Strain differences in response to acute hypoxia: CD-1 versus C57BL/6J mice

Charles F. Zwemer,1,3* Michael Y. Song,2* Katari A. Carello,3 and Louis G. D’Alecy3,4,5
1Department of Biology, Dickinson College, Carlisle, Pennsylvania; 2College of Literature, Science, and The Arts, University of Michigan, Ann Arbor; 3Department of Molecular and Integrative Physiology and 4Department of Surgery (Vascular), University of Michigan Medical School, Ann Arbor; and 5William Beaumont Hospital, Department of Surgery, Royal Oak, Michigan

Submitted 12 May 2006; accepted in final form 9 August 2006

Zwemer CF, Song MY, Carello KA, D’Alecy LG. Strain differences in response to acute hypoxia: CD-1 versus C57BL/6J mice. J Appl Physiol 102: 286–293, 2007. First published August 17, 2006; doi:10.1152/japplphysiol.00536.2006.—Some mammals respond to hypoxia by lowering metabolic demand for oxygen and others by maximizing efficiency of oxygen usage: the former strategy is generally held to be the more effective. We describe within the same species one outbred strain (CD-1) that lowers demand and another inbred strain (C57BL/6J) that maximizes oxygen efficiency to markedly extend hypoxic tolerance. Unanesthetized adult male mice (Mus musculus, CD-1 and C57BL/6J) between 20 and 35 g were used. Sham-conditioned (SC) C57BL/6J mice survived severe hypoxia (4.5% O2, balance N2) roughly twice as long as SC CD-1 mice (median 211 and 93.5 s, respectively; P < 0.0001). Following acute hypoxic conditioning (HC), C57BL/6J mice survived subsequent hypoxia 10 times longer than HC CD-1 mice (median 2,198 and 238 s respectively; P < 0.0001). Therefore, C57BL/6J mice are both naturally more tolerant to hypoxia and show a greater increase in hypoxic tolerance in response to hypoxic conditioning. Indirect calorimetry indicates that CD-1 mice lower mass-specific oxygen consumption (V̇O₂ in ml O2·kg⁻¹·min⁻¹) and carbon dioxide production (V̇CO₂ in ml CO₂·kg⁻¹·min⁻¹) in response to HC (P = 0.002 and P < 0.0001, respectively), but C57BL/6J mice maintain V̇O₂ and V̇CO₂ after HC. Respiratory exchange ratio and fluorometric assay of plasma ketones suggest that C57BL/6J mice rapidly switch to ketone metabolism, a more efficient substrate, while CD-1 mice reduce overall metabolic activity. We conclude that under severe hypoxia in mice, switching fuel, possibly to ketones, while maintaining V̇O₂ may confer a greater survival advantage than simply lowering demand.

hypoxic survival time; hypoxic conditioning; indirect calorimetry; respiratory exchange ratio; d-β-hydroxybutyrate

When faced with hypoxia, mammals tend to survive by either reducing metabolic demand to levels supported by oxygen delivery or by maximizing delivery and metabolic efficiencies to sustain utilization (14, 21). Hochachka et al. (21) and Gautier (14) hold that reducing demand most commonly confers the greatest tolerance. Furthermore, Frappell et al. (13) suggest that the degree of drop in metabolic rate is inversely related to body mass such that the smaller an adult mammal species, the more significant the drop in metabolic rate on exposure to hypoxic hypoxia. Our laboratory has studied hypoxia tolerance in Swiss CD-1 mice (5, 24, 27, 28, 30–34, 37), and we were the first to identify significant protection from hypoxia conferred by brief, intermittent hypoxia, now referred to as hypoxic conditioning (HC) (37). Our subsequent work linked much of this HC effect to activation of centrally active, δ-specific, opioid pathways (30–33). Decreases in core temperature and the seeking of cooler microenvironments in a thermocline in response to HC suggested that part of the conditioning effect may also be linked to induced decreases in metabolic demand (33). While tempting to ascribe a reduction in metabolic demand as a tolerance mechanism, linkage of hypometabolism to the mechanism of protection in our model has remained unresolved. Indeed, if the hypometabolic state is associated with reduced overall function, the overall utility for survival is limited. By contrast, a fuel shift with enhanced efficiency would be expected to maximize survival value of the acute changes.

In this study, we tested, using indirect calorimetry, the hypotheses that HC induces a rapid decrease in metabolic rate and that this hypometabolism could thus play a major role in improving hypoxic survival time. Swiss CD-1 outbred mice (CD-1) were chosen to further complement our previous work with the strain, while C57BL/6J inbred mice (C57) were chosen because of their popularity of use in studies of acute hypoxic tolerance (2, 7, 8, 20, 22, 36, 41) and induced hypometabolism (3). Additionally, C57 mice are the most common inbred strain used for deriving knockout models because of their robust tolerance of developmental manipulation and well-characterized genome (4, 6). The unexpected finding of marked strain differences in metabolic response to hypoxia opens the path to explore the genomic basis for the profound phenotypic difference within the same species.

