

## Niacin protects the isolated heart from ischemia-reperfusion injury

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**Trueblood, Nathan A., Ravichandran Ramasamy, Li Feng Wang, and Saul Schaefer.** Niacin protects the isolated heart from ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol* 279: H764–H771, 2000.—Nicotinic acid (niacin) has been shown to decrease myocyte injury. Because interventions that lower the cytosolic NADH/NAD<sup>+</sup> ratio improve glycolysis and limit infarct size, we hypothesized that 1) niacin, as a precursor of NAD<sup>+</sup>, would lower the NADH/NAD<sup>+</sup> ratio, increase glycolysis, and limit ischemic injury and 2) these cardioprotective benefits of niacin would be limited in conditions that block lactate removal. Isolated rat hearts were perfused without (Ctl) or with 1 μM niacin (Nia) and subjected to 30 min of low-flow ischemia (10% of baseline flow, LF) and reperfusion. To examine the effects of limiting lactate efflux, experiments were performed with 1) Ctl and Nia groups subjected to zero-flow ischemia and 2) the Nia group treated with the lactate-H<sup>+</sup> cotransport inhibitor α-cyano-4-hydroxycinnamate under LF conditions. Measured variables included ATP, pH, cardiac function, tissue lactate-to-pyruvate ratio (reflecting NADH/NAD<sup>+</sup>), lactate efflux rate, and creatine kinase release. The lactate-to-pyruvate ratio was reduced by more than twofold in Nia-LF hearts during baseline and ischemic conditions ( $P < 0.001$  and  $P < 0.01$ , respectively), with concurrent lower creatine kinase release than Ctl hearts ( $P < 0.05$ ). Nia-LF hearts had significantly greater lactate release during ischemia ( $P < 0.05$  vs. Ctl hearts) as well as higher functional recovery and a relative preservation of high-energy phosphates. Inhibiting lactate efflux with α-cyano-4-hydroxycinnamate and blocking lactate washout with zero flow negated some of the beneficial effects of niacin. During LF, niacin lowered the cytosolic redox state and increased lactate efflux, consistent with redox regulation of glycolysis. Niacin significantly improved functional and metabolic parameters under these conditions, providing additional rationale for use of niacin as a therapeutic agent in patients with ischemic heart disease.

ischemia; nicotinic acid; monocarboxylate transport; myocardium; lactate

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NICOTINIC ACID (niacin) is well known as an agent to treat dyslipidemias, specifically through its inhibition of lipolysis and very-low-density lipoprotein production while increasing high-density lipoprotein (12). Niacin

has also been shown to decrease ischemic events in patients with dyslipidemias (3). Although these findings are likely due in large part to the systemic effect of niacin on lipid metabolism, there is evidence that niacin may have cardiac effects that could limit ischemic injury independent of systemic lipids. Studies have investigated direct effects of niacin on myocardial metabolism (7, 16, 23, 36, 38), with several investigators noting that high concentrations of niacin limit the mobilization and accumulation of free fatty acids (FFA) from myocardial triglyceride stores during prolonged ischemia (7, 16, 36–38). Some of these studies also suggested benefits of niacin during myocardial ischemia and reperfusion, such as greater functional recovery (36) or lower ischemic injury (23). However, these studies used supratherapeutic concentrations of niacin and, as a literature base, did not demonstrate a consistent beneficial effect of niacin. Thus the true effects of niacin and the mechanism(s) limiting ischemic injury or modifying substrate metabolism are unknown.

Previous work in this laboratory has suggested that interventions that lower the cytosolic NADH/NAD<sup>+</sup> ratio, such as fasting or aldose reductase inhibition, limit ischemic injury (26, 31, 35, 39). For example, zopolrestat, an aldose reductase inhibitor, increased glycolysis in hearts from diabetic animals, presumably by limiting redox inhibition of glycolytic enzyme activity (35). Under low-flow ischemic conditions identical to those in the present study, zopolrestat also increased lactate efflux and lowered tissue lactate concentrations, changes consistent with increased glycolysis and increased lactate-H<sup>+</sup> cotransporter flux (27). Because niacin is a building block of NAD<sup>+</sup>, it is possible that its apparent beneficial effect in the setting of ischemia is via modulation of cellular NAD<sup>+</sup> levels. An increase in NAD<sup>+</sup> concentration would lower the cytosolic redox state and, on the basis of our earlier work (26, 31, 35, 39), should increase glycolysis and lactate efflux during low-flow ischemia and decrease ischemic injury. Such effects should also be independent of any impact of niacin on mobilizing myocardial triglyceride stores.

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Any cardioprotective intervention that acts by increasing glycolysis should ultimately be limited by the ability of the myocyte to limit the accumulation of glycolytic end products that act, through negative feedback, to limit glycolysis. Accordingly, it is possible that the beneficial effects of pharmacological stimulation of glycolysis under ischemic conditions can be modulated by techniques that limit lactate removal, such as zero-flow vs. low-flow ischemia and/or inhibitors of lactate- $H^+$  cotransport activity.

Therefore, we tested the hypotheses that 1) treating isolated hearts with therapeutic doses of niacin is associated with a decrease in the NADH/NAD<sup>+</sup> ratio (as reflected by the lactate-to-pyruvate ratio) and an increase in glycolysis and lactate efflux, 2) these metabolic effects will result in decreased ischemic injury independent of myocyte lipid stores, and 3) the extent of any protective effects of glycolysis stimulation under ischemic conditions is limited by inhibiting washout and efflux mechanisms. These hypotheses were tested by determining the effects of niacin on lactate efflux, high-energy phosphates, contractile function, and tissue injury under conditions of low- and zero-flow ischemia followed by reperfusion. To minimize any potentially confounding effects of niacin on myocardial FFA levels, hearts were perfused with glucose as the sole substrate.

