Failure of Glycogen Depletion to Improve Left Ventricular Function of the Rabbit Heart After Hypothermic Ischemic Arrest

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We tested the hypothesis that depletion of glycogen prior to myocardial ischemia diminishes lactate buildup and improves functional recovery on reperfusion in the isolated rabbit heart. Cardiac glycogen was reduced either by substituting N_2 for O_2 in the perfusate or by perfusion with substrate-free solution, before the onset of ischemia. Hearts were subjected to either 30 minutes of normothermic (37° C) or 60 minutes of hypothermic (4° C) ischemia followed by 30 minutes of reperfusion with oxygenated Krebs-Henseleit buffer. Function was assessed by measuring peak left ventricular pressure at end-diastolic pressures ranging from 0 to 20 mm Hg. N_2 perfusion for 15 minutes lowered myocardial glycogen by 60% and decreased ATP and phosphocreatine ($p<0.001$). Glycogen depletion did not decrease lactate accumulation during ischemia, but it impaired recovery with reperfusion (−46%, $p<0.05$). N_2 perfusion for 5 minutes also reduced glycogen by 60%, but energy-rich phosphates were not reduced and functional recovery was still impaired (−40%, $p<0.05$). Perfusion with substrate-free medium diminished glycogen by 33% ($p<0.05$). Although lactate accumulation was significantly reduced (−45%, $p<0.05$), recovery following reperfusion was not improved. The results suggest that preservation of glycogen stores, but not the prevention of lactate buildup during ischemia, is beneficial for the recovery of function with reperfusion. (*Circulation Research* 1988;63:81–86)

The myocardial cell contains endogenous substrates that may sustain the heart during periods of stress, ischemia, or ex vivo storage. Prominent among endogenous substrates is glycogen, which makes up approximately 1–2% of the myocardial cell volume[1] and which is rapidly broken down to lactate during ischemia in the course of anaerobic glycolysis.[2] The accumulation of lactate is thought to inhibit glycolysis[3] and to contribute to myocardial cell death.[4] Thus, although glycogen may be regarded as a potential source of energy from intracellular stores, rapid glycogenolysis and accumulation of lactate may be harmful for the function of the heart.

The starting point for the present work was the observation by Neely and Grotyohann[5] that accelerated glycogenolysis and the resulting high tissue lactate content are important factors contributing to the myocardial cell damage that occurs with normothermic ischemia. The authors reported that anoxic perfusion of the rat heart, prior to the onset of ischemia, depleted tissue glycogen stores, reduced accumulation of tissue lactate, and thereby improved functional recovery. They concluded that glycogen depletion protects the ischemic myocardium.

We thought that glycogen depletion might improve cardiac function after elective ischemic arrest. Because cardiac surgery and ex vivo storage of the heart are accomplished at low myocardial temperatures, we decided to test Neely and Grotyohann's findings first under normothermic and then under hypothermic conditions. We also tested different methods of glycogen depletion because we thought that anoxia itself might be harmful to the myocardium by some other mechanism. Contrary to our expectations, we found that depletion of tissue glycogen was associated with poor functional recovery of hearts subjected to either hypothermic or normothermic ischemia.

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Materials and Methods

Perfusions

Male New Zealand White rabbits (1.5–2.0 kg), maintained on a standard diet, were injected with heparin (1,000 IU i.v.) to prevent the formation of microemboli and were killed by cervical dislocation.

The heart was excised rapidly through a left thoracotomy and immersed in cold (4° C) bicarbonate buffer solution (pH 7.4) to arrest contractile activity. The aorta was secured to a stainless-steel cannula, the left ventricle was vented, and the heart was mounted on a modified nonrecirculating Langendorff apparatus.