MATERIALS AND METHODS

Adult male mice (CD-1, outbred and C57, inbred) of comparable age were obtained from Charles River Laboratories, Wilmington, MA, and subjected to either sham conditioning (SC) or hypoxic conditioning (HC) and then tested to determine hypoxic survival time (HST) or plasma d-β-hydroxybutyrate (BHB) concentrations. Additional cohorts of CD-1 and C57 were evaluated pre-, peri-, and post-hypoxic/sham conditioning for mass-specific oxygen consumption (V̇O₂), mass-specific carbon dioxide production (V̇CO₂), and respiratory exchange ratio (RER) but not tested for HST. All experiments were carried out during daylight hours (7:00 AM to 5:00 PM EST) at room temperature (21–23°C). The University of Michigan’s University Committee for Animal Care and Use approved all protocols (approval no. 8693 and no. 07124-V).

Hypoxic conditioning. Two or three mice were placed in either of two 500-ml airtight flow-through chambers and subjected to HC or SC before HST testing or blood sampling. Both chambers were initially flushed with room air (20.93% O₂) at 1.3 l/min. HC mice received...
four sequential exposures of 0.75, 1.5, 2, and 2.5 min of 4.6% O₂-balance N₂ separated by 5 min of room air including 5 min after the final hypoxic conditioning (see Fig. 1). SC mice received normoxic room air for the same duration (21.75 min) as the HC mice. A similar HC or SC protocol was followed for the cohort used in the indirect calorimetry protocols described below; however, one mouse at a time was conditioned (HC or SC) and subsequently evaluated with indirect calorimetry.

**Hypoxic survival time.** Groups of five (2 SC and 3 HC; or 3 SC and 2 HC) mice were transferred to five individual airtight flow-through chambers (3.7 cm ID × 10.5 cm in length) arranged in parallel for simultaneous HST testing. Hypoxic hypoxia was induced by flushing the five-chamber system for 20 s at 1.71 l/min with premixed 8.6% O₂-balance N₂, then by a constant flow of 4.6% O₂-balance N₂ at 1.71 l/min. HST, as used in this and all previous studies (11, 25, 26, 28, 30–33), is the time from onset of 4.6% O₂ to the cessation of spontaneous ventilation. Within 60–90 s of the onset of hypoxia, the animal falls into a state that is reported in humans as euphoric (16), loses consciousness, undergoes seizures, and then ceases spontaneous ventilation. At the cessation of spontaneous ventilation, the heart is still beating, and induction of artificial ventilation could resuscitate the animal. However, since we did not ventilate the mice, cessation of spontaneous ventilation in the unconscious mouse leads irrevocably to cardiac death and hence is a precise measurement of the time to death due to hypoxia. Hypoxic hypoxia is not asphyxia, and death by acute hypoxic exposure is humane and approved as an end point for use in this model by the university Institutional Animal Care and Use Committee that fully embraces all the articles of the recently published Principles for Establishment of Humane End Points (10).

**Indirect calorimetry.** \( \dot{V}O_2 \) (ml O₂·kg⁻¹·min⁻¹), \( \hat{V}CO_2 \) (ml CO₂·kg⁻¹·min⁻¹), and RER (\( \hat{V}CO_2/\dot{V}O_2 \); dimensionless) were calculated using an open-flow, indirect calorimeter designed to closely match conditions used in the HC, SC, and HST phases described above. Chamber inflow rate (\( \dot{V}_I \)) was measured using a standard temperature and pressure (STP) dry (STPD)-corrected mass flowmeter [National Institute of Standards and Technology-traceable, GF-M-17 (air, from 0 to 500 standard cubic centimeters per minute), Aalborg Instruments and Controls, Orangeburg, NY)]. Fractional inlet \( \hat{F}O_2 \) and fractional outlet oxygen concentrations (\( \hat{F}O_2 \)) were measured separately using two high-temperature zirconia electrochemical cells (model S-3A/II oxygen analyzer, AEI Technologies, Pittsburgh, PA). Fractional outlet carbon dioxide concentration (\( \hat{F}CO_2 \)) was measured using an infrared analyzer (CD-3A carbon dioxide analyzer, AEI Technologies) arranged in series before the \( \hat{F}O_2 \) measurement. The majority flow-through system was plumbed using 1/8-in. stainless steel or copper tubing to minimize diffusional equilibration with room air while the balance was plumbed with plastic (Tytong) tubing. For normoxic exposures, compressed room air was filtered and dried before entering the mouse test chamber. For hypoxic exposures, calibrated premixed gases (Cryogenic Gasses, Detroit, MI) were filtered and dried before entering the test chamber. Test chamber outlet gases were dried and filtered before entering gas analyzers. All measured and calculated variables were recorded continuously using a commercially available analog-to-digital data logger and software (MP150 system, Biopac Instruments, Santa Barbara, CA) and laptop computer (Apple G4 Powerbook, Cupertino, CA). The processed data were analyzed in 30-s average blocks bracketing standardized time points at preconditioning, 2, 4, 5, 6, 8, 10, 20, and 30 min. All calculated calorimetry data were determined using Haldane’s transformation of Fick’s consumption/production equations (1, 12, 18, 19). Please see APPENDIX for a complete derivation of these formulas.

