

Rat Strains Bred for Low and High Aerobic Running Capacity Do Not Differ in Their Survival Time to Hemorrhage

HAROLD G. KLEMCKE,^{*,1} KATHY L. RYAN,* STEVEN L. BRITTON,† LAUREN G. KOCH,†
MICHAEL A. DUBICK,* AND VICTOR A. CONVERTINO*

**U.S. Army Institute of Surgical Research, Fort Sam Houston, TX 78234; and †Department of Physical Medicine and Rehabilitation, University of Michigan, Ann Arbor, MI 48109*

Hemorrhagic shock reflects low tissue perfusion that is inadequate to maintain normal metabolic functions. Often associated with this condition are impairments in cellular oxygen delivery and utilization. Rat strains divergent in their running endurance have been artificially selected over 12 generations. As these rats bred for high (HCR) vs low (LCR) aerobic running capacity have greater tissue O₂ utilization capacity and improved cardiovascular function, we hypothesized that HCR would be more tolerant (i.e., have greater survivability) to the global ischemia of hemorrhagic shock than LCR. To address this hypothesis, survival time to a severe—as substantiated by dramatic changes in plasma lactate, HCO₃, and base deficit—controlled hemorrhage was measured. Male rats were catheterized and, ~24 h later, an estimated >35% of the calculated blood volume was removed during a 26-min period while the rats were conscious and unrestrained. Rats were observed for 6 h or until death. Contrary to our hypothesis, survival time in HCR (220 ± 63 min; *n* = 6) did not differ statistically (*P* = 0.46) from that in LCR (279 ± 53 min; *n* = 7). Similarly, there were no statistical differences (*P* ≥ 0.08) between rat lines in blood pH, lactate, HCO₃, and base deficit pre- or post-hemorrhage. In addition, few significant differences between lines in response to hemorrhage were detected by measures of cellular antioxidant status in heart, liver, or lung.

Since animals with genetically greater tissue oxygen utilization capacity failed to show longer survival times, our results suggest that other mechanisms must play a more dominant role in determining survivability to hemorrhage under conditions of this hemorrhage. *Exp Biol Med* 234:1503–1510, 2009

Key words: aerobic capacity rats; hemorrhagic shock; differential survival

Introduction

Hemorrhagic shock has been defined as “an abnormality of the circulatory system that results in inadequate organ perfusion and tissue oxygenation” (1) and results in an inability to maintain cellular metabolic functions (2). Subsequent to hemorrhagic shock, there is a continuum of biological responses that involves activation of hemodynamic and cellular processes in attempts to maintain homeostasis (3). A major component of these responses is the hemorrhage-induced hypoxia that cells experience as the blood loss and associated reduced tissue perfusion progress (3). Indeed, oxygen debt—when tissue O₂ requirements exceed O₂ availability (4)—has been shown to be strongly associated with a high probability of death after hemorrhage in dogs (5) and pigs (6), as is extraction efficiency in humans (7). In addition to cellular hypoxia, compromised cardiac output resulting from reduced central blood volume also contributes critically to decreased oxygen delivery to the tissue (3). However, it is unclear if individuals with larger cardiac outputs and/or tissue aerobic capacity would demonstrate greater survival time to hemorrhage.

Recently, two strains of rats have been selectively bred based on their aerobic running endurance (8, 9). These rats are sedentary during their lives, with the exception of one week for testing their aerobic running capacity. Hence, differences between the high- (HCR) and low- (LCR) capacity runners are intrinsic rather than due to effects of exercise training. In the most current literature, 16 generations of selection and breeding have been accom-

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¹ To whom correspondence should be addressed at U.S. Army Institute of Surgical Research, 3400 Rawley E. Chambers Avenue, Fort Sam Houston, TX 78234. E-mail: harold.klemcke@amedd.army.mil