Hearts were perfused at an aortic pressure of 100 mm Hg, with Krebs-Henseleit bicarbonate buffer (37° C) containing Ca^{2+} (2.5 mM) and glucose (5 mM). The perfusate was equilibrated with a gas mixture of 95% O_2:5% CO_2. Hearts were paced with 4 V for 10 msec at 200 beats/min with a Grass SD9 Stimulator (Grass Instruments, Quincy, Massachusetts). A high-compliance polyurethane balloon was inserted into the left ventricle through the left atrium and secured by a purse-string suture around the mitral annulus. A cannula attached to the balloon was connected to a Millar PC-350/TC-100 pressure-transducer system (Millar Instruments, Houston, Texas) for measurement of peak left ventricular pressure (PLVP). The signal was amplified by a Hewlett-Packard Model 8805-B carrier amplifier (Hewlett-Packard, Waltham, Massachusetts). Left ventricular function curves were generated by increasing left ventricular end-diastolic pressure (LVEDP) in increments of 2.5 mm Hg from 0 to 20 mm Hg and by measuring PLVP at each interval.

We used three experimental protocols: 1) pretreatment with nitrogen followed by normothermic ischemia and reperfusion; 2) pretreatment with nitrogen followed by hypothermic ischemia and reperfusion; and 3) pretreatment with substrate-free perfusion followed by normothermic ischemia and reperfusion. Each heart was perfused initially for 10 minutes with oxygenated Krebs-Henseleit buffer containing glucose (5 mM) to wash out blood and to attain a stable physiological state, and baseline function was assessed before the protocol was begun. Our isolated rabbit heart preparation is stable for a minimum of 90 minutes (data not presented).

Normothermic Ischemia and Reperfusion

Hearts of this protocol were divided into six groups ($n=5$ for each group; Table 1). After the washout period, hearts were perfused for either 10 minutes with buffer equilibrated with 95% O_2:5% CO_2 or for 15 minutes with buffer equilibrated with 95% N_2:5% CO_2 and assigned to one of the groups shown in Table 1. At the end of the protocol, hearts were freeze-clamped for metabolite determination. The protocol and experimental conditions used in these groups were the same as those used by Neely and Grotyohann[5] for rat hearts, with the notable exception that we used 2.5 rather than 1.25 mM [Ca^{2+}] throughout. This was necessary because of the strong dependence of the contractile force in rabbit heart on extracellular [Ca^{2+}] (data not presented).

Hypothermic Ischemia and Reperfusion

To test the effects of glycogen depletion on postischemic function after hypothermic (4° C) ischemic arrest, hearts were assigned to one of four groups ($n=5$ for each group; Table 1). After the washout period, hearts were perfused either with O_2 (10 minutes) or N_2 (5 minutes) prior to reperfusion, functional assessment, and metabolite determination.

Substrate-Free Perfusion

Hearts were assigned to one of three groups ($n=5$ for each group) to test whether tissue glycogen stores could be diminished without hypoxia (Table 1). After the washout period with standard buffer, hearts were perfused with substrate-free buffer (glucose = 0 mM) and assigned to one of the groups shown in Table 1.

Preparation of Tissue Extracts and Measurement of Metabolites

Aluminum tongs cooled in liquid nitrogen[6] were used to freeze-clamp hearts either at the end of the ischemic period or at the end of the perfusion period called for in the experiment. Tissue dry weights were determined, and perchloric acid extracts were prepared and neutralized as described earlier.[7] Hearts were assayed for ATP, ADP, AMP, phosphocreatine, glycogen, pyruvate, lactate, and alanine. The tissue metabolites were measured enzymatically in a Gilford 2600 spectrophotometer

TABLE 1. **Experimental Protocols**

Groups	Preischemic perfusion (minutes)	Ischemia (minutes)	Reperfusion (minutes)
Normothermic ischemia			
O_2	10 (O_2)	...	...
O_2 + ischemia	10 (O_2)	30 (37° C)	...
O_2 + ischemia + reperfusion	10 (O_2)	30 (37° C)	30
N_2	15 (N_2)	...	...
N_2 + ischemia	15 (N_2)	30 (37° C)	...
N_2 + ischemia + reperfusion	15 (N_2)	30 (37° C)	30
Hypothermic ischemia			
O_2	10 (O_2)	...	30
O_2 + ischemia + reperfusion	10 (O_2)	60 (4° C)	30
N_2	5 (N_2)	...	30
N_2 + ischemia + reperfusion	5 (N_2)	60 (4° C)	30
Substrate-free perfusion/normothermic ischemia			
SF	10 (O_2)	...	...
SF + ischemia	10 (O_2)	30 (37° C)	...
SF + ischemia + reperfusion	10 (O_2)	30 (37° C)	30

Each group consists of five hearts. Preischemic perfusions and reperfusions were carried out at 37° C; temperature during ischemia is given in parentheses. Buffer of preischemic perfusion was equilibrated with either 95% O_2:5% CO_2 (O_2) or 95% N_2:5% CO_2 (N_2). Reperfusion buffer was equilibrated with 95% O_2:5% CO_2. At the end of each perfusion protocol, hearts were immediately freeze-clamped while still being perfused.