**BHB assay.** Plasma concentration of BHB was determined for control and HC mice of both strains by using a fluorometry assay developed in our laboratory (29). Blood was collected by decapitation, and centrifugal effluent (mixed arterial and venous blood) was centrifuged at 3,000 rpm and 25°C to separate the plasma. Fifty microliters of plasma was deproteinized by using 200 μl of chilled 1 M HClO₄, vortexed, placed on ice for 10 min, and finally centrifuged at 3,000 rpm and 4°C. Two-hundred microliters of supernatant was neutralized to pH 5.8–6.5 using 163 μl (determined empirically) of 0.5 M K₂CO₃. One-hundred microliters of neutralized sample was added to a cuvette containing a solution containing hydrazine (H-0883, Sigma Chemical, St. Louis, MO). Using the change in fluorescence and a standard curve, plasma BHB concentration was calculated (29).

**Statistical analyses.** Sample sizes were sufficient to give a significance of \( P \leq 0.05 \) and power of 0.95 or greater. Survival curves were created and analyzed by using the Kaplan and Meier product limit method and the log-rank test. Indirect calorimetry variables were analyzed by using repeated measures, two-factor (conditioning treatment and strain) ANOVA. Specifically, interaction between conditioning treatments and interaction between strains was evaluated. Plasma BHB data were analyzed using two-factor ANOVA, and interaction between strains and sampling times was evaluated. One-factor ANOVA was used to evaluate changes over sampling time within strain. ANOVA of indirect calorimetry and plasma BHB data also included Bonferroni posttests to test for differences at selected time points between treatments and strains. Where appropriate, unpaired \( t \)-tests were used to ascertain individual differences either between strains or between treatments within strains. Linear regression of HC indirect calorimetry data were analyzed by using least-squares linear regression analysis to determine if the slope was not different from zero. Statistical software consisted of Microsoft Excel and Graphpad Prism 4.0.

**RESULTS**

**Strain differences in SC and HC hypoxic survival time.** Consistent with well-established strain data, CD-1 mice weighed ~15% more than comparably aged C57 mice (\( P < 0.01 \)). Survival curve analysis showed that SC CD-1 mice had a median HST of 94 s, and this was significantly lower (\( P < 0.0001 \), log-rank test) than SC C57 mice with a median survival time of 211 s (Fig. 2A). HC CD-1 mice had a median HST of 238 s, and this was significantly lower (\( P < 0.0001 \)) than HC C57 mice with a median survival time of 2,198 s (Fig. 2B). Survival curve analysis indicates HC prolongs survival in both CD-1 (\( P = 0.007 \)) and C57 (\( P < 0.001 \)) over SC control groups of the same strain (compare Fig. 2, A and B).

---

**Fig. 1.** Time course of the indirect calorimetry experiment is shown. The hypoxic conditioning (HC) period is expanded to highlight the normoxic and hypoxic periods. The end of the HC period is set as zero min. Both the preconditioning and post-conditioning periods were performed under normoxia. Five min post-HC is indicated (arrow) to show where hypoxic survival time (HST) testing would have started in a separate cohort of mice.
Strain differences during preconditioning. The preconditioning $V'\text{O}_2$, $V'\text{CO}_2$, and RER were analyzed by two-factor ANOVA, and the preconditioning values were found to be not different between treatments (SC or HC); therefore the preconditioning $V'\text{O}_2$, $V'\text{CO}_2$, and RER were combined between treatments to obtain an average strain value ($n=16$). While the RER was not different between strains, the $V'\text{O}_2$ and $V'\text{CO}_2$ were statistically higher in the CD-1 strain than in the C57 strain (Fig. 3).