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plished and reported (10). At generation 3, the cardiac output measured in an isolated heart preparation was 49% greater in HCR *vs* LCR, but HCR and LCR did not differ in resistance to myocardial ischemia (11). By generation 7, maximal oxygen consumption ($\text{Vo}_{2\text{max}}$) was 12% greater during normoxia and 20% greater during hypoxia in the HCR *vs* LCR (12). This enhanced $\text{Vo}_{2\text{max}}$ resulted from a higher capacity for O_2 transfer at the tissue level (a composite measure of all processes involved in movement of O_2 from capillaries to the mitochondria) rather than differences in cardiac output, and also occurred in the absence of differences in oxygen delivery. By generation 15, $\text{Vo}_{2\text{max}}$ was $\sim 40\%$ greater in HCR *vs* LCR, due to both increased oxygen delivery—resulting from an enhanced stroke volume—and oxygen tissue transfer (13). Such data strongly suggest that for the generation 12 rats used in the current study, similar trends for differences in oxygen consumption and cardiac function would exist.

We recently demonstrated 8-fold differences in mean survival time to a controlled hemorrhage among 15 inbred strains of rats (14). As part of that larger study, and in an anticipatory effort to identify mechanisms that might be important determinants of any measured differential survival time to hemorrhage, we took advantage of the intrinsic differences in aerobic capacity inherent in the HCR and LCR lines by comparing survival time of HCR and LCR lines to the same controlled hemorrhage. We hypothesized that a strain with improved $\text{Vo}_{2\text{max}}$ (i.e., HCR) would demonstrate an improved survival time to hemorrhage-induced hypoxia because of the enhanced cardiovascular function and/or tissue O_2 utilization capacity that have been thoroughly documented in both previous and subsequent generations of the HCR rat line. As hemorrhagic shock has been associated with generation of reactive oxygen and nitrogen species that have been linked to organ dysfunction (15, 16), we also examined indices of antioxidant status in heart, liver, and lung from these 2 strains of rats.

Materials and Methods

Animals. All rats were maintained in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. This study was approved by the Institutional Animal Care and Use Committee of the U.S. Army Institute of Surgical Research, Fort Sam Houston, TX. Animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 86-23, revised 1996). All rats were obtained from colonies maintained at the University of Michigan that had been artificially selected for intrinsic aerobic running capacity for 12 generations (17). All rats (males) were from generation 12, and were used at ~ 15 weeks of age. They were maintained individually in plastic cages ($27.3 \times 48.9 \times 27.3$ cm) at $19\text{--}23^\circ\text{C}$, with lights on from 06:00 to 18:00 h, and food (Harlan Global Teklad 2018; Madison, WI) and

water constantly available. Rats were randomly assigned to day of surgery, order of surgery on each day, and order of hemorrhage on each day. Eight rats from each line entered the study. However, results from 2 HCR rats and 1 LCR rat were not included in the analysis due to: 1) excessive blood loss during surgery (1.92 ml) and partial leg paralysis 24 h prior to the intended hemorrhage (1 HCR); 2) accidental disconnection of the catheter extension just prior to hemorrhage with excessive loss of blood (~ 1 ml) and additional perceived stress associated with reconnection of the extension (1 HCR); and 3) abnormal baseline values for blood lactate, base excess, and HCO_3^- just prior to hemorrhage concomitant with a low (36 mm Hg) mean arterial pressure (1 LCR).

Surgical Procedures. All surgical procedures were conducted under aseptic conditions, as previously described in detail (14). Prior to surgery, body weight and the weight of food and water provided to the rat for the next 24 h were recorded. Rats were anesthetized with 2–5% isoflurane (Forane, Baxter Healthcare Corporation, Deerfield, IL) in 100% oxygen. The left common carotid artery was catheterized, exteriorized in the intrascapular region, filled with sterile glycerol, and sealed, as previously described (14). Rats were injected with buprenorphine (2.5 $\mu\text{g}/100$ g body weight, s.c.) and with 10 ml of 0.9% saline (s.c.) to provide hydration during recovery.