SF, substrate-free buffer.

(Gilford Instruments, Oberlin, Ohio) using the techniques detailed by Bergmeyer.[8] Glycogen was measured with the amyloglucosidase method of Bartley and Dean.[9] Enzymes and cofactors for metabolite assays were obtained from Boehringer Mannheim (Indianapolis, Indiana), and other chemicals were supplied by Sigma Chemical, St. Louis, Missouri.

Data Analysis

All values are expressed as mean±SEM. Hemodynamic parameters and tissue metabolites were analyzed by Student's nondirectional t test. Differences were considered statistically significant when $p<0.05$.

Results

Normothermic Ischemia and Reperfusion

The recovery of ventricular function for hearts tested in this protocol is shown in Figure 1. Hearts subjected to ischemia and reperfusion showed a decrease in PLVP when compared with nonischemic control hearts (O_2), but the recovery of mechanical activity in hearts perfused initially with N_2 was significantly worse ($p<0.05$).

The tissue metabolite content at the end of each perfusion protocol is shown in Table 2. The content of energy-rich phosphate compounds was decreased with nitrogen perfusion, as was the total adenine nucleotide content ($p<0.05$, Table 2). After 15 minutes of nitrogen perfusion, ATP fell from 14.3 to 6.7 μmol/g dry wt ($p<0.001$) and phosphocreatine fell from 19.4 to 1.8 μmol/g dry wt ($p<0.001$). The nitrogen-perfused hearts showed a lower content of ATP and phosphocreatine after ischemia and reperfusion than the oxygen-perfused hearts, which may explain the diminished capacity to recover from ischemia in the group with N_2 plus ischemia and reperfusion.

Perfusion with 95% N_2:5% CO_2 for 15 minutes lowered glycogen by almost 60% compared with hearts perfused with O_2 (controls), but the decrease in glycogen had little effect on lactate accumulation in our model. The difference in lactate after 30 minutes of ischemia for the groups with O_2 plus ischemia and with N_2 plus ischemia was not significant. The amount of lactate formed during ischemia was greater than the amount of glycogen lost. A significant amount of lactate must, therefore, have come from the use of glucose present in the perfusate. This observation is further supported by the subsequent experiments with substrate-free perfusion (see below). Alanine paralleled the rise in lactate during ischemia, while pyruvate did not change appreciably (Table 2).

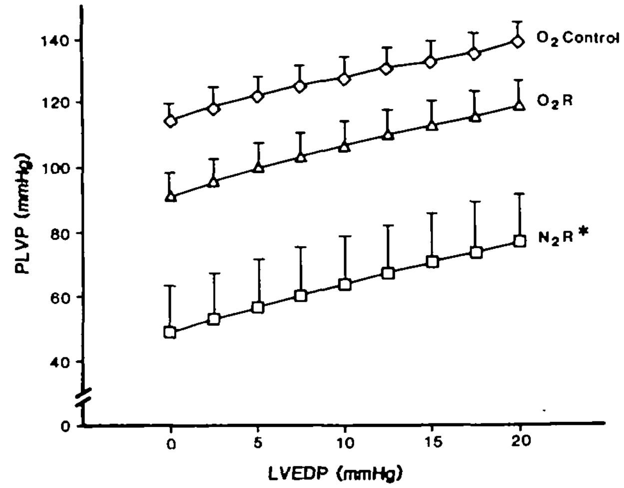

FIGURE 1. *Recovery of ventricular function following normothermic ischemia. Values are mean±SEM at left ventricular end-diastolic pressures (LVEDP) of 0–20 mm Hg in increments of 2.5 mm Hg. Peak left ventricular pressures (PLVP) for O_2 plus ischemia plus reperfusion (O_2R) and N_2 plus ischemia and reperfusion (N_2R) are compared with O_2 (O_2 Control). Experimental groups are defined in Table 1. (*p<0.05).*