Strain differences during HC. During HC (conditioning-timing illustrated in Fig. 1), $V'\text{O}_2$ and $V'\text{CO}_2$ appeared to decrease during each of the four sequential HC exposures before returning toward baseline with the restoration of normoxia after each exposure (data not shown). ANOVA of the final 30 s of the second, third, and fourth HC periods indicated no significant strain differences over subsequent conditioning steps for $V'\text{O}_2$ and RER but produced a $P<0.0001$ difference between strains for $V'\text{CO}_2$, suggesting CO$_2$ production was maintained higher in the C57 mice than the CD-1 mice (see Fig. 7). The longest HC exposure gave the most stable response, which was further analyzed for average values during the final 30 s of the fourth (final) HC period. During this final conditioning period, the CD-1 group had a significantly lower $V'\text{O}_2$ and $V'\text{CO}_2$ ($P=0.05$ and $P=0.0065$, by unpaired t-test) than the C57 mice. The $V'\text{O}_2$ and $V'\text{CO}_2$ of the CD-1 mice was $15.3 \pm 3.0$ ml O$_2$·kg$^{-1}$·min$^{-1}$ and $34.9 \pm 3.3$ ml CO$_2$·kg$^{-1}$·min$^{-1}$, respectively, and for the C57 was $25.5 \pm 3.7$ ml O$_2$·kg$^{-1}$·min$^{-1}$ and $48.7 \pm 2.8$ ml CO$_2$·kg$^{-1}$·min$^{-1}$, respectively.

Strain differences following HC. Analysis of the indirect calorimetry data between strains for the entire 30-min period following HC revealed that $V'\text{O}_2$, $V'\text{CO}_2$, and RER responded significantly differently (showed an interaction over time) in the CD-1 mice compared with the C57 mice, but with SC no such difference was detected (exact strain $P$ values are shown at left of symbol key in Figs. 4 and 5A). For the CD-1 mice HC Fig. 4. Indirect calorimetry measurement of mass-specific $V'\text{O}_2$ ($A$) and mass-specific $V'\text{CO}_2$ ($B$) in CD-1 ($\bullet$) and C57 mice ($\square$) are shown. Values are means ± SE; $n=8$ for each group. The solid lines represent the HC groups, and the dotted lines represent the SC groups. Five minutes postconditioning is indicated by a vertical dashed line that is the start time for HST testing in separate cohorts of mice with survival curves in Fig. 2. Two-factor (conditioning treatment and strain) ANOVA tested for interaction between treatments (HC vs. SC, exact $P$ values at right of symbol key) and interaction between strains (C57 vs. CD-1, exact $P$ values at left of symbol key).
altered the response of V′O₂ and V′CO₂ but not RER. For the C57 mice, HC altered the response of V′O₂ and RER but not V′CO₂ (exact treatment P values are shown at right of symbol key in Figs. 4 and 5A).

Plasma BHB levels were evaluated by using two-factor (strain and time) ANOVA. Significant interaction between the strains was detected (P = 0.0015). On the basis of this significant difference, we then tested differences between (unpaired t-tests) and within strains (one-way ANOVA) at all time points. No significant differences were detected between strains at control or 5 min postconditioning, but at 30 min postconditioning, CD-1 BHB was significantly higher (unpaired t-test, P = 0.006). Within strains, BHB in CD-1 at 30 min was more than double that of control and 5-min samples (1-factor ANOVA, overall P = 0.013 and Bonferroni’s posttest, P < 0.05 for each), while C57 BHB at 30 min was roughly one-half of the C57 BHB at the 5-min point (1-factor ANOVA, overall P = 0.011 and Bonferroni’s posttest, P < 0.05).

Treatment effect at start of HST. In Figs. 4 and 5, the 5-min postconditioning time point is indicated by a vertical dashed line indicating that it is the start time for HST testing. HST testing was done in separate cohorts of mice but was not done in the indirect calorimetry groups of mice. At this critical 5-min postconditioning time point, indirect calorimetry data (Fig. 6) revealed that HC in CD-1 mice produced significantly lower V′O₂S and V′CO₂S than SC. HC and SC values were not different in C57 mice. However, C57 mice significantly decreased RER 5 min post-HC, but CD-1 mice did not reduce their RER by 5 min post-HC.

