Intermittent Hypoxic Training Protects Canine Myocardium from Infarction

PU ZONG,*,[1] SRINATH SETTY,* WEI SUN,* RODOLFO MARTINEZ,* JOHNATHAN D. TUNE,* IGOR V. EHRENBURG,† ELENA N. TKATCHOUK,† ROBERT T. MALLET,* AND H. FRED DOWNEY*

Department of Integrative Physiology, University of North Texas Health Science Center, Fort Worth, Texas 76107; and †Clinical Research Laboratory of Hypoxia Medical Academy, Moscow 123367, Russia

This investigation examined cardiac protective effects of normobaric intermittent hypoxia training. Six dogs underwent intermittent hypoxic training for 20 consecutive days in a normobaric chamber ventilated intermittently with N_2 to reduce fraction of inspired oxygen (F_{IO_2}) to 9.5%–10%. Hypoxic periods, initially 5 mins and increasing to 10 mins, were followed by 4-min normoxic periods. This hypoxia-normoxia protocol was repeated, initially 5 times and increasing to 8 times. The dogs showed no discomfort during intermittent hypoxic training. After 20 days of hypoxic training, the resistance of ventricular myocardium to infarction was assessed in an acute experiment. The left anterior descending (LAD) coronary artery was occluded for 60 mins and then reperfused for 5 hrs. At 30 mins of LAD occlusion, radioactive microspheres were injected through a left atrial catheter to assess coronary collateral blood flow into the ischemic region. After 5 hrs reperfusion, the heart was dyed to delineate the area at risk (AAR) of infarction and stained with triphenyl tetrazolium chloride to identify infarcted myocardium. During LAD occlusion and reperfusion, systemic hemodynamics and global left ventricular function were stable. Infarction was not detected in 4 hearts and was 1.6% of AAR in the other 2 hearts. In contrast, 6 dogs sham-trained in a chamber ventilated with compressed air and 5 untrained dogs subjected to the same LAD occlusion/reperfusion protocol had infarcts of 36.8% ± 5.8% and 35.2% ± 9.5% of the AAR, respectively. The reduction in infarct size of four of the six hypoxia-trained dogs could not be explained by enhanced collateral blood flow to the AAR. Hypoxia-trained dogs had no ventricular tachycardia or ventricular fibrillation. Three sham-trained dogs had ventricular tachycardia and two had ventricular fibrillation. Three untrained dogs had ventricular fibrillation. In conclusion, intermittent hypoxic training protects canine myocardium from infarction and life-threatening arrhythmias during coronary artery occlusion and reperfusion. The mechanism responsible for this potent cardioprotection merits further study. Exp Biol Med 229:806–812, 2004

Key words: cardiac protection; intermittent hypoxia; myocardial infarction; collateral blood flow

A lower incidence of myocardial infarction and mortality from coronary heart disease had been observed in populations living in areas of high altitude (1, 2). In 1966, Poupa *et al*. demonstrated cardioprotective effect of hypobaric hypoxia against isoproterenol-induced myocardial necrosis in rats (3), and in 1973, Meerson *et al*. reported that exposure to simulated high altitude for 5 hrs/day, 5 days/week, reduced the mortality rate of rats with coronary artery ligation by 84% and the size of myocardial infarction by 35% (4). Later, Meerson *et al*. reported that ischemia/reperfusion–induced ventricular arrhythmias were reduced and ventricular contractile function was better preserved in rats exposed to intermittent hypobaric hypoxia (5). In rats of widely varying ages, McGrath *et al*. demonstrated that cardiac resistance to anoxia was increased after exposure to intermittent hypobaric hypoxia (6). More recently, other studies have confirmed that intermittent hypobaric hypoxia is cardioprotective in rats (7–11). Xi *et al*. (12) and Cai *et al*. (13) examined ischemia/reperfusion injury in isolated perfused hearts of mice sacrificed 24 hrs after normobaric intermittent hypoxia. Both studies found that several cycles of hypoxia reduced myocardial infarction by about 50%. To date, however, no research has demonstrated cardioprotective effects of intermittent systemic hypoxia in a large animal.

There is increasing interest in intermittent hypoxia training (IHT) to improve exercise performance, enhance acclimatization to high altitude, and prevent and treat various illnesses (14–18). This training involves multiple cycles of brief (~5 mins), moderate hypoxia interspersed with normoxia, often on a daily basis for several weeks.

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[1] To whom correspondence should be addressed at Department of Integrative Physiology, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107-2699. E-mail: pzong@hsc.unt.edu

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Table 1. Intermittent Hypoxia Training Protocol[a]

Session	F_{IO_2} (%)	Hypoxia (mins)	Normoxia (mins)	Replications	Σ Hypoxia (mins)
1	10	5	4	5	25
2	10	5	4	6	30
3	10	5	4	7	35
4	10	5	4	8	40
5	10	5	4	8	40
6	9.5	6	4	7	42
7	9.5	6	4	8	48
8	9.5	6	4	8	48
9	9.5	7	4	7	49
10	9.5	8	4	7	56
11–20	9.5	10	4	7	70

[a] Replications = number of cycles of hypoxia/normoxia per daily session. Σ Hypoxia = total minutes of hypoxia per session. F_{IO_2}, fraction of inspired oxygen.