Hypothermic Ischemia and Reperfusion

Figure 2 shows the recovery of ventricular function of hearts after 1 hour of hypothermic (4° C) ischemia after initial perfusion with either O_2 or N_2. Both groups showed a decline in function when

TABLE 2. **Normothermic Ischemia and Reperfusion: Tissue Metabolites**

Group	Metabolite content (μmol/g dry wt)								
	ATP	ADP	AMP	Total adenine nucleotides	Phosphocreatine	Glycogen	Pyruvate	Lactate	Alanine
O_2 (5)	14.3±0.50	2.36±0.15	0.56±0.04	17.3±0.35	19.5±1.67	51.3±5.16	0.10±0.03	3.80±0.94	2.07±0.28
O_2 + ischemia (5)	9.97±0.79	4.31±0.16	1.63±0.24	15.9±0.79	5.80±0.80	34.5±3.07	0.10±0.08	62.8±9.07	9.56±0.91
O_2 + ischemia + reperfusion (5)	12.1±0.75	2.88±0.20	0.52±0.03	15.4±0.88	19.2±1.18	25.5±5.57	0.21±0.09	0.67±0.40	3.41±0.15
N_2 (5)	6.75±1.12*	4.50±0.30*	2.17±0.09*	13.4±1.37‡	1.85±0.40*	21.2±5.07†	0.67±0.51	9.01±1.03†	3.77±0.69
N_2 + ischemia (5)	4.75±0.72†	3.51±0.29‡	1.38±0.15	9.64±1.13†	1.13±0.25*	16.1±4.34†	0.18±0.06	47.8±4.86	4.86±0.33†
N_2R + ischemia + reperfusion (4)	8.16±2.13	3.39±0.41	0.74±0.16	12.3±2.64	13.1±2.22‡	18.0±4.54	0.40±0.13	4.35±1.12‡	0.90±0.43*

Hearts from fed rabbits were perfused according to protocols described in text. See Table 1 for definition of groups. Each value is mean±SEM of number of experiments given in parentheses. N_2 groups are compared with O_2 groups.
*$p<0.01$, †$p<0.02$, ‡$p<0.05$.

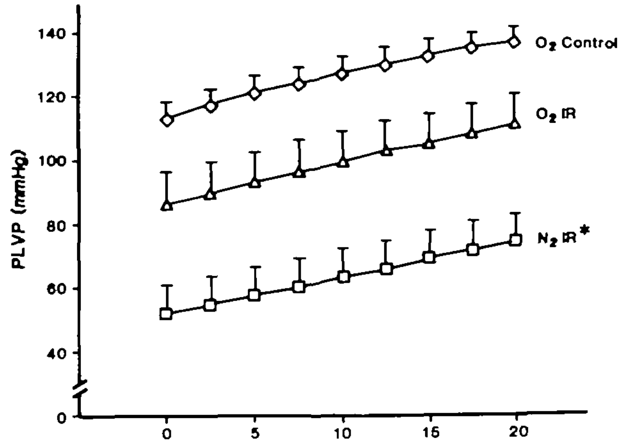

FIGURE 2. *Recovery of ventricular function following hypothermic ischemia. Peak left ventricular pressures (PLVP) for O_2 hearts, never exposed to ischemia, serve as baseline (O_2 Control). Group O_2 plus ischemia and reperfusion (O_2IR, n=5) underwent 10 minutes of O_2 perfusion, 1 hour (4° C) of ischemia, and 30 minutes of O_2 reperfusion. Group N_2 plus ischemia and reperfusion (N_2IR, n=5) underwent 5 minutes of N_2 perfusion, 1 hour (4° C) of ischemia, and 30 minutes of O_2 perfusion. Last two groups are compared with O_2 control (*p<0.05). Values are mean±SEM. LVEDP, left ventricular end-diastolic pressure.*