Treatment effect during HC. V′O₂, V′CO₂, and RER perihypoxic values were analyzed by using standard least-squares linear multiple regression analysis (Fig. 7). Data for each variable were taken at control, over the last 30 s of each 5-min normoxic recovery period during HC, and 5 min following cessation of HC (Fig. 1). Linear regression of perihypoxic C57 mice V′O₂ (Fig. 7A) revealed a slope no different from zero, suggesting no measurable conditioning effect. The same treatment of C57 mice V′CO₂ data (Fig. 7B) suggested a moderately negative slope (−0.32 ± 0.35 ml CO₂·kg⁻¹·min⁻², r² = 0.22) that was also not significantly different from zero. Over the same time period, however (Fig. 7, A and B), CD-1 mice demonstrated a significant conditioning-dependent decrease in V′O₂ and V′CO₂ (V′O₂ slope: −1.29 ml ± 0.21 ml O₂·kg⁻¹·min⁻², r² = 0.93; V′CO₂ slope: −1.33 ± 0.26 ml CO₂·kg⁻¹·min⁻², r² = 0.90). RER (Fig. 7C) decreased significantly with HC in C57 mice at a rate of −0.005 ± 0.0005 dimensionless units/min (r² = 0.97) and was significantly different from a zero slope (P = 0.0028). CD-1 mice RER values tended to decrease at roughly one-half the rate of C57 (−0.0025 ± 0.0019 dimensionless units/min, r² = 0.37) and did not statistically separate from a slope of zero.

DISCUSSION

The main findings of this study are 1) the inbred C57 strain was both naturally and hypoxia-induced more tolerant to hypoxia than the outbred CD-1 strain, and 2) since both strains displayed different metabolic changes when subjected to hypoxic conditioning, the metabolic strategy of the inbred strain, i.e., a switching toward another metabolic fuel (possibly ketones), may be more efficient than the one adopted by the outbred strain, i.e., a lowering of the metabolic demand to face the severe hypoxic challenge.

Fig. 5. A: RER in CD-1 (○, n = 8) and C57 mice (●, n = 8). The solid lines represent the HC groups, and the dotted lines the SC groups. Five min postconditioning is indicated by a vertical dashed line that is the start time for HST testing in separate cohorts of mice with survival curves in Fig. 2. B: plasma β-hydroxybutyrate concentration ([BHB]) for C57 and CD-1 mice at control and 5 and 30 min postconditioning. Values are means ± SE with sample size from 6–10 mice in each group. In CD-1 mice, [BHB] was unchanged from control at 5 min but increased significantly at 30 min. In C57 mice, [BHB] was unchanged from control at 5 min but decreased significantly at 30 min. *Statistically significant difference (P = 0.006) between CD-1 and C57 mice [BHB] at 30 min.

Fig. 6. Differences in indirect calorimetry data at 5 min postconditioning are shown for C57 and CD-1 mice. The 5-min postconditioning values were analyzed separately in that this is the time when HST testing (Fig. 1) would have been initiated in a separate cohort of mice. Mass-specific VO₂ and mass-specific VCO₂ decrease with HC in the CD-1 mice but are not different with HC in the C57 mice. RER is unchanged with HC in CD-1 mice and significantly reduced in the C57 mice.
The hypothesis that CD-1 mice would decrease metabolic demand, as measured by indirect calorimetry, following HC was supported. As seen in Fig. 2, HC conferred roughly a 2.5-fold increase in median HST in CD-1 mice, and that was accompanied by a 40% and 57% decrease in $\dot{V}O_2$ and $\dot{V}CO_2$, respectively, at 5 min following HC, the time at which mice would be subjected to HST testing (Fig. 6). This observation is also supported by the deceleration (negative slope) of $\dot{V}O_2$ and $\dot{V}CO_2$ of CD-1 mice over the hypoxic conditioning period (Fig. 7, A and B). Extrapolating these data, a rough halving of aerobic demand as reflected by $\dot{V}O_2$ could reasonably account for much of this improved HST in CD-1 mice. Using the same model system and mouse strain, Mayfield and D’Alecy (30) showed that protection afforded by HC is mediated through $\delta$-selective opioid activity. The current study confirms this protection and increased HT induced by HC in CD-1 mice and extends the basic observation to a second strain of mice (C57).

Additional recent work in this area suggests that hibernation and many survival-related responses in mammals may exploit a similar set of mechanisms via activation of $G_{i}$-protein receptors ultimately opening sarcolemmal and mitochondrial ATP-sensitive K channels (40). The resultant hyperpolarization of the cell membrane (in excitable tissues) putatively serves to reduce probability of depolarization and thus spares overall cell energy demand. It seems likely, then, that the Swiss CD-1 strain, when hypoxically conditioned, initiates a series of metabolic changes aimed at reducing total aerobic demand to a level supportable by delivery. This torporlike state has survival utility but only in a protected setting where minimal activity does not increase susceptibility to environmental or predatory threats.