Because neither sojourns to high altitude nor hypobaric chambers are required for normobaric IHT, it can readily be implemented in the clinic. Considering the demonstrated cardioprotective effects of hypoxia in rodents, it seemed conceivable that a clinically relevant IHT protocol would be cardioprotective in dogs. Thus, the current investigation was designed to test this hypothesis. We found that IHT was remarkably effective in protecting canine hearts from infarction and arrhythmias due to coronary artery occlusion and reperfusion.

Materials and Methods

This investigation was approved by the institutional animal care and use committee and was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996). Seventeen adult mongrel dogs of either sex, free of clinically evident disease, were used for this study. Six dogs completed a 20-day IHT protocol and then were subjected to acute experimentation to assess cardiac responses to coronary artery occlusion and reperfusion. To provide control data, this acute experimentation was also performed on 6 dogs that had completed a 20-day sham IHT protocol and also on five untrained dogs.

Intermittent Hypoxia Training Protocol. Dogs were exposed to intermittent, normobaric hypoxia according to the protocol described in Table 1. Dogs were subjected to one session per day for 20 consecutive days. For this training, the dogs were placed in a Plexiglas chamber (interior dimensions: $114 \times 33 \times 71$ cm), and N_2 was introduced into the chamber to reduce fraction of inspired oxygen (F_{IO_2}) to the prescribed level (Table 1). Chamber O_2 was monitored with an Alpha Omega Instruments, Series 2000 O_2 analyzer (Cumberland, RI). The dogs showed no distress during hypoxic training. For sham IHT, the 20-day IHT protocol was followed, except instead of N_2, compressed air was introduced into the chamber to keep the F_{IO_2} at 20%.

Assessment of Protection Against Myocardial Infarction. *Surgical Procedures.* On the day following completion of the hypoxia or sham training protocols, the dogs were subjected to an acute myocardial ischemia/reperfusion experiment. Untrained dogs were also subjected to this acute experiment.

The dogs were fasted overnight and then anesthetized with sodium pentobarbital (30 mg/kg, iv). The dogs were intubated and mechanically ventilated with room air containing supplemental O_2. Arterial blood samples were collected at frequent intervals and analyzed for PO_2, PCO_2, and pH, which were kept within normal physiological limits by adjusting supplemental O_2, tidal volume, and respiratory rate. Supplemental pentobarbital was administered as needed to maintain stable anesthesia through a vinyl catheter positioned in a femoral vein. A saline-filled vinyl catheter was inserted into the thoracic aorta via a femoral artery to measure aortic pressure. In the other femoral artery, two Tygon catheters were placed to collect reference blood samples required for measuring coronary collateral flow with the radioactive microsphere technique (19). The heart was exposed through a left thoracotomy in the fifth intercostal space and suspended in a pericardial cradle. The left anterior descending (LAD) coronary artery was isolated near its origin, and a silk snare was passed around it. A Millar catheter-tip pressure transducer (Millar Instruments, Houston, TX) was inserted through the left atrium and advanced to the left ventricle to measure left ventricular pressure and dP/dt. Another vinyl catheter was positioned in the left atrium for injecting microspheres. Limb lead II of the electrocardiogram was recorded along with pressures and dP/dt on a Grass polygraph (Grass Medical Instruments, Quincy, MA). Body temperature was monitored with a hypodermic needle probe and maintained at 36.5°–37.5°C with a circulating H_2O heating pad.

When surgical preparations were complete and the animal stable, the LAD was occluded for 1 hr by tightening the snare and then allowed to reperfuse for 5 hrs after releasing the snare. Lidocaine (1.0 mg/kg, iv) was administered 1 min before LAD occlusion and 1 min before LAD reperfusion.

Hemodynamic and cardiac function variables were measured before and at the midpoint of the LAD occlusion

coincident with microsphere injection, at 60 mins of LAD occlusion, and at 1, 3, and 5 hrs of LAD reperfusion. At 5 hrs of reperfusion, heparin (500 U/kg, iv) was administered to facilitate coronary artery perfusion to demarcate the LAD perfusion territory at risk of infarction (see below).

Coronary Collateral Blood Flow Measurement. Because the extent of myocardial infarction is highly dependent on the amount of collateral flow, which varies among dogs, radioactive microspheres were injected at the midpoint of the LAD occlusion period to measure coronary collateral blood flow into the LAD region and in the normally perfused left circumflex region (19). The microspheres were agitated on a vortex mixer and in an ultrasonic bath for at least 15 mins before use. Microspheres (5 million; 15-μm diameter) labeled with ^{46}Sc, ^{85}Sr, or ^{141}Ce were injected into the left atrium followed by a gentle 10-ml saline flush. Beginning just before and continuing for 3 mins after microsphere injection, duplicate reference arterial blood samples were withdrawn from the thoracic aorta at a constant rate of 3 ml/min. Adequacy of microsphere mixing in the blood perfusate was verified by comparing radioactivities in the duplicate reference blood samples.