Hemorrhage Procedures. Approximately 24 h later, the rats as well as the remaining food and water were weighed. A controlled, conscious hemorrhage of unrestrained rats breathing room air was conducted. Briefly, the rat was restrained while a tubing extension was attached to the indwelling catheter. The rat was released into a standard rat cage, and the extension tubing was connected to a transducer (Deltrans IV, Utah Medical Products, Midvale, UT) that in turn was connected to a Differential DC Amplifier and Signal Conditioner (Ectron Corp, San Diego, CA). Blood pressure was measured and recorded at 5-sec intervals in the freely moving rat. Baseline measures were recorded for 5 min before the transducer was disconnected and the rat was subjected to hemorrhage through the same catheter. The rat's blood volume was calculated using the stable body weight just prior to surgery and the figure of 5.83 ml/100 g of body weight, as used previously (14). Fifty-five percent of this calculated blood volume was then removed: 25% of the blood volume to be removed was withdrawn at a constant rate during the first 4 min, while the remaining 75% of the blood volume to be removed was withdrawn at a constant rate during the next 22 min (14). The first (initiation or "pre" sample) and last (termination or "post" sample) 0.5 ml of the total blood volume removed were used for measures. At the end of hemorrhage, total blood loss was obtained by weight. When rats displayed agonal breathing, cessation of breathing, or reached 6 h post-initiation of hemorrhage, they were euthanized with an intravascular injection of sodium pentobarbital (15 mg/100 g

body weight). Blood samples were analyzed using an AVL Omni 1–9 Blood gas analyzer (Basel, Switzerland).

Antioxidant Measures. Lung, liver, and heart tissues were removed from rats at death, rapidly frozen in liquid nitrogen, and stored at -80°C until subsequent analysis. Tissues were homogenized in 50 mM potassium phosphate buffer at pH 7.4. Thiobarbituric acid reactive substances (TBARS), expressed as nanomoles of malondialdehyde per mg of protein, were determined in the butanol phase, as described by Naito et al. (18). Total antioxidant capacity (FRAP) was determined spectrophotometrically by evaluating the iron-reducing capacity of the tissue (19). Antioxidant enzyme activities, glutathione peroxidase (GP), glutathione reductase (GR), superoxide dismutase (SOD), and catalase were determined spectrophotometrically, as previously described (20). Reduced glutathione was determined spectrophotometrically using the enzymatic assay described by Anderson (21). Myeloperoxidase activity in lung, as an index of neutrophil infiltration, was determined by a modification of the method of Trush et al. (22) using o-dianisidine as substrate. Total nitrates/nitrites as an estimate of nitric oxide concentration in tissues was determined by a commercial assay (Stressgen, Assay Design, Ann Arbor, MI). Arginase activity in liver was determined, as previously described (23). Protein concentrations were determined with a commercial kit (BioRad Laboratories, Richmond, CA).

Statistics. Data were analyzed using the Statistical Analysis System package (SAS; Cary, NC). Surgical and bleeding-associated measures were analyzed using a single-way analysis of variance (ANOVA). Differences among individual means were examined using the *a posteriori* Student-Newman-Keuls test. Blood variables measured at the beginning and at the end of hemorrhage (e.g., pH, pCO_2 , pO_2 , and lactate) were analyzed using a two-way ANOVA with repeated measures. Means separation tests were conducted using robust, orthogonal contrasts and the conservative *t*-test with Bonferroni adjustment for multiple comparisons. Because antioxidant measures were made in tissues taken at multiple times (based on time of death) after initiation of the hemorrhage, these data were analyzed using a two-way ANOVA, wherein the factors of rat strain (two levels; HCR vs LCR) and time of death (two levels; prior to 360 min vs 360 min) were included. All data were tested for homogeneity of variance (Levene's test) and normality of distribution with the Kolmogorov-Smirnov test. Data were transformed where necessary to meet assumptions of ANOVA. Differences in percent of rats surviving were examined using the Fisher's exact test. Both rat lines had rats that survived the complete 6 h and were euthanized. The true survival time of these rats is unknown, and such data are therefore said to be "censored." These survival data with censored observations were analyzed using the Kaplan-Meier procedure for estimating survivor functions and log-rank test for determining differences among survivor functions was used to compare these rat lines without