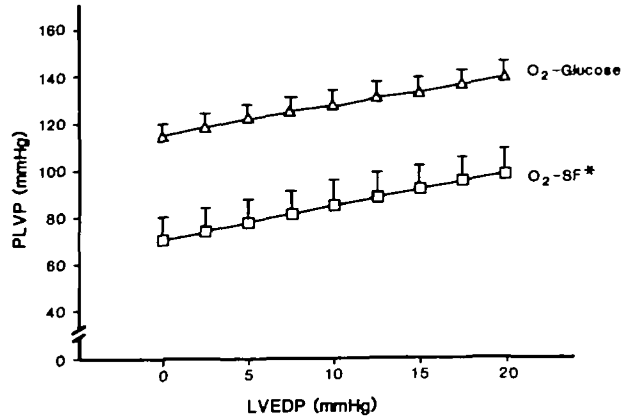

FIGURE 3. *Ventricular function in oxygen and substrate-free control hearts. Ventricular performance of glucose-perfused (5 mM) control hearts (O_2-Glucose) is compared with substrate-free control hearts (O_2-SF). Perfusion of hearts with substrate-free medium for 20 minutes lowered peak left ventricular pressure (PLVP) significantly (*p<0.02). Values are mean±SEM. LVEDP, left ventricular end-diastolic pressure.*

compared with nonischemic control hearts (O_2), but the recovery of nitrogen-perfused hearts was less than the recovery of oxygen-perfused hearts ($p<0.05$).

We found that an abbreviated nitrogen exposure (5 minutes) diminished glycogen stores to the same extent as the longer N_2 exposure of 15 minutes (compare "Glycogen" in Table 3, line 3, with Table 2, line 4). At the same time, the 5-minute N_2 exposure did not affect ATP, phosphocreatine, and total adenine nucleotide levels. However, after reperfusion, hearts that were initially perfused with N_2 showed a decrease in ATP (Table 3; 5.2 vs. 9.9 μmol/g dry wt, $p<0.001$), phosphocreatine (5.7 vs. 8.7 μmol/g dry wt, $p<0.05$), and total adenine nucleotides (9.9 vs. 16.5 μmol/g dry wt, $p<0.001$), which may explain the decrease in function. Lactate, alanine, and pyruvate were not different after ischemia and reperfusion.

Substrate-Free Perfusion

Because nitrogen perfusion per se could have been deleterious to postischemic ventricular recovery, we attempted to lower tissue-glycogen stores and diminish lactate accumulation during a time of ischemia by preperfusing hearts with substrate-free medium.

The ventricular performance of hearts perfused for 10 minutes with either substrate-free or standard medium (glucose) is compared in Figure 3. The two groups are identical to groups SF and O_2 listed in Table 1. Perfusion without glucose prior to ischemia diminished PLVP at all levels of LVEDP ($p<0.02$).

As was the case with N_2, perfusion of hearts with a substrate-free medium significantly lowered glycogen

TABLE 3. Hypothermic Ischemia and Reperfusion: Tissue Metabolites

	Metabolite content (μmol/g dry wt)								
Group	ATP	ADP	AMP	Total adenine nucleotides	Phosphocreatine	Glycogen	Pyruvate	Lactate	Alanine
O_2	14.5±0.27	3.95±0.27	1.61±0.20	20.0±0.38	19.3±2.24	80.6±16.5	0.20±0.05	2.22±0.58	1.36±0.09
O_2+ischemia +reperfusion	9.91±0.66	5.14±0.32	1.43±0.09	16.5±1.03	8.68±0.82	27.2±9.38	0.86±0.07	1.12±0.45	1.51±0.19
N_2	13.9±1.24	3.54±0.30	0.70±0.08*	18.2±1.57	24.4±1.27	25.0±4.58§	0.10±0.05	3.03±0.69	1.20±0.18
N_2+ischemia +reperfusion	5.20±0.53†	3.34±0.25*	1.40±0.53	9.94±0.71†	5.68±0.71‡	14.9±1.49	0.81±0.06	1.76±1.32	1.43±0.48

Hearts from fed rabbits were perfused according to protocols defined in text. See Table 1 for definition of groups. Each value is the mean±SEM of number of five experiments. N_2 groups are compared with O_2 groups.
*$p<0.01$, †$p<0.001$, ‡$p<0.05$, §$p<0.02$.

stores (compare "Glycogen" in Table 2, line 1, with Table 4, line 1, and Table 2, line 4). The decreases in ATP and phosphocreatine were less pronounced with substrate-free perfusion than with nitrogen perfusion. Perfusion without substrate reduced lactate buildup following ischemia (34.8 vs. 62.8 μmol/g dry wt, $p<0.05$; compare Tables 4 and 2). The lower lactate content in substrate-free hearts was similar to the results seen with the N_2 perfusion.