## MATERIALS AND METHODS

**Isolated heart protocol.** Age-matched male Sprague-Dawley rats ( $n = 94$  for the entire study) were pretreated with heparin (100 U ip) followed by pentobarbital sodium (65 mg/kg). As soon as deep anesthesia was achieved, as evidenced by lack of eye-blink and foot-withdrawal reflexes, hearts were rapidly isolated and retrogradely perfused with a modified Krebs-Henseleit (KH) buffer equilibrated with 95% O<sub>2</sub>-5% CO<sub>2</sub> [buffer contained (in mM) 118 NaCl, 4.7 KCl, 1.2 CaCl<sub>2</sub>, 1.2 MgSO<sub>4</sub>, 25 NaHCO<sub>3</sub>, and 11 glucose]. After 20 min of baseline perfusion (12.5 ml/min), hearts were subjected to 1) 30 min of low-flow ischemia (10% of baseline flow) followed by 30 min of reperfusion (LF conditions) or 2) 20 min of zero-flow ischemia followed by 30 min of reperfusion (ZF conditions). This laboratory has characterized both of these ischemia protocols previously (27, 31, 32).

In addition to hearts perfused with only the modified KH buffer described above (Ctl group), the buffer (in LF groups) also contained 1  $\mu$ M niacin (Nia group) or 1 mM  $\alpha$ -cyano-4-hydroxycinnamate (CHC) in addition to 1  $\mu$ M niacin (Nia + CHC group). This concentration of niacin was used to approximate concentrations in the therapeutic range of humans (25). CHC and nicotinic acid (Sigma Chemical) were dissolved directly into the buffer. Treatment with CHC and nicotinic acid was started 10 min before ischemia and continued throughout the protocol. A preliminary CHC dose-response analysis was performed, and 1 mM CHC + niacin was found to decrease ischemic lactate efflux rates to approximately those of hearts without niacin. Cardiac function [left ventricular end-diastolic pressure (EDP), systolic pressure, and heart rate] was measured throughout the protocol via a balloon inserted into the left ventricle connected to a pressure transducer and Gould Windowgraf recorder. Rate-pressure product (heart rate  $\times$  systolic pressure) was used as an index of cardiac oxygen consumption.

**Tissue lactate and pyruvate assays for NADH/NAD<sup>+</sup> ratio.** To determine changes in the cytosolic redox state, parallel experiments were performed using hearts in the Ctl and Nia groups ( $n = 6$  in each group). At the end of the baseline perfusion period and at the end of ischemia, hearts were freeze-clamped in liquid nitrogen. Perchloric acid was used to extract lactate and pyruvate from the freeze-clamped tissue, and lactate and pyruvate were measured using standard biochemical assays (2).

**Effluent lactate measurements.** Lactate concentration was measured in the coronary effluent by standard biochemical techniques (2). The sample concentrations were normalized to heart weight, multiplied by coronary flow rate, and expressed as efflux rates ( $\mu$ mol  $\cdot$  min<sup>-1</sup>  $\cdot$  g dry wt<sup>-1</sup>).

**<sup>31</sup>P NMR spectroscopy.** <sup>31</sup>P NMR spectra were obtained every 5 min during baseline, ischemia, and reperfusion with GE Omega-300 or Bruker AMX 400 vertical-bore spectrometers. Spectra were obtained using 248 acquisitions of 45° pulse width and 1.21-s interpulse delay. Spectra were processed using an exponential multiplication of 10 Hz and manual phasing. Metabolites were referenced to their baseline value and expressed as a fraction of baseline. The pH was calculated from the chemical shift of P<sub>i</sub> and phosphocreatine (PCr) by use of a titration curve developed in this laboratory (15, 32).

**Creatine kinase measurement.** Creatine kinase (CK) release into the coronary effluent was assessed spectrophotometrically (CK kit 47-20, Sigma Chemical) for Nia and Ctl groups. Because of a reaction between CHC and the contents of the CK reagent of this kit, CK kit 661-TB (Sigma Chemical) was used to measure CK in the Nia + CHC group effluent. Both CK kits measured control samples to establish a conversion equation between the different kits. Values were also corrected for coronary flow rate, time between samples (5 min), and heart weight and are expressed as total integrated release per gram dry weight (IU/g dry wt) (31).

**Isolation and quantification of myocardial FFA.** Myocardial lipids were extracted using a modified Folch method (11) as described below. At the end of baseline perfusion and at the end of low-flow ischemia, Ctl and Nia hearts ( $n = 4$  for both groups and time points) were freeze-clamped in liquid nitrogen. One gram of heart tissue was then homogenized in 5 ml of Folch's reagent (2:1 chloroform-methanol, vol/vol) by use of a Tissue Tearor homogenizer. This homogenate was filtered through Na<sub>2</sub>SO<sub>4</sub> crystal to remove water from the homogenate and then dried in a rotary evaporator at 35°C. This dried sample was reconstituted in 0.25 ml of Folch's reagent. Total polar and neutral lipids were isolated from the tissue extract by using TLC plates coated with silica gel. Chloroform-methanol-acetic acid-water (in ml, 90:8:1:0.8) was used as developing solvent. The lipid bands were visualized using iodine crystals, and the neutral lipid band was scraped into a sintered funnel (phospholipids were excluded). Folch's reagent was used to elute the FFA from the substrate, which was dried under nitrogen gas. These FFA samples were *trans*-methylated in 3 ml of 6% MeOH  $\cdot$  HCl (along with 25  $\mu$ g of 17:0 fatty acid as internal standard) for 12 h at 120°C and then extracted in petroleum ether. After evaporation under nitrogen gas, the sample was dissolved in dichloromethane. Gas chromatography was performed at 210°C (model GL17A, Shimadzu), with helium as the carrier gas (50 cm/s free induction decay) and with the use of a DB-225 column (J & W Scientific, 30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m). FFA content was quantified by comparison with the internal standard and is expressed in nanomoles per gram wet weight.