After slicing the ventricle and determining the area at risk (AAR) of infarction and the infarct size (see below), ventricular samples were cut from the central ischemic region and from the left circumflex region. Lateral border zones were excluded to avoid errors associated with measuring blood flow in samples of heterogeneous composition. The tissue samples were divided into endocardial, mid-myocardial, and epicardial thirds (~1 g each). Radioactivities of tissue and blood reference samples were measured in a Packard gamma counter (Packard Instrument Company, Meriden, CT). Blood flow in these tissue samples ($ml \cdot min^{-1} \cdot g^{-1}$) was calculated as previously described (19, 20).

Collateral flow in the AAR was evaluated in two ways. An average collateral flow was computed by averaging the endocardial and mid-myocardial flows of all samples of the AAR of each heart. This average collateral flow in the central region of the AAR has previously been used to evaluate cardioprotective interventions (21–24). A minimum collateral flow was also computed by averaging the endocardial and mid-myocardial flows in the slice of the AAR with the lowest collateral flow.

Determination of Myocardial Infarct Size (IS). The size of the AAR was determined with a dual-perfusion technique applied *in situ* (21, 25). The descending aorta and the brachiocephalic artery were ligated, and a large-bore cannula was advanced into the root of the aorta through the left subclavian artery. The LAD was cannulated at the site of occlusion. Small-bore catheters within the aortic and LAD cannulas were connected to pressure transducers, so aortic root and LAD pressures could be monitored during the dual-perfusion procedure. The aortic and LAD cannulas were connected to pressurized reservoirs containing 2.5% Evans blue dye and normal saline, respectively. The left and right ventricles were vented to atmospheric pressure by cannulas inserted through the apex of the heart. When these preparations were complete, the left and right coronary arteries were perfused from the aorta with saline containing Evans blue dye, whereas the LAD was perfused with saline alone. These solutions were infused simultaneously for 1–2 min at constant pressures of 85 mm Hg. This procedure delineated the ischemic area of the LAD perfusion territory at risk of infarction, as blue dye was excluded from this region. The heart was excised for measurements of infarct size and regional myocardial blood flow.

After excision of the atria and right ventricle, the left ventricle (LV) was frozen and stored overnight before being cut into four to six transverse slices approximately 1-cm thick. The weight of the ventricular slices was measured (LV), and then these slices were incubated in triphenyl tetrazolium chloride (1% w/v) in phosphate buffer (0.1 mol/l, pH 7.4) at 37°C for 20 mins, which imparts a deep red color to non-infarcted tissue (26). Undyed, infarcted tissue was resected and weighed, and then the remaining red tissue was cut away from the adjacent blue tissue and weighed. The weight of the red tissue plus the weight of the infarcted tissue equaled the AAR. IS/AAR and AAR/LV were computed.

Statistical Analyses. Values are expressed as mean ± SE. Hemodynamic data were analyzed with a two-way, repeated measures analysis of variance (ANOVA) to detect effects of (i) treatment (i.e, IHT, sham training, no training) and (ii) time period during the acute experimental protocol (i.e., baseline, 30 mins ischemia, 60 mins ischemia, 1 hr reperfusion, 3 hrs reperfusion, and 5 hrs reperfusion). Infarct size/area at risk of infarction, AAR/LV, regional coronary blood flow, and arterial hemoglobin and O_2 content were analyzed with completely randomized ANOVA to detect differences between IHT, sham training, and no training. When significance ($P < 0.05$) was detected by ANOVA, a Student-Newman-Keuls multiple comparison test was performed. Statistical procedures were performed with GB-Stat statistical software, version 9.0 (Dynamic Microsystems, Silver Spring, MD).

Results

Hemodynamic variables are presented in Table 2. Mean arterial pressure, heart rate, and global left ventricular function were stable during LAD occlusion and reperfusion. There were no significant differences in any hemodynamic variable between IHT group and sham-trained or untrained groups. Heart rate was elevated during the baseline condition due to the vagolytic action of sodium pentobarbital anesthesia and remained elevated throughout the experiment. Rate-pressure product, an index of myocardial oxygen consumption, was also similar among the groups. At the acute experiment following 20 days of hypoxic training, arterial hemoglobin and arterial O_2 content in IHT dogs were similar to those observed in sham-trained or untrained dogs (Table 3).