By contrast, naive (SC) C57 mice had a median HST only 11% lower than CD-1 mice that had already increased their HST by hypoxic conditioning (Fig. 2, A and B). Furthermore, HC C57 mice survived almost 10 times longer than HC-CD-1 mice (with 2 C57 mice survivors lasting more than 1.5 h at $F_{I02} = 4.5\%$). In stark contrast to the response in CD-1 mice, C57 mice dramatically increased HC survival time without decreases in $\dot{V}O_2$ and $\dot{V}CO_2$ measured during conditioning (Fig. 7, A and B) or at 5 min following HC (Fig. 6) when HST testing would have started. We conclude from these data that C57 mice do not respond to HC by reducing metabolic demand; rather they must be relying on increases in delivery and/or increased efficiencies in carbon fuel utilization. It is as if the C57 strain responds as though it were experiencing significantly less relative stress despite having been exposed to exactly the same $F_{I02}$.

An alternative approach to assessing HT would be to expose the different strains of mice to differing $F_{I02}$s and record at what $F_{I02}$ spontaneous ventilation ceased. This would presumably yield a different $F_{I02}$ for the CD-1 and the C57 mouse strains, reflecting their difference in overall hypoxic tolerance. This is a very different set of experiments exploring a graded hypoxic stress rather than the fixed hypoxic challenge we used and perhaps could be evaluated in future protocols.

Work on hypoxic ventilatory responses in inbred mouse strains by Tankersley et al. (42) shows that C57 mice markedly increase ventilation by increasing tidal volume ($V_T$) and breathing frequency in response to hypoxia ($F_{I02} = 10\% O_2$) and hypercapnic hypoxia ($F_{I02} = 10\%$ and $F_{I02CO} = 8\%$), and these were among the largest responses of eight inbred strains studied. Comparative morphology of lung structure suggests C57 mice have relatively large mass-specific lung volume compared with other inbred strains (23). Using a similar hypoxia depth and time course of acute exposure per Tankersley et al. (42), Campen et al. (8) have recently shown that C57 mice decrease low-frequency (LF) power heart rate variability, while high-frequency power (HF) heart rate variability remains unchanged. Traditional interpretation of these power data suggests a diminishing sympathetic LF response along with an unchanging parasympathetic HF response and may explain the relative drop in mean arterial blood pressure and heart rate with hypoxia that these authors described (7, 8). Using an HST model similar in design to our previously established HC-HST model, Zhang et al. (45) have recently shown that HC resulted in significantly improved blood gas profiles when C57 mice were exposed to $F_{I02} = 7\%$. These authors also showed that HC significantly reduced pulmonary vascular permeability,
suggesting a protection of pulmonary diffusive capacitance (45). Taken together, these strain-specific differences may work to improve hypoxic ventilatory response in C57 mice, but the exact role here remains highly speculative as measuring these differences was beyond the scope of our study. Furthermore, while an increase in oxygen delivery may have played a part in the modest enhancement in hypoxia tolerance of SC C57 mice, it is unlikely that this increase in delivery could account for a 10-fold increase in hypoxia tolerance with HC in C57 mice.

Prass et al. (35) also identified strain differences in mice; however, they focused on differences between C57 and SV129 mice. Using a 5-day exposure to hyperbaric 100% oxygen (HBO), they demonstrated that HBO induced tolerance to permanent but not transient focal ischemia in SV129 mice; however, HBO did not induce tolerance against permanent or transient focal cerebral ischemia in C57BL/6 mice. While this is relevant in that it represents a strain difference, it is not fully comparable in that they did not, in an acute setting, use an intermittent hypoxic hypoxia to induce an increase in hypoxic survival time.

In addition to potential increases in oxygen delivery, changes in metabolic fuel selection provoked by either HC or simply in response to acute hypoxia may also have contributed to greater HST seen in C57 mice. The RER of C57 mice was depressed well below 0.6 (see proposed explanation below) in the first 2 min following HC (Fig. 5A). This was driven by a large decrease in $V_{CO2}$ and no change in $V_{O2}$ (Fig. 4, A and B). By 5 min post-HC, this suppression was gone, and $V_{CO2}$ in C57 mice was no different from the control value (Fig. 6). CD-1 mice did not demonstrate the same magnitude of change immediately following HC. Instead, CD-1 mice had decreased RER significantly by 10 min after the conditioning, in contrast to C57 mice that were increasing RER to mid-0.80 levels. We infer that C57 mice may be more agile in rate of change of fuel mobilization and selection than CD-1 mice and that ketones may be the fuel of choice in hypoxia and following HC.