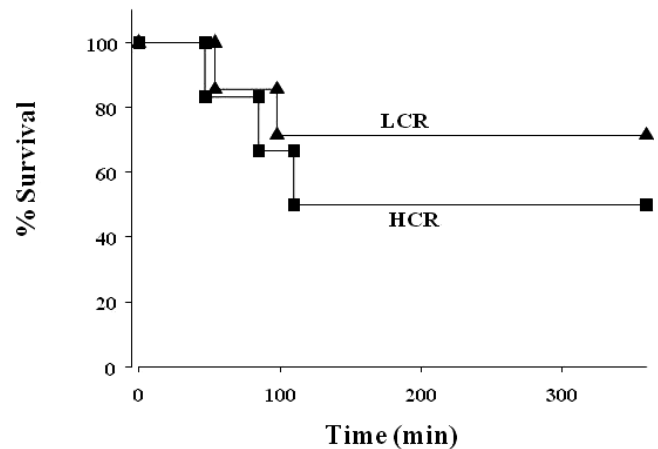


Figure 1. Kaplan-Meier survival curves for high running capacity (HCR, $n = 6$) and low running capacity (LCR, $n = 7$) rats. There were no differences between the two rat lines in their survival responses ($P = 0.46$).

covariates. The Cox regression and its associated proportional hazards model (24) were used with covariates. All covariates considered were initially included in the overall model but then, through an iterative process, covariates that were found not to be significant were dropped from the model. Similarly, interactions between each significant covariate and rat lines were tested and retained in the final model if found to be significant. Validity of the proportional hazard assumption was examined by testing the interaction of the covariates with time (24). Data are presented as arithmetic means $\pm$ SEM. Survival times and their SEM are underestimates due to censored data; however, statistical procedures noted above allow for correct interpretation of such data.

Results

Mean survival times were 220 ± 63 and 279 ± 53 min for HCR and LCR, respectively. Kaplan-Meier survival graphs and associated log-rank test indicate statistically indistinguishable responses ($P = 0.46$) throughout the 6-h observational period, whether or not covariates were considered (Fig. 1). No differences were present ($P = 0.59$) in percent survival between HCR (46.2%) and LCR (53.9%) rats.

Duration of surgery (47.8 ± 2.4 vs 49.1 ± 1.4 min, mean $\pm$ SEM, HCR vs LCR, $P = 0.64$), blood loss during surgery ($.07 \pm .02$ vs $.08 \pm .03$ ml/100 grams of body weight, $P = 0.83$), time interval between surgery and hemorrhage (23.4 ± 0.4 vs 23.6 ± 0.5 h, $P = 0.83$), time period of restraint prior to hemorrhage (4.5 ± 0.3 vs 4.4 ± 0.2 min, $P = 0.86$), and measured percent hemorrhage volume (54.8 ± 0.5 vs $54.8 \pm 0.4\%$, $P = 0.94$) did not differ between HCR and LCR. When tested as covariates for survival time in rat-line comparisons using the Cox proportional hazard model, these variables were found not

Table 1. Independent, Rat Line–Related (HCR vs LCR) Variables Potentially Related to Survival Time to Hemorrhage^a

Measure	Rat line		ANOVA comparison of rat lines
	HCR (<i>n</i> = 6)	LCR (<i>n</i> = 7)	
BW 24 h prior to surgery (grams)	312 ± 14	357 ± 8	<i>P</i> = 0.02
BW at surgery (grams)	318 ± 11	366 ± 9	<i>P</i> < 0.01
BW at hemorrhage (grams)	296 ± 12	361 ± 7	<i>P</i> < 0.01
Weight change during 24 h prior to surgery as percent BW	+2.2 ± 1.1	+2.7 ± 2.7	<i>P</i> = 0.72
Weight change between surgery and bleed as percent BW at surgery	−4.4 ± 0.9	−1.6 ± 0.8	<i>P</i> = 0.03
Water consumed (grams) during 24 h prior to surgery per 100 gram body weight (BW)	4.5 ± 0.7	5.1 ± 0.5	<i>P</i> = 0.53
Water consumed (grams) post surgery per 100 grams BW	1.9 ± 0.6	3.5 ± 0.2	<i>P</i> = 0.03
Food consumed (grams) during 24 h prior to surgery per 100 grams BW	4.4 ± 0.4	4.3 ± 0.2	<i>P</i> = 0.79
Food consumed (grams) post surgery per 100 grams BW	3.6 ± 0.8	5.0 ± 0.3	<i>P</i> = 0.12