**Statistical methods.** Values are means  $\pm$  SE. Differences in data between two groups were analyzed using unpaired

two-sided Student's *t*-test. When the differences between more than two groups were compared, ANOVA with Student-Newman-Keuls multiple comparisons posttests was used.  $P < 0.05$  was considered significant.

## RESULTS

**Effects of niacin on cardiac function.** Table 1 indicates heart rate and left ventricular developed pressure and EDP at baseline, end-ischemic, and end-reperfusion time points. Figure 1 demonstrates changes in the rate-pressure product over time in the different LF groups. Niacin had no significant effect on developed pressure, heart rate, or rate-pressure product during baseline perfusion compared with Ctl hearts.

The LF protocol did not reveal any significant differences between groups in EDP. Heart rate and developed pressure were greater in Nia-LF than in Ctl hearts in the second half of ischemia ( $P < 0.05$ ). As a result, rate-pressure product was also greatest in Nia-LF hearts, with end-ischemic values of  $659 \pm 290$  in Ctl-LF,  $2,281 \pm 339$  in Nia-LF, and  $644 \pm 294$  in Nia + CHC ( $P < 0.01$ ).

On reperfusion, Nia-LF hearts exhibited normalization of developed pressure and rate-pressure product (3-fold greater in Nia-LF), changes that were significantly different from Ctl hearts. There was a trend toward normalization of EDP and recovery of heart rate after ischemia in Nia-LF hearts. Although the EDP and heart rate values approached significance at  $P < 0.05$ , they were not different by ANOVA ( $P = 0.051$  for heart rate,  $P = 0.092$  for EDP). Despite not quite reaching significance, this limitation of contracture may be of biological importance.

Table 1. Heart rates and left ventricular pressures at baseline, end low-flow ischemia, and end reperfusion

|                              | Ctl-LF        | Nia-LF         | Nia+CHC      |
|------------------------------|---------------|----------------|--------------|
| End-diastolic pressure, mmHg |               |                |              |
| Baseline                     | $3.6 \pm 0.7$ | $3.8 \pm 0.4$  | $6.7 \pm 1$  |
| End ischemia                 | $23 \pm 6$    | $14 \pm 6$     | $27 \pm 7$   |
| End reperfusion              | $49 \pm 11$   | $5.8 \pm 3.1$  | $31 \pm 16$  |
| Developed pressure, mmHg     |               |                |              |
| Baseline                     | $93 \pm 11$   | $100 \pm 7$    | $64 \pm 6^a$ |
| End ischemia                 | $22 \pm 9$    | $33 \pm 5^b$   | $12 \pm 6$   |
| End reperfusion              | $35 \pm 20$   | $110 \pm 9^c$  | $22 \pm 12$  |
| Heart rate, beats/min        |               |                |              |
| Baseline                     | $302 \pm 15$  | $312 \pm 20$   | $313 \pm 6$  |
| End ischemia                 | $17 \pm 8$    | $76 \pm 13^d$  | $34 \pm 17$  |
| End reperfusion              | $109 \pm 67$  | $297 \pm 22^e$ | $209 \pm 67$ |

Values are means  $\pm$  SE;  $n = 6$  in each group, with comparisons by ANOVA. End-diastolic pressures tended to be lower in group treated with niacin and subjected to low-flow ischemia (Nia-LF) than in control group subjected to low-flow ischemia (Ctl-LF) or Nia group also treated with  $\alpha$ -cyano-4-hydroxycinnamate (Nia+CHC) during reperfusion ( $P = 0.092$ ). Developed pressure was depressed in Nia+CHC hearts at baseline ( $^aP < 0.05$ ). End-ischemic developed pressure was greater in Nia-LF than in the Nia+CHC hearts ( $^bP < 0.05$ ). Developed pressure was greater in the Nia-LF group throughout reperfusion than in all groups ( $^cP < 0.01$ ). Heart rate was greater in Nia-LF hearts at the end of ischemia and reperfusion than in Ctl-LF or Nia+CHC hearts ( $^dP < 0.05$  at end ischemia;  $^eP = 0.051$  at end reperfusion).