Table 2. Hemodynamic Data Measured During the Myocardial Ischemia/Reperfusion Protocol[a]

	Baseline	30 mins ischemia	60 mins ischemia	1 hr reperfusion	3 hrs reperfusion	5 hrs reperfusion	ANOVA treatment
Mean aortic pressure (mm Hg)							$P = 0.0797$
IHT ($n = 6$)	119 ± 8	119 ± 9	119 ± 7	100 ± 8	102 ± 8	98 ± 9	
Sham ($n = 6$)	133 ± 6	135 ± 5	133 ± 4	125 ± 4	120 ± 6	117 ± 6	
Untrained ($n = 5$)	119 ± 7	106 ± 9	114 ± 5	112 ± 6	112 ± 4	117 ± 6	
Heart rate (bpm)							$P = 0.3222$
IHT ($n = 6$)	143 ± 15	145 ± 14	149 ± 16	161 ± 5	171 ± 8	164 ± 5	
Sham ($n = 6$)	162 ± 6	169 ± 6	165 ± 5	165 ± 4	175 ± 3	177 ± 3	
Untrained ($n = 5$)	161 ± 9	164 ± 14	169 ± 11	170 ± 10	174 ± 8	180 ± 8	
Left ventricular pressure (mm Hg)							$P = 0.1975$
IHT ($n = 6$)	138 ± 8	138 ± 9	141 ± 7	127 ± 9	125 ± 10	129 ± 8	
Sham ($n = 6$)	144 ± 4	147 ± 2	143 ± 3	136 ± 4	137 ± 4	133 ± 5	
Untrained ($n = 5$)	126 ± 8	119 ± 9	126 ± 6	123 ± 3	129 ± 6	134 ± 5	
Left ventricular dP/dt_{max} (mm Hg/sec)							$P = 0.3473$
IHT ($n = 6$)	1707 ± 221	1847 ± 244	1828 ± 257	1765 ± 271	1796 ± 258	1790 ± 332	
Sham ($n = 6$)	2253 ± 216	2435 ± 193	2275 ± 166	2128 ± 116	2234 ± 137	2000 ± 127	
Untrained ($n = 5$)	2216 ± 235	1858 ± 368	2020 ± 248	1804 ± 149	1826 ± 92	1926 ± 88	
Rate pressure product (mm Hg × bpm × 10^{-3})							$P = 0.4854$
IHT ($n = 6$)	21.5 ± 2.5	21.8 ± 2.4	22.9 ± 2.1	20.6 ± 1.9	21.2 ± 2.3	21.1 ± 1.9	
Sham ($n = 6$)	23.3 ± 1.4	24.8 ± 1.1	23.6 ± 1.2	22.4 ± 0.9	23.9 ± 1.0	23.6 ± 1.2	
Untrained ($n = 5$)	20.3 ± 1.8	19.7 ± 2.6	21.3 ± 1.9	21.0 ± 1.3	22.5 ± 1.7	24.1 ± 1.4	

[a] Values are mean ± SE. ANOVA, analysis of variance; IHT, intermittent hypoxic trained; sham, sham trained.

Left anterior descending coronary artery occlusion produced clearly visible cyanosis in the myocardium distal to the occlusion. Ventricular premature contractions were observed in some dogs upon LAD occlusion and in all dogs upon LAD reperfusion. No cases of ventricular tachycardia or ventricular fibrillation (VF) occurred in the IHT dogs. In comparison, 5 out of 6 sham-trained dogs and 3 out of 5 untrained dogs developed ventricular tachycardia or VF during the same LAD occlusion/reperfusion protocol (Table 4). All cases of VF were successfully defibrillated, and the protocol was completed.

Figure 1 illustrates the infarct size and AAR determined after 5 hrs of reperfusion. In IHT dogs, 32% ± 2% of the left ventricle was ischemic, and sham-trained and untrained dogs had ischemic zones of 27% ± 3% and 30% ± 3%, respectively. No infarcted myocardium was detected in four IHT dogs. Two IHT dogs had infarcts weighing 0.5 g each, which was 1.6% of the AAR. In sham-trained and untrained dogs subjected to the same acute protocol, 36.8% ± 5.8% and 35.2% ± 9.5% of the AAR infarcted, respectively.

Coronary blood flow in the normally perfused, left circumflex (LC) region and in the AAR were computed from radioactivity resulting from tissue trapping of radioactive microspheres injected into the left atrium at 30 mins of LAD occlusion. Left circumflex flow did not differ significantly among the groups (Fig. 2). The average and minimum collateral flows in the AAR of all 6 hypoxia-trained dogs were 0.36 ± 0.16 $ml \cdot min^{-1} \cdot g^{-1}$ and 0.20 ± 0.09 $ml \cdot min^{-1} \cdot g^{-1}$, respectively. These mean values were affected by unusually high average collateral flows (>0.70 $ml \cdot min^{-1} \cdot g^{-1}$) of 2 IHT dogs, so mean collateral flows of the other 4 dogs were computed and are presented in Figure 2. For these four dogs, coronary collateral flow to the ischemic LAD region was similar to that observed in the ischemic region of six sham-trained and five untrained dogs.

