H. NELLIS, AND J. R. NEELY. Effects of excess free fatty acids on mechanical and metabolic function in normal and ischemic myocardium in swine. *Circ. Res.* 43: 652–661, 1978.
19. LOCHNER, A., J. C. N. KOTZE, AND W. GEVERS. Mitochondrial oxidative phosphorylation in myocardial anoxia: effects of albumin. *J. Mol. Cell. Cardiol.* 8: 465–480, 1976.
20. MALLOY, C. R., A. D. SHERRY, AND F. M. H. JEFFREY. Carbon flux through citric acid cycle pathways in perfused heart by ^{13}C NMR spectroscopy. *FEBS Lett.* 212: 58–62, 1986.
21. MJØS, O. D. Effect of free fatty acids on myocardial function and oxygen consumption in intact dogs. *J. Clin. Invest.* 50: 1386–1389, 1971.
22. MOLAPARAST-SALESS, F., A. J. LIEDTKE, AND S. H. NELLIS. Effects of fatty acid blocking agents, oxfenicine and 4-bromocrotonic acid, on performance in aerobic and ischemic myocardium. *J. Mol. Cell. Cardiol.* 19: 509–520, 1987.
23. MOON, R. B., AND J. H. RICHARDS. Determination of intracellular pH by 31P nuclear magnetic resonance. *J. Biol. Chem.* 248: 7276–7278, 1973.
24. MOST, A. S., M. H. LIPSKY, P. A. SZYDLIK, AND C. BRUNO. Failure of FFA to influence myocardial oxygen consumption in the intact anaesthetised dog. *Cardiology* 58: 220–228, 1973.
25. NEELY, J. R., K. M. WHITMER, AND S. MOCHIZUKI. Effects of mechanical activity and hormones on myocardial glucose and fatty acid utilization. *Circ. Res.* 38: 122–130, 1976.
26. PRESSMAN, B. C., AND H. A. LARDY. Effect of surface active agents on the latent ATPase of mitochondria. *Biochim. Biophys. Acta* 21: 458–466, 1956.
27. ROGERS, W. J., H. MCDANIEL, R. MORASKI, C. E. RACKLEY, AND R. O. RUSSEL. Effect of heparin-induced free fatty acid elevation on myocardial oxygen consumption in man. *Am. J. Cardiol.* 40: 365–372, 1977.
28. SAGAWA, K. The ventricular pressure-volume diagram revisited. *Circ. Res.* 43: 677–687, 1978.
29. SPITZER, J. J., A. A. BECHTEL, L. T. ARCHER, M. R. BLACK, L. J. GREENFIELD, AND L. B. HINSHAW. Effects of coronary hypotension on myocardial substrate utilization. *Am. J. Physiol.* 228: 365–368, 1975.
30. STARNES, J. W., D. F. WILSON, AND M. ERECINSKA. Substrate dependence of metabolic state and coronary flow in perfused rat heart. *Am. J. Physiol.* 249 (*Heart Circ. Physiol.* 18): H799 H806, 1985.
31. SUGA, H. Total mechanical energy of a ventricle model and cardiac oxygen consumption. *Am. J. Physiol.* 236 (*Heart Circ. Physiol.* 5): H498–H505, 1979.
32. SUGA, H. Ventricular energetics. *Physiol. Rev.* 70: 247–277, 1990.
33. SUGA, H., R. HISANO, Y. GOTO, O. YAMADA, AND Y. IGARASHI. Effect of positive inotropic agents on the relation between oxygen consumption and systolic pressure-volume area in canine left ventricle. *Circ. Res.* 53: 306–318, 1983.
34. VIK-MO, H., AND O. D. MJØS. Influence of free fatty acids on myocardial oxygen consumption and ischemic injury. *Am. J. Cardiol.* 48: 361–365, 1981.
35. WILLEBRANDS, A. F., AND K. J. VAN DER VEEN. Influence of substrate on oxygen consumption of isolated perfused rat heart. *Am. J. Physiol.* 212: 1529–1535, 1967.
36. WOJTCZAK, L. Effect of fatty acids and acyl-CoA on the permeability of mitochondrial membranes to monovalent cations. *FEBS Lett.* 44: 25–30, 1974.
37. ZWEIER, J. L., AND W. E. JACOBUS. Substrate-induced alterations of high energy phosphate metabolism and contractile function in the perfused heart. *J. Biol. Chem.* 262: 8015–8021, 1987.