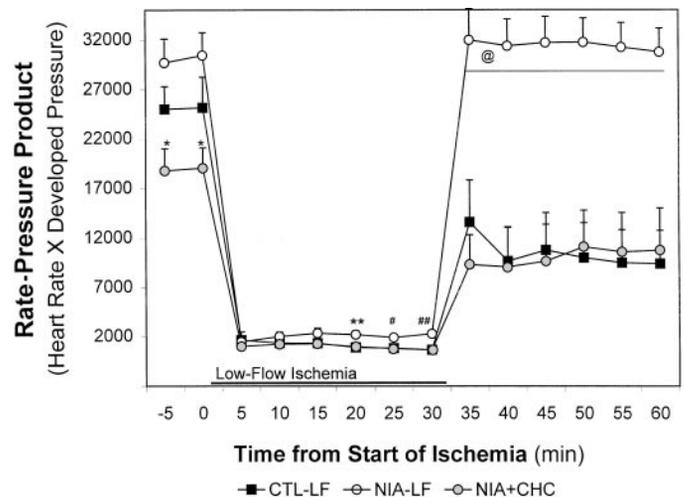


Fig. 1. Rate-pressure product (RPP) during low-flow ischemia (LF) protocol. Values are means  $\pm$  SE ( $n = 6$  in each group). RPP was reduced at baseline in group treated with niacin (Nia) +  $\alpha$ -cyano-4-hydroxycinnamate (CHC) vs. control (Ctl)-LF and Nia-LF groups ( $*P < 0.05$ ). RPP was reduced in all groups during LF. RPP was greater in the second half of ischemia in the Nia-LF group:  $**P < 0.05$  vs. Ctl-LF and Nia + CHC;  $^#P < 0.05$  vs. Ctl-LF;  $^{##}P < 0.01$  vs. Ctl-LF and Nia + CHC. With reperfusion, only the Nia-LF group recovered:  $^@P < 0.01$  vs. Ctl-LF;  $P < 0.001$  vs. Nia + CHC. There were no RPP differences between Ctl or Nia hearts at any time point during the zero-flow (ZF) protocol.

Nia + CHC hearts demonstrated a reduction in developed pressure and rate-pressure product under baseline conditions ( $P < 0.05$  compared with Nia-LF). There were no differences in hemodynamic recovery between the Ctl and Nia + CHC groups. There were no differences between Nia and Ctl hearts at any time during the ZF protocol with regard to heart rate, developed pressure, or EDP.

Rate-pressure product at baseline was  $25,244 \pm 2,332$  in Ctl-ZF and  $24,300 \pm 3,021$  in Nia-ZF hearts. At the end of ischemia, rate-pressure product was  $0 \pm 0$  in both ZF groups. At the end of reperfusion, rate-pressure product was  $6,637 \pm 6,127$  in Ctl-ZF and  $8,685 \pm 8,685$  in Nia-ZF hearts (not significant).

**Effects of niacin on tissue lactate-to-pyruvate ratio.** The tissue lactate-to-pyruvate ratio, reflecting the cellular NADH/NAD<sup>+</sup> ratio, was decreased more than twofold in Nia hearts under baseline and LF conditions ( $P < 0.001$  vs. Ctl hearts; Table 2). Similarly, the lactate-to-pyruvate ratio was reduced by niacin in the ZF protocol ( $P < 0.005$ ).

**Effects of niacin on lactate release.** Lactate efflux rate was increased by niacin under baseline conditions ( $5.3 \pm 1.7$  in Ctl hearts vs.  $12.4 \pm 2.3 \mu\text{M} \cdot \text{min}^{-1} \cdot \text{g dry wt}^{-1}$ ,  $P < 0.05$ ), an effect that was negated by CHC ( $6.5 \pm 3.0 \mu\text{M} \cdot \text{min}^{-1} \cdot \text{g dry wt}^{-1}$ ). The lactate efflux rates were significantly higher for Nia than for Ctl hearts for the last 20 min of the LF protocol ( $P < 0.05$ ). At the end of the LF protocol, the lactate release rate in Nia hearts was twice that in Ctl hearts (Fig. 2). The addition of CHC to Nia hearts eliminated the difference in lactate release between the Ctl and Nia groups

Table 2. Effects of niacin, low-flow ischemia, and zero-flow ischemia on tissue lactate and lactate-to-pyruvate ratio ( $NADH/NAD^+$ )

|  | Ctl-LF         | Nia-LF          | Ctl-ZF         | Nia-ZF          |
|--|----------------|-----------------|----------------|-----------------|
| Tissue lactate, $\mu\text{mol/g}$ dry wt |                |                 |                |                 |
| Baseline                                 | $5.6 \pm 0.6$  | $2.1 \pm 0.3^a$ | $5.8 \pm 0.3$  | $2.2 \pm 0.4^a$ |
| End ischemia                             | $60 \pm 8$     | $23 \pm 2^b$    | $102 \pm 13$   | $41 \pm 6^b$    |
| Lactate-to-pyruvate ratio                |                |                 |                |                 |
| Baseline                                 | $12.6 \pm 0.8$ | $4.5 \pm 0.7^c$ | $14.7 \pm 1.6$ | $4.4 \pm 0.3^c$ |
| End ischemia                             | $188 \pm 34$   | $76 \pm 10^d$   | $335 \pm 55^e$ | $109 \pm 12^d$  |

Values are means  $\pm$  SE;  $n = 6$  for each group and time point. ZF, zero flow. Tissue lactate was lower at baseline with Nia ( $^aP < 0.001$ , Ctl-LF vs. Nia-LF and Ctl-ZF vs. Nia-ZF). Tissue lactate was also lower at the end of ischemia with Nia ( $^bP < 0.05$ , Ctl-LF vs. Nia-LF;  $P < 0.001$ , Ctl-ZF vs. Nia-ZF). Baseline lactate-to-pyruvate ratios were lower in Nia groups ( $^cP < 0.05$  vs. Ctl groups). At the end of 20 min of zero-flow ischemia, as well as at the end of 30 min of low-flow ischemia, lactate-to-pyruvate ratio was lower in Nia than in Ctl hearts ( $^dP < 0.01$  in LF comparison,  $P < 0.001$  in ZF comparison). Lactate-to-pyruvate ratios were higher in Ctl-ZF than in Ctl-LF hearts ( $^eP < 0.01$ ).

and resulted in a delay in lactate release compared with Ctl hearts.