The extent of myocardial infarction in this canine model of ischemia/reperfusion varies inversely with collateral flow, such that infarct size may be small even in the absence of a cardioprotective intervention (21–25). How-

Table 3. Arterial Blood Hemoglobin and O_2 Content Measured During the Myocardial Ischemia/Reperfusion Protocol[a]

	IHT ($n = 6$)	Sham trained ($n = 6$)	Untrained ($n = 5$)
Arterial hemoglobin (g/100 ml blood)	12.6 ± 0.4	13.8 ± 0.5	13.3 ± 0.7
Arterial O_2 content (ml O_2/100 ml blood)	16.6 ± 0.8	18.5 ± 0.6	17.6 ± 1.0

[a] Values are mean ± SE. IHT, intermittent hypoxic trained.

Table 4. Ventricular Arrhythmias Recorded During the Myocardial Ischemia/Reperfusion Protocol[a]

	PVC	VT	VF
IHT ($n = 6$)	6	0	0
Sham trained ($n = 6$)	6	3	2
Untrained ($n = 5$)	5	0	3

[a] PVC, premature ventricular contractions; VT, ventricular tachycardia; VF, ventricular fibrillation; IHT, intermittent hypoxic trained.

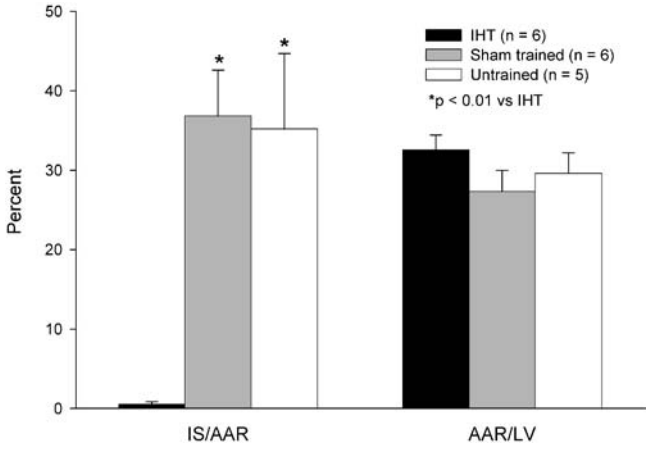

Figure 1. Left ventricular (LV) infarct size (IS) expressed as percentage of the area at risk (AAR), and AAR expressed as percentage of total LV mass.

ever, 4 IHT dogs had low average (0.12 ± 0.004 $ml \cdot min^{-1} \cdot g^{-1}$) and minimum ($0.075 \pm 0.018$ $ml \cdot min^{-1} \cdot g^{-1}$) collateral flows, so absence of significant infarction in these dogs cannot be explained by enhanced collateral flow. Figure 3 shows myocardial infarct size as a function of average (Panel A) and minimum (Panel B) collateral flows to the inner two-thirds of the ischemic myocardial wall, after excluding animals with average collateral flow >0.20 $ml \cdot min^{-1} \cdot g^{-1}$ (21–25). Using this criteria, Figure 3 compares data from four IHT dogs with those from five sham-trained dogs and from four untrained dogs. It is clear from Figure 3 that the degree of infarction in these four IHT dogs was much less than what would have been expected if intermittent hypoxia had conferred no cardioprotective effect.

Discussion

The major findings of this investigation are that IHT prevented significant myocardial infarction and lethal ventricular tachyarrhythmias during canine myocardial ischemia and reperfusion. This is the first report of cardioprotective effects of IHT in a large animal model.

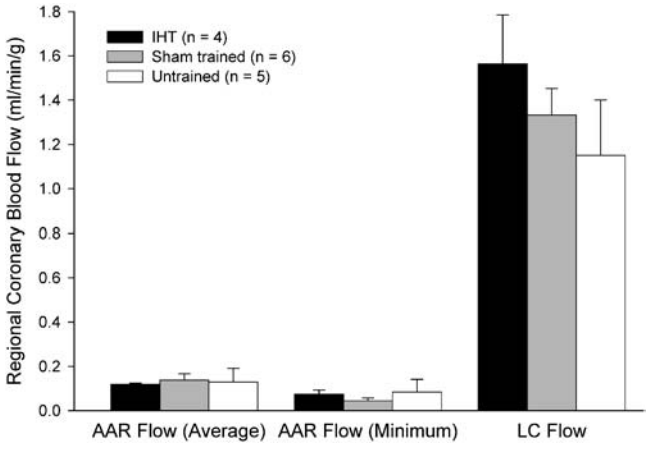

Figure 2. Coronary blood flow in the normally perfused left circumflex (LC) region and collateral blood flow in the left anterior descending (LAD) region. Data from two IHT dogs with high average collateral flow (>0.70 ml/min/g) are not included.