Figure 4 plots the recovery of ventricular function for the reperfusion groups. Neither substrate-free medium nor nitrogen perfusion enhanced functional recovery on reperfusion.

Discussion

The purpose of the present study was to test a potentially useful method for preservation of left ventricular function following prolonged hypothermic ischemia. We were guided by the report of Neely and Grotyohann,[5] who depleted glycogen stores in isolated rat hearts by preperfusion with nitrogen-gassed anoxic buffer and found that this method prevented accumulation of lactate during ischemia and also enhanced postischemic recovery.

In an effort to improve recovery of cardiac function after prolonged ischemia, we manipulated endogenous glycogen stores following three different protocols, one of which was the protocol used by Neely and Grotyohann. In contrast to Neely and Grotyohann, we found that none of the methods showed an improvement in ventricular performance in hearts rendered ischemic and then reperfused. We were also unable to correlate functional recovery with lactate levels during ischemia. Instead, we found that hearts depleted of glycogen prior to ischemia functioned less well when reperfused than hearts in which glycogen stores were preserved.

According to Neely and Grotyohann,[5] a second major determinant of postischemic function is the concentration of Ca^{2+} in the perfusate (i.e., in the extracellular space). The authors have pointed out that functional recovery at low (1.25 mM) [Ca^{2+}] was inversely related to lactate accumulation in the tissue. We used a [Ca^{2+}] of 2.5 mM because of the negative inotropic effect of lower Ca^{2+} concentrations in the rabbit heart in contrast to the rat heart (C.F. Lagerstrom and H. Taegtmeyer, unpublished observations). Therefore, discrepancies between the present study and the earlier study may be a Ca^{2+}-related phenomenon.

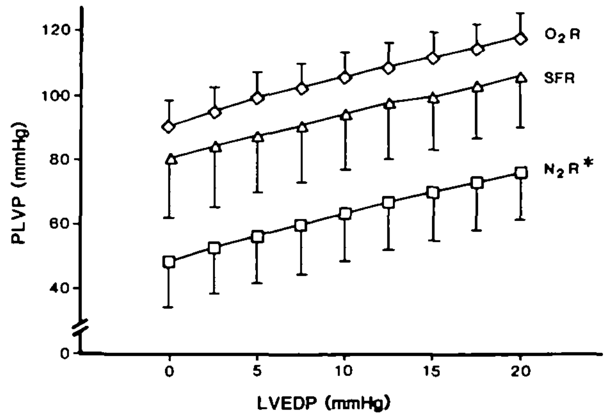

FIGURE 4. *Recovery of ventricular function following normothermic ischemia. Recovery of left ventricular function is compared for groups O_2 plus ischemia plus reperfusion (O_2R), substrate-free plus ischemia plus reperfusion (SFR), and N_2 plus ischemia plus reperfusion (N_2R). See Table 1 for definition of groups. Difference in peak left ventricular pressure (PLVP) between O_2 plus reperfusion and N_2 plus reperfusion was significant (*$p<0.05$). LVEDP, left ventricular end-diastolic pressure.*

The present findings suggest that a loss of glycogen is detrimental to the heart and that endogenous glycogen may be important in sustaining myocardial viability during ischemia. Glycogen may provide a critical amount of glucose for ATP production, which may be important for maintaining membrane function[10] and supporting the survival and recovery of the myocardium.[11,12] In our experiments, hearts with lower glycogen levels before normothermic ischemia had ATP values between 28% and 50% less than controls (Tables 3 and 4), perhaps due to impaired ATP synthesis and turnover or, more likely, due to the loss of adenine nucleotides by degradation to adenosine and its metabolites, which readily leave the cell. The function of hearts with