^a Results of rat line comparisons by analysis of variance (ANOVA) for the various measures are presented. BW, body weight; HCR, high capacity runners; LCR, low capacity runners.

to be significant and were subsequently dropped from the analytical model. Hence, survival analysis in the absence of covariates was considered the most appropriate statistical model.

Some additional intrinsic variables (Table 1) showed line-dependent differences (*P* < 0.05). HCR rats weighed less than LCR rats at a comparable age (14.7 weeks). In the 24 h prior to surgery, HCR and LCR consumed comparable quantities of water and food per 100 g body weight (Table 1). Subsequent to surgery, HCR drank less water and lost more body weight than did LCR (Table 1). While HCR took longer than LCR to recover (regain consciousness) from the surgical anesthetic (14.7 ± 2.4 vs 5.4 ± 1.1 min; *P* < 0.01), anesthetic recovery in both strains occurred >23 h before the hemorrhage.

Evaluation of variables that reflect a mixture of hematological, respiratory, metabolic, and cardiovascular functions indicates an absence of differences between the rat lines for most variables at either the initiation or termination of the bleed 26 min later (Table 2). There were consistent marked changes in most variables between initial and final measures. In general, these changes between the initial and final values (pre vs post) were not different between strains. Exceptions to these broad statements include: 1) arterial plasma K⁺ concentration ([K⁺]_a) was greater in LCR than in HCR at the end of the bleed; 2) arterial plasma glucose concentrations were greater (*P* = 0.023) in HCR than in LCR at the beginning of the bleed; 3) plasma pH (for both HCR and LCR) and ([K⁺]_a) in HCR remained constant during the time periods measured; 4) changes in base deficit were greater in HCR vs LCR (14.6 ± 1.5 vs 11.1 ± 0.6 mmol/l; *P* = 0.048). It is noteworthy that hematocrit was not different between rat lines at either measurement period, but decreased 25% in both rat lines between the beginning and the end of hemorrhage (Table 2).

Table 2. Arterial Blood Measures During Hemorrhage in Rat Lines Selected for High Running Capacity Running (HCR) and Low Running Capacity (LCR)^a

Variable	Rat line	Initial sample (pre)	Final sample (post)
pO ₂ (mm Hg)	HCR	89 ± 1	136 ± 4*
	LCR	92 ± 2	126 ± 6*
Hb (g/dl)	HCR	12.7 ± 0.5	10.3 ± 0.6*
	LCR	11.8 ± 0.3	9.9 ± 0.4*
Hct (%)	HCR	41.1 ± 1.5	30.5 ± 0.7*
	LCR	39.1 ± 1.1	29.4 ± 0.8*
O ₂ content (ml %)	HCR	16.7 ± .8	13.3 ± 0.7*
	LCR	15.5 ± .4	12.6 ± .5*
pCO ₂ (mm Hg)	HCR	36.8 ± 1.4	15.5 ± 2.0*
	LCR	37.2 ± 1.0	18.2 ± 1.5*
Lactate (mmol/l)	HCR	2.0 ± .3	15.5 ± .8*
	LCR	2.1 ± 0.3	14.7 ± .5*
HCO ₃ (mmol/l)	HCR	26.4 ± 1.2	10.5 ± 1.7*
	LCR	25.3 ± .4	12.5 ± 0.8*
pH	HCR	7.47 ± .01	7.43 ± .04
	LCR	7.45 ± .01	7.46 ± .02
Base deficit (mmol/l)	HCR	−2.9 ± 1.0	11.7 ± 2.1*
	LCR	−1.4 ± .4	9.5 ± .7*
Glucose (mmol/l)	HCR	11.7 ± 0.7†	24.5 ± 1.4*
	LCR	9.8 ± .3†	22.6 ± 1.8*
K ⁺ (mmol/l)	HCR	4.0 ± 0.1	4.5 ± 0.3†
	LCR	3.9 ± 0.1	4.8 ± 0.4*,†
MAP (mm Hg)	HCR	128 ± 7	89 ± 3*
	LCR	115 ± 5	91 ± 15*