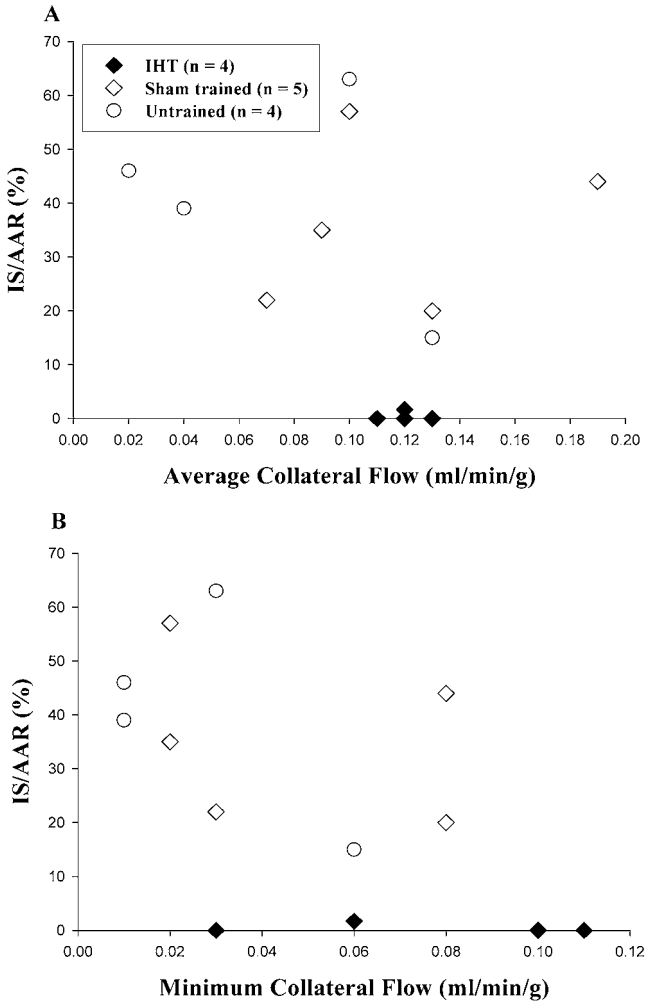

Figure 3. Myocardial infarct size is plotted as a function of average (Panel A) and minimum (Panel B) coronary collateral flow to the inner 2/3 of the ischemic myocardial wall. Each graph shows data from dogs with average collateral flow <0.20 ml/min/g.

Many investigations of interventions to protect ischemic myocardium have been stimulated by the observation in 1986 by Murry *et al*. that a brief period of acute ischemia reduced the extent of myocardial infarction resulting from subsequent, more prolonged ischemia (24, 27). In fact, the cardioprotective effect of hypobaric hypoxia had been reported many years earlier (3, 4, 28). Potentially beneficial effects of hypoxia for cardiac protection have received much less attention compared to ischemic preconditioning. This seems somewhat surprising because hypoxic exposure occurs normally at high altitude and can readily be accomplished in the laboratory or clinic. However, recently a hypobaric IHT protocol was employed to treat 46 patients with coronary heart disease and dyslipidemia; 37 patients were followed for 10 months, and none developed myocardial infarction (18).

To date, experimental investigations of cardioprotective

effects of simulated high altitude or intermittent normobaric hypoxia have been performed only in small animals (mice, rats, guinea pigs). Neckar *et al*. subjected rats to intermittent hypobaric exposures simulating 5000–7000 m altitude for 8 hrs/day, 5 days a week. After 24–32 exposures, the rats were anesthetized and subjected to 20–30 mins LAD occlusion followed by 4 hrs reperfusion. They found that adaptation of rats to intermittent hypobaric hypoxia decreased IS/AAR by 15%–25% (9, 10). It should be noted that in the current study, 20 days of intermittent normobaric hypoxic training produced more substantial protection against myocardial infarction in dogs than the protection observed in rodents adapted to more severe intermittent hypobaric hypoxia. Furthermore, the current study also indicates that IHT is effective in protecting canine myocardium from infarction when the duration of coronary artery occlusion has been extended to 60 mins compared with the 20–30 mins regional myocardial ischemia produced by Neckar *et al*. in rats (9, 10). However, it must be acknowledged that dogs have greater native coronary collateral flow than rats, and this factor could have contributed to the smaller infarcts observed in the current study.

Xi *et al*. found that 4 hrs acute normobaric systemic hypoxia (FIO_2 = 10%) protected isolated mice hearts from infarction when the hearts were subjected to ischemia/reperfusion 24 hrs after treatment (12). Similar findings were reported by Cai *et al*., who found this cardioprotection present at 24 hrs but not at 30 mins after hypoxia (13). We did not test the resistance of myocardium to ischemia immediately after IHT, but our results are consistent with the myocardial protection observed by others 1 day after IHT (9, 10, 12, 13). The results of Cai *et al*. (13) suggest that the protective mechanism activated by IHT may differ from that activated by ischemic preconditioning, because ischemic preconditioning can induce both early and delayed phases of resistance to ischemic injury (24, 27, 29, 30). The minimum duration of IHT required to produce significant protection against myocardial infarction and the duration of this protection in the canine model of ischemia/reperfusion remains to be determined.