We sampled whole blood for D-β-hydroxybutyrate (BHB), arguably the most relevant of ketones, at control, 5 min, and 30 min post-HC (Fig. 5B). BHB in C57 mice tended to increase at 5 min following HC but by 30 min had decreased (by ~46%) significantly from 5-min values. In opposition to this response, BHB in CD-1 mice remained unchanged at 5 min but by 30 min following HC had increased by more than 1.5 times that of control. Previous studies from our laboratory (24–28) have drawn a consistent correlation between increased plasma BHB and increased hypoxic tolerance; however, regardless of the consistency and strength of the correlation, this does not establish causality.

Together, these plasma ketone and RER data suggest that C57 mice may be producing and consuming BHB rapidly during and immediately following HC. Placing this in context, Simonson and DeFronzo (39) noted that when BHB is produced from palmitate (one of the most common mammalian fatty acid chains), the calculated RER is zero since oxygen is used but no carbon dioxide is produced. If a systemic switch to palmitate-based BHB production was forced by HC, it is possible that this was happening in C57 mice at 2 min post-HC and earlier, and thus the RER was depressed well below 0.6 (Fig. 5A). By 5 min post-HC, consumption of BHB was nearly matched by production. By 10 min, BHB consumption rate slightly surpassed production rate such that between 10 and 30 min, the RER of C57 mice closely approximated the theoretical BHB RER of 0.89 (39). By 30 min, consumption surpassed production, resulting in the decrease in BHB in the plasma of C57 mice; alternatively, a production decrease could also account for the same. Along a similar line of reasoning, reducing $V_{O2}$ (and no change in RER), CD-1 mice were likely not producing BHB as early as C57 mice. By 10 min post-HC, however, they may have begun BHB production, as suggested by sub-0.8 RERs. Thus, by 30 min, net production of BHB may have exceeded consumption (RER still <0.8), and thus its concentration in the blood was elevated from control. Simple plasma concentration measurements cannot resolve this, and radiolabeled tracers would be required. It is noteworthy that the reduction in $V_{CO2}$ even during the hypoxic phases of HC in the C57 mice was statistically less compared with the changes CD-1 mice. This is most likely interpreted as a better maintenance of carbon dioxide production by oxidative metabolism in the C57 mice.

Alternatively, it is possible that BHB levels increased in CD-1 mice because of HC stress-induced systemic lipolysis. Since beta-oxidation of free fatty acids can decrease measured aerobic metabolism (oxygen consumption) this could have resulted in acetyl-CoA accumulation and thus increased total acetocetate and BHB by mass action. C57 mice, by virtue of their relatively constant metabolic rate, may have utilized ketones at roughly the same rate ketones were made. Since we did not measure plasma free fatty acid concentrations and because BHB was massively increased by 30 min post-HC, and not earlier, it is difficult to accept this alternative as a mechanism in an animal with such a high mass-specific resting metabolic rate.

Ketones as primary fuel sources are highly efficient, and only recently has their unique role in stress and survival physiology been appreciated. In an elegant study utilizing an isolated/prefused working rat heart model, Sato et al. (38) showed that addition of BHB to the perfusion buffer increased pumping-work-to-$V_{O2}$ ratio by ~30%. The authors attributed much of the ketone effect to an increase in the free energy ($\Delta G'$) of cytosolic ATP hydrolysis, the result of a BHB-dependent increase in redox energy of electron transport chain couples between sites I and II (38, 43, 44). BHB use as a fuel increased work output by roughly one-third while holding $V_{O2}$ unchanged or slightly decreased (34). Veech et al. (43) suggest that beyond the increased efficiency of work, the greater $\Delta G'$ of ATP hydrolysis at the Na$^+$-K$^+$ ATPase pump widens the potential energy of extra/intracellular ionic gradients and thus hyperpolarizes highly excitable cell membranes and reduces their unwanted discharge. Whether BHB-mediated increases in $\Delta G'$ of ATP hydrolysis is at work in our model remains speculative. Additionally, other possible mechanisms have recently been reviewed and include the role of activation of several different signaling pathways and modulation of potential second messengers that could contribute to the preconditioning response (15).

Our analysis of the data also suggests that the rate change of RER [$d(RER)/dt$ in dimensionless units/min$^2$] during HC or immediately following HC (first 5 min) may be mechanistically linked to two strategies we observed between the strains, with a greater relative $d(RER)/dt$ indicating greater metabolic agility and perhaps enhanced hypoxic tolerance. It holds, then,
that measured $|d(RER)/dt|$ on hypoxic challenge could indicate which strategy of hypoxic tolerance is to be used by the animal, and it may be a relatively sensitive metric predictive of qualitative HST.