^a For most variables and for both initial and final samples, there were no differences between HCR and LCR lines. Blood pH was not different between LCR and HCR, nor between pre and post measures. MAP measures were taken 1 min prior to initiation of hemorrhage and 1 min after termination of hemorrhage.

* Variables within a rat line were different between pre and post measures (*P* < 0.05).

† Variables were different between strains at the observational period noted (*P* < 0.05).

Table 3. Antioxidant Measures in Heart, Liver, and Lung from High (HCR) and Low (LCR) Aerobic Running Capacity Rats

Antioxidant Measure	Organ					
	Heart			Lung		
	HCR (n)	LCR (n)	P	HCR (n)	LCR (n)	P
Antioxidant capacity ($\mu\text{mol}/\text{mg protein}$)	.011 $\pm$.001 (6)	.015 $\pm$.001 (7)	0.0049	.018 $\pm$.001 (6)	.022 $\pm$.002 (7)	0.003
TBARS (units/ml)	.039 $\pm$.002 (6)	.037 $\pm$.003 (7)	0.39	.794 $\pm$.056 (6)	.535 $\pm$.064 (7)	0.0224
Mn-SOD (units/mg protein)	1.313 $\pm$.078 (6)	1.274 $\pm$.096 (7)	0.74	8.393 $\pm$.582 (6)	8.125 $\pm$.592 (7)	0.74
CuZn-SOD (units/mg protein)	.850 $\pm$.126 (6)	1.141 $\pm$.136 (7)	0.18	14.179 $\pm$ 1.646 (6)	12.338 $\pm$ 1.013 (7)	0.26
Glutathione reductase (units/mg protein)	.024 $\pm$.002 (6)	.024 $\pm$.002 (6)	0.53	.087 $\pm$.006 (6)	.074 $\pm$.004 (6)	0.09
Glutathione peroxidase (units/mg protein)	.131 $\pm$.008 (6)	.121 $\pm$.008 (6)	0.63	.068 $\pm$.008 (6)	.064 $\pm$.005 (6)	0.35
Catalase (units/mg protein)	6.791 $\pm$.292 (6)	5.121 $\pm$.492 (6)	0.048	352.1 $\pm$ 66.9 (6)	342.5 $\pm$ 50.0 (6)	0.84
Nitric oxide (nmol/mg protein)	8.913 $\pm$.581 (6)	8.487 $\pm$ 1.249 (6)	0.72	34.7 $\pm$ 4.1 (6)	47.4 $\pm$ 3.0 (6)	0.018
Reduced glutathione ($\mu\text{mol}/\text{mg protein}$)	.184 $\pm$.009 (6)	.154 $\pm$.010 (6)	0.038	1.226 $\pm$.163 (4)	1.133 $\pm$.043 (4)	0.43
Arginase (units/mg protein)	—	—	—	1440 $\pm$ 102	1318 $\pm$ 120	0.54
				—	—	—

TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase.

In heart and liver, total antioxidant capacity (FRAP) was 36% and 22% higher, respectively, in LCR than HCR rats (Table 3). In liver, this was associated with 48% higher TBARS levels, an index of lipid peroxidation, in the HCR rats compared with the LCR rats. Heart catalase activity and GSH levels were significantly lower in LCR than HCR rats. In contrast, none of the antioxidant status variables was significantly different between groups in lung. Also, lung myeloperoxidase activity, as an index of neutrophil infiltration, was not different between groups (data not shown).