It has been noted that adaptation to hypobaric hypoxia protects the rat heart against ischemic ventricular tachyarrhythmias (5, 7, 8, 10). Meerson *et al*. reported that the duration of extrasystole and VF induced by acute coronary ligation in conscious rats adapted to hypobaric hypoxia was decreased 2- to 3-fold compared to that of control rats (5). In open-chest rats exposed to intermittent hypobaric hypoxia, Neckar *et al*. observed no VF, compared with the 9.1% incidence of VF in normoxic control rats (10). In the current study, VF did not occur in any of the 6 IHT dogs subjected to 60 mins LAD occlusion and 5 hrs reperfusion. In contrast, two of six sham-trained dogs and three of five untrained dogs developed VF during the same acute experimental protocol. The apparent antiarrhythmic effect of IHT cannot be attributed to the cardioprotective effect of lidocaine (31), because the same dose of lidocaine was used in all animals during the acute experiment.

Acute hypoxia-induced myocardial protection of the canine heart has been reported by Shizukuda *et al*., who perfused the LAD of anesthetized dogs with severely hypoxic blood (<1 ml O_2/100 ml blood) for 5 mins in a protocol to mimic ischemic preconditioning. After 10 mins of normoxic perfusion, the LAD was then occluded for 1 hr and reperfused for 5 hrs, as in the current study. Infarct size in these hypoxic preconditioned hearts was 7.2% of the AAR compared to 22.4% in untreated control hearts (21). In the current study, 20 days of IHT was more cardioprotective than acute hypoxic preconditioning. Furthermore, Shizukuda *et al*. found that acute hypoxic preconditioning provided no protection against VF (21). Therefore, the protective mechanism activated acutely by hypoxic preconditioning may differ from that activated by more prolonged IHT.

As with ischemic preconditioning, there is currently no definitive mechanism to explain intermittent hypoxia-induced cardioprotection. Kolar reviewed putative mechanisms of hypoxic adaptation of myocardium (32). These mechanisms include altered (i) myocardial vascularity and coronary blood flow, including collateral flow, (ii) blood hematocrit and hemoglobin content, (iii) myocardial myoglobin concentration, (iv) energy metabolism, (v) neurohumoral factors, (vi) antioxidant enzymes, (vii) stress proteins, (viii) prostaglandins, and (ix) adenosine release. Recently, Asemu *et al*. (7), Neckar *et al*. (9), and Zhu *et al*. (11) reported evidence that ATP-dependent potassium channels are involved in hypoxia-mediated cardioprotection. Xi *et al*. demonstrated that the infarct-limiting effect of acute systemic hypoxia is triggered and mediated by inducible nitric oxide synthase but not by endothelial nitric oxide synthase or cyclooxygenase-2 (12). Cai *et al*. found that erythropoietin protected rodent hearts in a manner similar to intermittent hypoxia, and that this protection was critically dependent on activation of hypoxia-inducible factor 1 (13). Thus, redundant mechanisms may be involved in the cardioprotection conferred by IHT, and more research is required to further clarify the contributions of these and possibly other mechanisms.

Data from this study do permit comment on two potential protective mechanisms. First, the hemoglobin and arterial O_2 contents of IHT dogs were not different from those of sham-trained and untrained dogs, so the amount of O_2 transported in blood flowing through collateral vessels was not enhanced by IHT. These results do not exclude a role for erythropoietin but suggest that its effect would have been independent of its stimulation of red blood cell production. Second, augmented coronary collateral flow is not required for IHT-induced cardioprotection, as essentially no infarction occurred in four IHT dogs that had very low collateral flow (Fig. 1). However, we did not measure collateral flow prior to IHT, so we cannot exclude an effect of IHT on collateral vessel development.

In summary, 20 consecutive days of IHT provided remarkable protection against myocardial infarction and ventricular tachyarrhythmias in a canine model of 60 mins

coronary artery occlusion and 5 hrs reperfusion. This cardioprotection did not result from increased arterial O_2 carrying capacity or increased coronary collateral blood flow.