We conclude that CD-1 mice improve HST following HC primarily by lowering metabolic demand as measured by indirect calorimetry. Quite differently, C57 mice did not decrease metabolic demand during HST testing to match oxygen delivery but instead appear to switch metabolic fuel, perhaps to BHB, and thereby gain putative efficiencies not possible with other substrates. This fuel change, combined with a putative increase in oxygen delivery as suggested by Zhang et al. (45), may explain the dramatic improvement in HST seen in HC C57 mice (41) compared with CD-1 mice. This profound phenotypic strain difference within the same species presents an excellent opportunity of exploring the genotypic basis for hypoxic tolerance. In addition, the demonstrated capacity of C57 mice for agile switching of fuel choice suggests enhanced ketone metabolism should be evaluated as a potential therapeutic target for management of acute low oxygen states.

APPENDIX

The following are the abbreviations used in derivations of the $V'O_2$, $V'CO_2$, and RER equations found below:

Glossary

- $V'O_2$: volumetric rate (STP) of oxygen consumption (ml O2/min)
- $V'O_2$: mass-specific volumetric rate (STP) of oxygen consumption (ml O2·kg⁻¹·min⁻¹)
- $V'CO_2$: volumetric rate (STP) of carbon dioxide production (ml CO2/min)
- $V'CO_2$: mass-specific volumetric rate (STP) of carbon dioxide production (ml CO2·kg⁻¹·min⁻¹)
- $V_I$: volumetric rate (STP) of chamber inlet airflow (ml air/min)
- $V_E$: volumetric rate (STP) of chamber outlet airflow (ml air/min)
- $F'O_2$: chamber inlet fraction of oxygen (decimal %)
- $F'O_2$: chamber outlet fraction of oxygen (decimal %)
- $F'CO_2$: chamber inlet fraction of carbon dioxide (decimal %)
- $F'CO_2$: chamber outlet fraction of carbon dioxide (decimal %)
- $F'N_2$: chamber inlet fraction of nitrogen (decimal %)
- $F'N_2$: chamber outlet fraction of nitrogen (decimal %)
- RER: respiratory exchange ratio (dimensionless)

Haldane’s transformation of Fick’s equation for oxygen consumption proceeds as follows, where

$$V'O_2 = V_F'O_2 - V_E'F'O_2 \quad (1)$$

and

$$F'O_2 = 1.0 - F'O_2 - F'CO_2 \quad (2)$$

and

$$F'N_2 = 1.0 - F'N_2 - F'CO_2 \quad (3)$$

Thus if

$$V'E = \frac{V_F'E_N_2}{F_E'N_2} \quad (4)$$

and

$$F'E_N_2 = 1.0 - F'E_O_2 - F'E_C O_2 \quad (5)$$

then by substitution

$$V'E = \frac{Vf(1.0 - F'E_O_2 - F'E_C O_2)}{1.0 - F'E_O_2 - F'E_C O_2} \quad (6)$$

So,

$$V'O_2 = \frac{Vf(1.0 - F'E_O_2 - F'E_C O_2)}{1.0 - F'E_O_2 - F'E_C O_2} F'E_O_2 \quad (8)$$

or in mass-specific terms and using distributive property for $V_I$:

$$V'I = \frac{(1.0 - F'O_2 - F'CO_2)}{1.0 - F'O_2 - F'CO_2} F'E_O_2 \quad (9)$$

For mass-specific carbon dioxide production,

$$V'CO_2 = \frac{VfF'E_C O_2 - VfF'E_O_2}{V_I} \quad (10)$$

and, making the same corrections [Haldane transformation (17–19)] as used above for oxygen and dividing through by mass, then:

$$V'I = \frac{(1.0 - F'O_2 - F'CO_2)}{1.0 - F'O_2 - F'CO_2} F'E_C O_2 - F'E_O_2 \quad (11)$$

Respiratory exchange ratio was calculated as the following:

$$RER = \frac{V'CO_2}{V'O_2} \quad (12)$$

ACKNOWLEDGMENTS

We thank Steven E. Whitesall and Mary C. Lloyd for expert and excellent advice and assistance throughout this project. We also thank Prof. Henry D. Prange for help with revisions of this manuscript.

GRANTS

C. F. Zwemer was supported in part by a sabbatical supplement grant from the Dickinson College Dean’s Office. M. Y. Song was supported by the University of Michigan Undergraduate Research Opportunities Program.

REFERENCES


13. Frappell P, Lanthier C, Baudinette RV, Mortola JP.

12. Fick A.

11. Eiger SM, Kirsch JR, D’Alecy LG.

17. Gidday JM.

16. Gautier H.

18. Haldane JS.


24. Kirsch JR, D’Alecy LG.

23. Kirsch JR, D’Alecy LG.

25. Kirsch JR, D’Alecy LG.