Discussion

Significant reductions in blood O_2 content and HCO_3^- , with increased lactate and base deficit, substantiate that our hemorrhage model resulted in a tissue hypoxia typical of that observed in circulatory shock (3). Contrary to our hypothesis, we found that survival time and most blood measurements associated with tissue metabolic responses to hemorrhage did not differ between HCR and LCR rats at the time period measured. Thus, a major and unique finding of this study is that, for these rat strains, physiological mechanisms not specifically related to systemic delivery and tissue utilization of O_2 are important determinants of survival after this degree of hemorrhage.

Our original hypothesis evolved from a number of previously documented observations. First, marked increases of $\text{Vo}_{2\text{max}}$ in HCR vs LCR were present in generations 7 (12) and 15 (13), which bracket the rats used in the current study (generation 12). Second, maximal cardiac output was 24% (generation 7) and 28% (generation 15) greater in HCR vs LCR under hypoxic conditions (which would be applicable to hemorrhage), and was commensurate with 21% and 30% increases in $\text{Vo}_{2\text{max}}$ (12, 13). At generation 7, this increased $\text{Vo}_{2\text{max}}$ was accompanied by a 32% increase in the number of capillaries per unit mass of skeletal muscle, which was correlated with functional muscle O_2 diffusive capacity and was associated with increased activities of the muscle oxidative enzymes citrate synthase and β -hydroxyacyl-CoA dehydrogenase (25). These enzymes are important to aerobic production of ATP via the citric acid cycle and β oxidation of fatty acids, respectively (25). Finally, in generation 11, protein expression for multiple mitochondrial enzymes associated with mitochondrial biogenesis and function was increased in HCR vs LCR (26). Given these documented characteristics in rat line generations that bracket the currently used generation, we thought it reasonable to assume that similar characteristics would apply to generation 12 rat lines. We therefore hypothesized that, at generation 12, the HCR would be better able than LCR to withstand the challenge of severe hemorrhage.

Against expectations, the HCR and LCR demonstrated similar survival times in this model of severe hemorrhage. To our knowledge, this study is the first to examine the

response to hypovolemia in animals specifically bred for increased aerobic capacity. In earlier work, however, responses to either hemorrhage or central hypovolemia induced by lower body negative pressure (LBNP) have been investigated in outbred rats with increased $\text{Vo}_{2\text{max}}$ produced by exercise training, with mixed results. Bond et al. (27) demonstrated that exercise training produced a protective effect when anesthetized rats were subjected to a severe hemorrhage. Conversely, Tipton and his colleagues showed that exercise training exacerbated the hypotension induced by mild hemorrhage and/or LBNP in anesthetized rats, leading to their suggestion that trained animals could be at a disadvantage in hemorrhaging experiments (28, 29). While it is unclear from these conflicting data whether increases in aerobic capacity induced by exercise training alter the response to hemorrhage in anesthetized rat models, the data presented herein suggest that innate increases in $\text{Vo}_{2\text{max}}$ produced by genetic selection do not alter the ability to survive severe hemorrhage in this conscious rodent model. Interestingly, aerobic fitness (i.e., an increase in $\text{Vo}_{2\text{max}}$) has also not reliably predicted orthostatic tolerance in humans (30, 31). Of course, unlike earlier studies, rats of the current study did not undergo training to improve $\text{Vo}_{2\text{max}}$, but were instead selected for genetic enhancement of cardiovascular function and tissue oxygen utilization. Hence, the possibility exists that the two procedures used to attain improved $\text{Vo}_{2\text{max}}$ may involve somewhat different mechanisms.

Closely linked to the above-noted observations is the question that led to our hypothesis: Would individuals with larger cardiac outputs and/or tissue aerobic capacity demonstrate greater survival time to hemorrhage? A positive response to this question might involve an adaptive cross-tolerance or cross-adaptation. As a well-recognized example of such a phenomenon, heat acclimation is associated with cross-tolerance to cerebral hypoxia, hyperoxia, and traumatic brain injury (32). Perhaps more germane to this study, exercise training provides protection against a subsequent hemorrhage and is therefore another example of cross-tolerance (27).