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1. Hurtado A. Some clinical aspects of life at high altitudes. Ann Intern Med 53:247–258, 1960.
2. Mortimer EA, Monson RR, MacMahon B. Reduction in mortality from coronary heart disease in men residing at high altitude. N Engl J Med 296:581–585, 1977.
3. Poupa O, Krofta K, Prochazka J, Turek Z. Acclimation to simulated high altitude and acute cardiac necrosis. Fed Proc 25:1243–1246, 1966.
4. Meerson FZ, Gomzakov OA, Shimkovich MV. Adaptation to high altitude hypoxia as a factor preventing development of myocardial ischemic necrosis. Am J Cardiol 31:30–34, 1973.
5. Meerson FZ, Ustinova EE, Orlova EH. Prevention and elimination of heart arrhythmias by adaptation to intermittent high altitude hypoxia. Clin Cardiol 10:783–789, 1987.
6. McGrath JJ, Prochazka J, Pelouch V, Ostadal B. Physiological response of rats to intermittent high altitude stress: effect of age. J Appl Physiol 34:289–293, 1973.
7. Asemu G, Papousek F, Ostadal B, Kolar F. Adaptation to high altitude hypoxia protects the rat heart against ischemia-induced arrhythmias. Involvement of mitochondrial K_{ATP} channel. J Mol Cell Cardiol 31:1821–1831, 1999.
8. Asemu G, Neckar J, Szarszoi O, Papousek F, Ostadal B, Kolar F. Effects of adaptation to intermittent high altitude hypoxia on ischemic ventricular arrhythmias in rats. Physiol Res 49:597–606, 2000.
9. Neckar J, Szarszoi O, Koten L, Papousek F, Ostadal B, Grover GJ, Kolar F. Effects of mitochondrial K_{ATP} modulators on cardioprotection induced by chronic high altitude hypoxia in rats. Cardiovasc Res 55:567–575, 2002.
10. Neckar J, Papousek F, Novakova O, Ostadal B, Kolar F. Cardioprotective effects of chronic hypoxia and ischaemic preconditioning are not additive. Basic Res Cardiol 97:161–167, 2002.
11. Zhu HF, Dong JW, Zhu WZ, Ding HL, Zhou ZN. ATP-dependent potassium channels involved in the cardiac protection induced by intermittent hypoxia against ischemia/reperfusion injury. Life Sci 73:1275–1287, 2003.
12. Xi L, Tekin D, Gursoy E, Salloum F, Levasseur JE, Kukreja RC. Evidence that NOS2 acts as a trigger and mediator of late preconditioning induced by acute systemic hypoxia. Am J Physiol 283:H5–H12, 2002.
13. Cai Z, Manalo DJ, Wei G, Rodriguez ER, Fox-Talbot K, Lu H, Zweier JL, Semenza GL. Hearts from rodents exposed to intermittent hypoxia or erythropoietin are protected against ischemia-reperfusion injury. Circulation 108:79–85, 2003.
14. Roach RC. Hypoxia: From genes to the bedside. New York: Kluwer Academic/Plenum Publishers, pp377–399, 2001.
15. Levine BD. Intermittent hypoxic training: fact and fancy. High Alt Med Biol 3:177–193, 2002.
16. Powell FL, Garcia N. Physiological effects of intermittent hypoxia. High Alt Med Biol 1:125–136, 2000.
17. Boning D. Altitude and hypoxia training – a short review. Int J Sports Med 18:565–570, 1997.
18. Tin'kov AN, Aksenov VA. Effects of intermittent hypobaric hypoxia on blood lipid concentrations in male coronary heart disease patients. High Alt Med Biol 3:277–282, 2002.
19. Heymann MA, Payne BD, Hoffman JIE, Rudolph AM. Blood flow measurements with radionuclide-labeled particles. Prog Cardiovasc Dis 20:55–79, 1977.
20. Zong P, Sun W, Setty S, Tune JD, Downey HF. α-Adrenergic vasoconstrictor tone limits right coronary blood flow in exercising dogs. Exp Biol Med 229:312–322, 2004.
21. Shizukuda Y, Mallet RT, Lee SC, Downey HF. Hypoxic preconditioning of ischaemic canine myocardium. Cardiovasc Res 26:534–542, 1992.
22. Reimer KA, Jennings RB, Cobb FR, Murdock RH, Greenfield JC Jr, Becker LC, Bulkley BH, Hutchins GM, Schwartz RP Jr, Bailey KR, Passamani ER. Animal models for protecting ischemic myocardium: results of the NHLBI cooperative study. Comparison of the unconscious and conscious dog models. Circ Res 56:651–665, 1985.
23. Murry CE, Richard VJ, Jennings RB, Reimer KA. Myocardial protection is lost before contractile function recovers from ischemic preconditioning. Am J Physiol 260:H796–H804, 1991.
24. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74:1124–1136, 1986.
25. Li GC, Vasquez JA, Gallagher KP, Lucchesi BR. Myocardial protection with preconditioning. Circulation 82:609–619, 1990.
26. Nachlas MM, Shnitka TK. Macroscopic identification of early myocardial infarcts by alterations in dehydrogenase activity. Am J Pathol 42:379–405, 1963.
27. Reimer KA, Murry CE, Yamasawa I, Hill ML, Jennings RB. Four brief periods of myocardial ischemia cause no cumulative ATP loss or necrosis. Am J Physiol 251:H1306–H1315, 1986.
28. McGrath JJ, Bullard RW. Altered myocardial performance in response to anoxia after high-altitude exposure. J Appl Physiol 25:761–764, 1968.
29. Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. Physiol Rev 83:1113–1151, 2003.
30. Tajima M, Katayose D, Bessho M, Isoyama S. Acute ischaemic preconditioning and chronic hypoxia independently increase myocardial tolerance to ischaemia. Cardiovasc Res 28:312–319, 1994.
31. Dobson GP, Jones MW. Adenosine and lidocaine: a new concept in nondepolarizing surgical myocardial arrest, protection, and preservation. J Thorac Cardiovasc Surg 127:794–805, 2004.
32. Kolar F. Cardioprotective effects of chronic hypoxia: relation to preconditioning. In: Wainwright CL, Parratt JR, Eds. Myocardial Preconditioning. Austin, Tex: RG Landes Co., pp261–275, 1996.