TABLE 4. **Substrate-Free Perfusion: Tissue Metabolites**

	Metabolite content (μmol/g dry wt)								
Group	ATP	ADP	AMP	Total adenine nucleotides	Phosphocreatine	Glycogen	Pyruvate	Lactate	Alanine
SF	11.0±1.06*	4.08±0.16†	0.87±0.05‡	15.9±1.20	8.98±1.40‡	34.2±4.15§	0.36±0.04	1.33±0.80	0.96±0.24
SF+ischemia	7.20±0.59*	5.19±0.39	2.27±0.50	14.7±0.52	2.20±0.38‡	28.6±6.95	0.46±0.09	34.8±5.12*	8.18±0.59
SF+ischemia +reperfusion	8.61±0.93§	3.80±0.08‡	0.88±0.10‡	13.3±1.00	11.3±1.29‡	32.4±7.06	0.57±0.04	1.08±0.39	1.90±0.17

Hearts from fed rabbits ($n=5$ for each group) were initially perfused with perfusate containing no glucose (refer to text for explanation of groups). Ischemia was for 30 minutes at 37° C, and 30-minute reperfusion was with oxygenated buffer containing glucose (5 mM). For statistical analysis, metabolites from substrate-free hearts were compared with time-matched oxygenated hearts perfused with standard buffer (Groups O_2, O_2+ischemia, O_2+ischemia and reperfusion in Table 2).

*$p<0.05$, †$p<0.001$, ‡$p<0.01$, §$p<0.02$.

low glycogen and low adenine nucleotide content was also depressed (see Figures 1–3). Ichihara et al[13] have shown that glycogen stores preserved by hypothermia (10° C) were used during the early phase of reperfusion at 37° C in the isolated rat heart. Thus, tissue glycogen and its relation to energy-rich phosphate compounds may be especially important during recovery from hypothermic ischemia.

It is accepted that depletion of adenine nucleotides, together with an inability to restore ATP on reperfusion, leads to irreversible injury,[14] although recovery of the heart following reversible ischemia does not closely correlate with residual ATP levels.[15] Jennings et al[16] found that myocardial cells were injured irreversibly when ATP levels were less than 10% of control values. We reported that when residual ATP levels were less than 20% of control values, none of the working rat hearts used in that study regained function.[15] The present study shows an even greater interdependence between function and residual adenine nucleotide content of reperfused hearts pretreated with N_2. The residual adenine nucleotide content had dropped by 50% (Table 3) when left ventricular function was depressed by approximately 50% as well (Figure 2). These data are different from those reported by Neely and Grotyohann,[5] who observed ATP levels declining to as low as 3% of control levels after ischemia, yet their particular hearts showed return of function on reperfusion to 92% of controls. We have no explanation for the discrepancy of our present findings with those of the earlier investigators other than that 1) we used rabbits instead of rats (and that there may be substantial interspecies differences), and 2) the amount [Ca^2] in our perfusion medium was higher, as discussed above. We attempted to follow the protocol of Neely and Grotyohann[5] exactly by using a lower [Ca^{2+}] in the perfusate (1.25 mM). In our model, left ventricular peak systolic pressure was only 50–60 mm Hg, whereas with the higher [Ca^{2+}] of 2.5 mM, pressures were in the physiological range of 100–110 mm Hg (data not shown).

It is clear that many complex and interrelated biochemical changes occur in myocardium exposed to ischemia and/or hypoxia. Increased rates of glycolysis and lactate accumulation are only one facet. Katz and Hecht[17] proposed that ischemia caused a buildup of protons and intracellular acidosis that decreased contractility because protons displaced calcium from binding sites on the thin filaments. According to Gevers,[18] protons arise from ATP hydrolysis in the cytoplasm. ATP hydrolysis is not only part of the contractile process but also intimately linked to the reactions of glycogen, glucose, and lipid metabolism. Another hypothesis, by Kübler and Katz,[19] states that the buildup of inorganic phosphate (released from ATP and phosphocreatine) deprives the cytosol of calcium by forming calcium phosphate. The data presented here on the glycogen-depleted heart exposed to hypothermic ischemic arrest are consistent with either of the two hypotheses. We have shown that prevention of lactate buildup does not improve cardiac function following ischemia. In contrast, preservation of myocardial glycogen stores seems to correlate with improved postischemic recovery.

Acknowledgments

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KEY WORDS • hypothermic ischemic arrest • perfused heart • left ventricular function • glycogen • lactate • energy-rich phosphates