**Myocardial high-energy phosphates.** Figure 3 illustrates changes in ATP levels throughout the LF protocol in the different groups. ATP levels were significantly higher in the Nia-LF group during ischemia than in the Ctl group ( $P < 0.05$ ). End-ischemic ATP levels (as a percentage of baseline) were  $65 \pm 7\%$  in Ctl,  $88 \pm 3\%$  in Nia, and  $77 \pm 5\%$  in Nia + CHC hearts ( $P < 0.05$ , Nia vs. Ctl). Nia hearts maintained significantly greater ATP levels at every time point during reperfusion than Ctl hearts ( $P < 0.05$ ). ATP levels at the end of reperfusion were  $60 \pm$

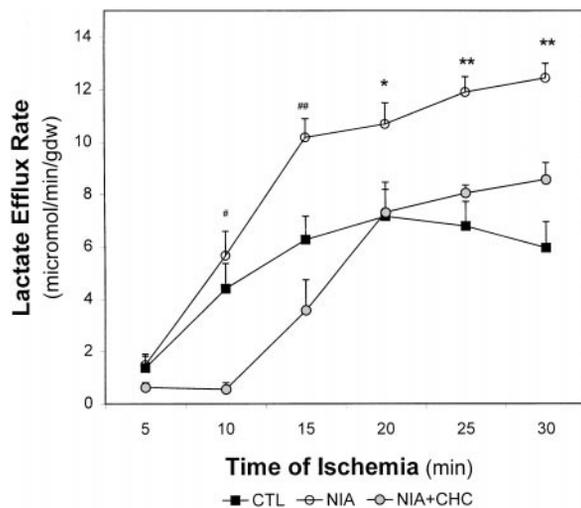


Fig. 2. Ischemic lactate efflux rates. Coronary effluent samples were taken every 5 min during the LF protocol and assayed for lactate. Lactate concentrations were normalized to the dry weight of the heart and multiplied by coronary flow rate.  $^{\#}P < 0.01$ , Nia and Ctl vs. Nia + CHC.  $^{##}P < 0.01$ , Nia vs. Ctl;  $P < 0.001$ , Nia vs. Nia + CHC.  $^*P < 0.05$ , Nia vs. Ctl and Nia + CHC;  $P < 0.05$ , Nia vs. Ctl and Nia + CHC.  $^{**}P < 0.001$ , Nia vs. Ctl;  $P < 0.01$ , Nia vs. Nia + CHC.

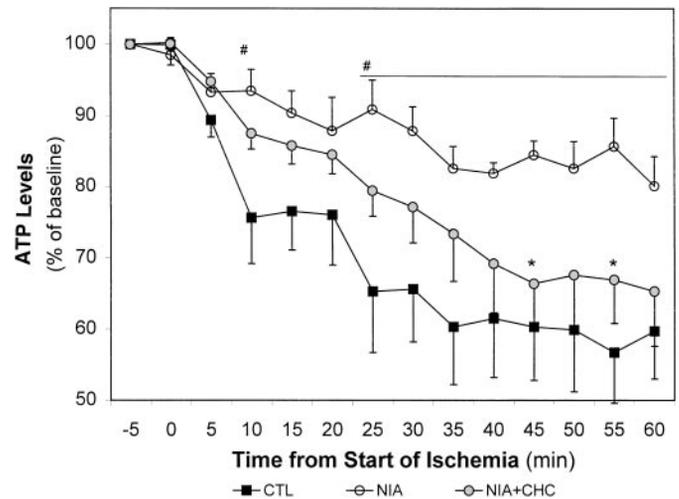


Fig. 3.  $^{31}\text{P}$  NMR assessment of ATP levels in hearts during LF protocol.  $^{\#}P < 0.05$ , Ctl vs. Nia.  $^*P < 0.05$ , Nia vs. Nia + CHC. There were no differences in ATP levels between Ctl and Nia hearts at any time point during the ZF protocol.

6% in Ctl,  $80 \pm 4\%$  in Nia, and  $65 \pm 7\%$  in Nia + CHC hearts ( $P < 0.05$ , Nia vs. Ctl). The reperfusion levels of ATP in Nia + CHC hearts were within a standard deviation of the Ctl group and were significantly lower than in Nia hearts at 15 and 25 min of reperfusion ( $P < 0.05$ ). ATP levels in Nia hearts were not different from those in Ctl hearts at any time point during the ZF protocol.

The PCr recovery was greater for Nia-LF than for Ctl-LF and Nia + CHC hearts at the first 5 min of reperfusion. Specifically, as a percentage of baseline levels, PCr was  $59 \pm 5\%$  in Ctl-LF,  $85 \pm 3.7\%$  in Nia-LF, and  $73.8 \pm 3.7\%$  in Nia + CHC hearts at 5 min into reperfusion ( $P < 0.01$ , Nia-LF vs. Ctl-LF;  $P < 0.05$ , Nia-LF vs. Nia + CHC). End-ischemic PCr levels (also as a percentage of baseline) were  $37 \pm 10\%$  in Ctl,  $53 \pm 2.7\%$  in Nia, and  $47.6 \pm 4\%$  in Nia + CHC hearts (not significant). PCr levels at the end of reperfusion were  $75 \pm 6\%$  in Ctl,  $93 \pm 2.3\%$  in Nia, and  $84 \pm 7.4\%$  in Nia + CHC hearts. There were no differences in PCr recovery in the two ZF groups.