We cannot dismiss the possibility that different stress responses between the two rat lines subsequent to catheterization surgery may have influenced our results. Reductions in nutrient consumption and body weight after surgery are well documented (e.g., 33–35). In the current study, such reductions may also represent a differential response to the single administration of buprenorphine, as this analgesic is known to decrease food and water intake in rats (35). In addition, HCR may have had an increased sensitivity to isoflurane as measured by their recovery time from this anesthetic. Irrespective of cause, differences in water consumption and changes in body weight between the strains may influence the interpretation of survival time observations, since fasting and associated decreased water consumption shorten survival time to hemorrhage (36, 37).

In the present study, of the antioxidant status variables measured, total antioxidant capacity in liver and heart was

higher in LCR than HCR rats. Nevertheless, as a whole, there did not appear to be consistent differences between strains in antioxidant status measures in any of the tissues assayed. Also, there were no differences in neutrophil infiltration in the lung between groups. While LCR rats had a slight increase in antioxidant capacity following hemorrhage, this possible advantage did not manifest itself in increased survival. Hence, if organ dysfunction had occurred, these measures did not indicate substantial differences between strains.

A major homeostatic mechanism during hemorrhagic shock is plasma refill from interstitial and intracellular sources (37). Hence, if the two rat lines were differentially hydrated, then this plasma refill and survival time might be compromised (38). To prevent potential dehydration, all rats were injected with isotonic saline after surgery, and rats were not food- or water-restricted. Further, alterations in hematocrit (an index of plasma refill; 6, 12) and blood glucose concentration (a source of increased plasma osmolality that drives refill; 6, 12) during hemorrhage did not differ between rat lines. Both observations suggest that at the end of the hemorrhage, movement of fluid from the interstitium into the capillaries was not altered by differences in water consumed or in body weight lost.

Indeed, in a follow-up study, we measured actual plasma and blood volumes in these two rat lines under conditions almost identical to those of our initial study, ($n = 3$ HCR; 4 LCR; generation 14). Plasma volumes did not differ between rat lines or between day of surgery and day of hemorrhage ($P > 0.1$). Similarly, blood volumes did not differ ($P = 0.63$) between rat lines on either day of surgery (8.7 ± 0.5 vs 7.7 ± 0.5 ml/100 g body weight; HCR vs LCR) or day of hemorrhage (7.1 ± 0.6 vs 7.0 ± 0.4 ml/100 g body weight). By extrapolation of these blood volumes to rats used in the current study, it was determined that HCR rats were bled to $36.6 \pm 6\%$ of total blood volume, whereas LCR rats were bled $41.8 \pm 0.3\%$ ($P < 0.01$). With the assumption that the two generations had similar blood volumes, such results suggest that LCR received a more challenging hemorrhage and further argue for mechanisms other than O_2 delivery and utilization being involved in comparable survival times.

It is possible that the hemorrhage challenge of this study was too severe to fully distinguish physiological differences between the two rat lines. However, $\sim 50\%$ of rats in both rat lines survived the hemorrhage during a 6-h observation period, suggesting that the hemorrhage was appropriate to distinguish the strains with a high level of sensitivity. While lactate levels lower than those observed in this study are associated with mortality in human trauma patients (39), our lactate levels are similar to those reported previously in a severe hemorrhage model in rats that survived (40). Furthermore, we have previously demonstrated profound differences in survival from a similar hemorrhage in different inbred rat strains, suggesting that

genetic components are not overwhelmed by this severe challenge (14).

In summary, this study provides the first evidence for a dissociation between the ability to survive global ischemia and greater intrinsic aerobic capacity when that capacity has been obtained through genetic selection. Either previously documented enhancements in $\text{VO}_{2\text{max}}$ and associated cardiovascular characteristics in HCR were insufficient to compensate for the marked O_2 deficits occurring during severe hemorrhage or the hemorrhagic challenge was not of sufficient magnitude to manifest these innate characteristics.

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