In summary, the overall recovery of high-energy phosphates and functional parameters were significantly greater in Nia-LF than in Ctl-LF hearts. This marked functional and metabolic recovery was, in general, not seen in Nia-ZF or Nia + CHC hearts.

**Intracellular pH.** Figure 4 demonstrates intracellular pH in the LF and ZF experiments. In LF experiments, the pH nadir occurred after 15 min of ischemia and corresponded with pH values of  $6.41 \pm 0.12$  in Ctl,  $6.74 \pm 0.11$  in Nia, and  $6.66 \pm 0.07$  in Nia + CHC hearts (not significant by ANOVA). These pH data reflect a twofold difference in  $\text{H}^+$  concentration between Nia and Ctl hearts during LF. In ZF experiments, the pH nadir occurred at the end of the 20-min ischemic period and corresponded with pH values of  $5.89 \pm 0.03$  in Ctl-ZF hearts and  $6.05 \pm 0.06$  in Nia-ZF hearts ( $P < 0.05$ , by  $t$ -test).

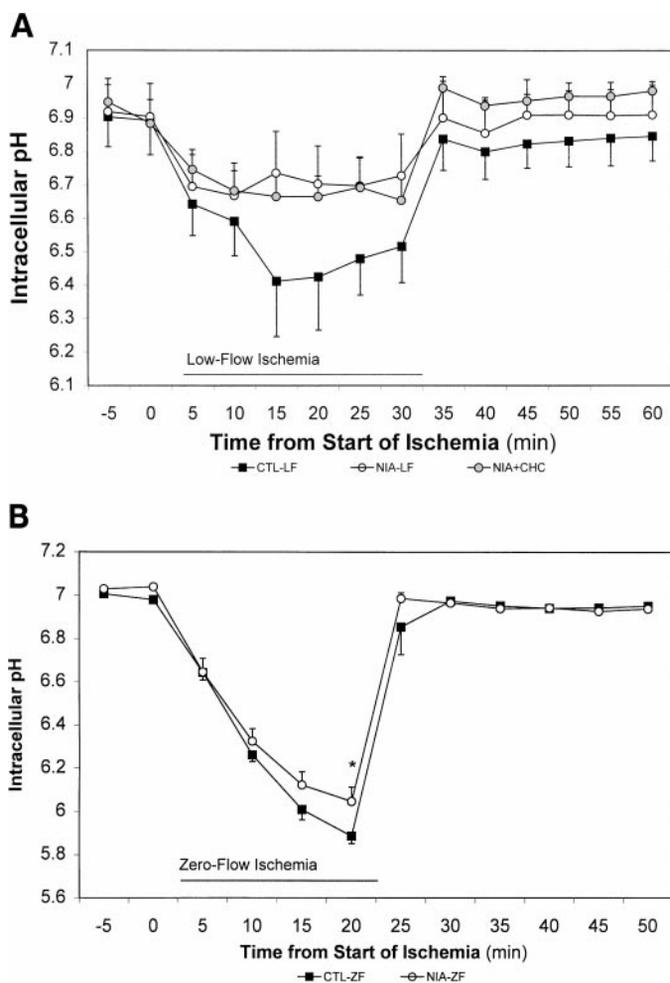


Fig. 4. Intracellular pH calculated from shift in  $P_i$  peak by  $^{31}P$  NMR. Although Nia appears to have limited acidosis with ischemia in LF (A) and ZF (B) groups, this difference only reached significance in the ZF protocol, in part because of smaller within-group variability ( $*P < 0.05$ ).

**CK release.** As shown in Fig. 5, total integrated CK release was  $103.2 \pm 22.5$ ,  $17.2 \pm 5.6$ , and  $95.8 \pm 6.3$  IU/g dry wt in Ctl, Nia, and Nia + CHC hearts, respectively ( $P < 0.01$ , Nia vs. Ctl;  $P < 0.01$ , Nia vs. Nia + CHC). The marked decrease in CK release in the Nia group indicates a decrease in cellular injury with niacin. Furthermore, this effect of niacin was negated by Nia + CHC. CK release was  $774 \pm 141$  and  $231 \pm 50$  IU/g dry wt in Ctl-ZF and Nia-ZF hearts, respectively ( $P < 0.01$ ).

**Myocardial FFA.** Hearts perfused with niacin had no significant differences in total myocardial FFA levels compared with controls at baseline ( $1.3 \pm 0.2$  and  $0.9 \pm 0.1$   $\mu\text{mol/g}$  wet wt in Ctl and Nia, respectively, not significant). End-ischemic FFA levels were not different ( $1.1 \pm 0.2$  and  $1.3 \pm 0.1$   $\mu\text{mol/g}$  wet wt in Ctl and Nia hearts, respectively; not significant). Furthermore, FFA levels did not appear to increase due to LF in Ctl hearts, and niacin did not appear to inhibit myocardial FFA mobilization from triglyceride stores with ischemia.

## DISCUSSION

We have shown for the first time that therapeutically relevant concentrations of niacin directly affect lactate efflux rates and the cytosolic redox  $\text{NADH}/\text{NAD}^+$  ratio. The effect of niacin on lactate efflux is consistent with increased glycolysis and increased lactate- $\text{H}^+$  cotransport flux. This effect of niacin on glycolytic metabolism likely contributed to the greater tolerance to low-flow ischemia, as demonstrated here by improved contractile function and bioenergetic status. These effects of niacin appear to be independent of any inhibition of FFA mobilization from triglyceride stores. Furthermore, the significant reversal of the effects of niacin under conditions that inhibit lactate washout (zero-flow ischemia) or efflux (CHC) gives additional support to a role of the lactate- $\text{H}^+$  cotransporter and/or lactate washout in mediating the beneficial effect of niacin. These data provide further support for the concept that modulation of cytosolic redox state may be an important mechanism for the amelioration of ischemic injury likely through maintenance of glycolytic ATP production.

**Niacin effects on redox state and metabolism.** The effect of niacin on the cytosolic redox state, glycolysis, and lactate metabolism is controversial. For example, Vik-Mo et al. (38) showed no effect of niacin on lactate production, but Datta et al. (7) demonstrated significant increases in glycolysis with no change in the  $\text{NADH}/\text{NAD}^+$  ratio. We postulated that niacin, by providing a building block of  $\text{NAD}^+$ , would lower the redox state. This postulate was supported by the tissue lactate-to-pyruvate ratios, which were lower in Nia hearts under baseline and ischemic conditions.

**Cytosolic redox modulation of glycolysis.** Because previous studies using interventions that lower the cytosolic redox state have been associated with increased glycolytic flux (35), we postulated that niacin would increase glycolysis under low-flow ischemic conditions. The putative mechanism for the cytosolic redox modulation of glycolysis is that  $\text{NAD}^+$  is a cofactor

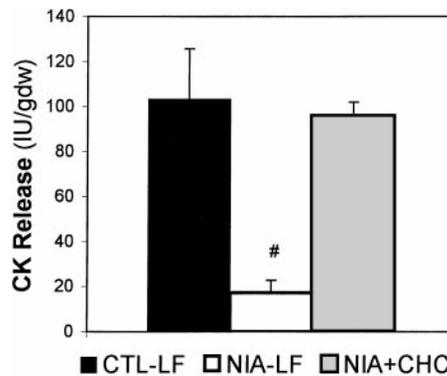


Fig. 5. Effects of Nia and CHC on creatine kinase (CK) release. Effluent samples were collected at 5-min intervals during reperfusion, and CK concentration was normalized to heart weight. CK data are presented as total integrated CK release (IU/g dry wt). CK release was lower in Nia-LF than in CTL-LF or Nia + CHC group ( $\#P < 0.01$ ). CK release was lower in Nia-ZF than in Ctl-ZF group ( $P < 0.01$ ) (data not shown).

required for glycolytic enzymes (such as glyceraldehyde-3-phosphate dehydrogenase). Because cytosolic  $\text{NAD}^+$  is reduced to NADH with glycolysis and without oxygen NADH cannot be reoxidized,  $\text{NAD}^+$  becomes limited in oxygen- or flow-limited conditions (such as ischemia) and in metabolic disease states (such as diabetes). Furthermore, Mochizuki and Neely (19a) clearly demonstrated inhibition of a key glycolytic enzyme (glyceraldehyde-3-phosphate dehydrogenase) with increasing concentrations of NADH.

Previous studies have demonstrated increases in exogenous glucose utilization in humans treated with niacin (34), and niacin treatment in pig hearts resulted in significant increases in glycolysis (7). Vik-Mo et al. (38), however, showed no effect of niacin on lactate efflux. The present study further supports a significant role for niacin (and a lower  $\text{NADH}/\text{NAD}^+$  ratio) in increasing glycolysis under these conditions, since the lactate efflux rate, as shown in Fig. 2, was increased by niacin during low-flow conditions. These observations also support the important role for glucose and glycolysis under low-flow conditions and are consistent with observations across many laboratories that interventions that increase glycolytic metabolism (6, 8, 32) result in improved functional and metabolic status. As well, these data suggest that studies aimed at glycolytic modulation may be most effective if performed under low-flow conditions.

*Effect of niacin on ischemic mobilization of myocardial triglyceride stores.* One postulated mechanism for the effects of niacin is that its antilipolytic actions result in a myocardial substrate shift from fatty acid oxidation to glycolysis. Van Bilsen et al. (36) demonstrated that FFA accumulate from myocardial triglyceride stores with ischemia. Importantly, they also showed that 10  $\mu\text{M}$  niacin limited this ischemia-induced FFA accumulation. Stone et al. (34) also showed an increase in glucose utilization and a decrease in fatty acid levels with niacin.

Although the heart has a demonstrated preference for fatty acids as an energy substrate under normoxic conditions (20, 21), it is also clearly an opportunistic tissue in that it uses whatever substrates are available (14). Accordingly, any intervention that inhibits the use of fatty acids by the heart will result in a shift toward glycolysis, and interventions that stimulate glycolytic enzymes result in decreased fatty acid oxidation (1, 18, 30, 35, 37). This shift in substrate use has been shown in experiments with inhibitors of fatty acid oxidation (mepacrine, etomoxir, and niacin) and stimulators of pyruvate dehydrogenase (dichloroacetate, ranolazine, and insulin) (1, 2, 19, 28, 36). However, all these studies have been in the presence of perfusate or plasma fatty acids (and/or other substrates beyond glucose). In the present study, we used concentrations of niacin unlikely to have significant effects on lipolysis and performed the experiments with glucose as the sole substrate for the isolated hearts. Under these conditions, glucose provides up to 90% of the energy for oxidative metabolism (4). Therefore, a priori, the sub-

strate shift theory is not likely to be the mechanism for the beneficial effects of niacin in these experiments.

Additional support for the theory that mobilization of FFA was not a component of the observed effects of niacin is provided by the tissue measurements of FFA. These data indicate that LF conditions did not result in an increase in total FFA levels in control hearts and, furthermore, that niacin did not inhibit triglyceride mobilization. Therefore, in these experiments, the effects of niacin are not likely due to inhibition of fatty acid mobilization and appear to be primarily mediated by the other effects of niacin.

*Effect of niacin on lactate- $\text{H}^+$  cotransport flux.* In addition to an increase in glycolysis, the increases in lactate efflux rates during baseline and ischemic conditions with exposure to niacin may have also been due to a direct effect of niacin on lactate efflux through the lactate- $\text{H}^+$  cotransporter. Several studies have demonstrated the existence and kinetics of lactate- $\text{H}^+$  cotransporters in myocardium (9, 10, 13, 17, 24, 40). There are at least two isoforms of monocarboxylate transporters (MCTs) in myocardium (13), and the myocardial MCTs are reported to be operating near maximal velocity under conditions of ischemia. Accordingly, any intervention that increases MCT maximal velocity should increase the lactate removal capacity and, therefore, ischemic tolerance of the heart (29). Transgenic animals overexpressing cardiac MCT may yield valuable information with regard to this hypothesis.

*Effects of CHC + niacin.* In this study we have demonstrated that 1 mM CHC inhibits lactate efflux during low-flow ischemia. This concentration of CHC was used to decrease the lactate efflux rates of the Nia group to the level of the Ctl group, thereby isolating the role of greater lactate removal (and concurrent tissue lactate accumulation) under ischemic conditions. Inhibition of lactate release in Nia hearts by CHC negated some of the beneficial effects of niacin, suggesting that greater lactate efflux and lower tissue accumulation were important components of the beneficial effect of niacin. This postulate was further supported by the zero-flow ischemia data, which similarly suggest that residual flow and, therefore, metabolite (e.g., lactate and  $\text{H}^+$ ) washout are important for the success of interventions that modulate glycolysis. It has long been established that the ischemic accumulation of lactate has deleterious effects, including decreased contractility, increased mitochondrial damage, shortening of the action potential, and inhibition of glycolysis (22). These observations were supported by recent experiments in this laboratory in which increased glucose entry (using glucose + insulin) before and during zero-flow ischemia increased  $\text{NADH}/\text{NAD}^+$  and resulted in greater ischemic injury (33). Accordingly, interventions that increase lactate efflux (as suggested here with niacin) should limit ischemic injury, and interventions that limit lactate efflux (as suggested here with CHC) or washout (as suggested here with zero-flow ischemia) should be detrimental.

Another possible effect of CHC could be on nonspecific substrate transport. Although CHC is a specific

inhibitor of lactate-H<sup>+</sup> cotransport, Halestrap et al. (13) showed that MCTs could also transport other substrates, including  $\beta$ -hydroxybutyrate, pyruvate, and acetate. Although this could potentially be a confounding effect, pyruvate has been shown to be a poor substrate under low-flow ischemic conditions (32); thus, combined with the preference for glucose under these conditions, inhibition of pyruvate entry should have minimal effect on myocyte function or recovery. In addition, because there was a marked increase in lactate and proton concentrations during low-flow ischemia (in contrast to minimal changes in pyruvate concentrations), the predominant effect of CHC was likely on lactate and protons rather than other substrates.

**Limitations.** These data must be interpreted with several conditions in mind. First, the concentration of CHC (1 mM) depressed baseline left ventricular developed pressure, consistent with findings reported by Elliot et al. (10) using 4 mM CHC. Accordingly, it is possible that the role of lactate efflux may be somewhat obscured by the functional effect of CHC. However, because there was minimal evidence of ischemic contracture, i.e., a modest degree of heart rate recovery, and because CK release was similar to that of Ctl hearts, we do not believe that the baseline left ventricular developed pressure depression from CHC represents a significant toxic or beneficial effect of the inhibitor. Because little is known about how CHC decreases baseline function, the possibility remains that calcium mobilization or uptake may be altered. For example, CHC may impair function by increasing the NADH/NAD<sup>+</sup> ratio, which may directly inhibit calcium release from the sarcoplasmic reticulum (5). Second, the use of the isolated rat heart with glucose as the sole substrate allowed examination of the effects of niacin without confounding systemic effects of the compound or other substrates. However, extension of these findings to the in situ animal model or to a human with ischemic heart disease should be done with caution. Third, the concentration of niacin used in these experiments (1  $\mu$ M) was chosen on the basis of the plasma concentrations typically found in patients with coronary artery disease. It is possible that other effects (positive or negative) could result from different concentrations.

**Conclusions.** This study showed that, under low-flow ischemia-reperfusion conditions, niacin lowered the cytosolic redox state and increased the lactate efflux rate, consistent with redox regulation of glycolysis. These metabolic changes were associated with total functional recovery and marked reductions in CK release and significantly improved maintenance and recovery of high-energy phosphate concentrations. However, the beneficial effect of niacin was markedly reduced when lactate washout or efflux was limited. These beneficial effects of niacin during ischemia may provide additional rationale for the use of niacin as a therapeutic agent in patients with ischemic heart disease.

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