Normobaric hypoxia impairs human cardiac energetics

Cameron Holloway,*,†,[1] Lowri Cochlin,*,† Ion Codreanu,*,† Edward Bloch,* Marzieh Fatemian,* Cezary Szmigielski,† Helen Atherton,* Lisa Heather,* Jane Francis,† Stefan Neubauer,† Peter Robbins,* Hugh Montgomery,‡,§ and Kieran Clarke*

*Department of Physiology, Anatomy, and Genetics and †University of Oxford Centre for Clinical Magnetic Resonance Research, University of Oxford, Oxford, UK; and ‡University College London Institute for Human Health and Performance and §Institute for Sport, Exercise and Health; University College London, London, UK

ABSTRACT **Hypoxia causes left ventricular dysfunction in the human heart, but the biochemical mechanism is poorly understood. Here, we tested whether short-term normobaric hypoxia leads to changes in cardiac energetics and early cardiac dysfunction. Healthy male volunteers (n=12, age 24±2 yr) were exposed to normobaric hypoxia in a purpose-built hypoxic chamber. The partial pressure of oxygen during end-tidal expiration ($P_{ET}O_2$) was kept between 50 and 60 mmHg, and peripheral oxygen saturation (Sa_{O_2}) was kept above 80%. Cardiac morphology and function were assessed using magnetic resonance imaging and echocardiography, both before and after 20 h of hypoxic exposure, and high-energy phosphate metabolism [measured as the phosphocreatine (PCr)/ATP ratio] was measured using ^{31}P magnetic resonance spectroscopy. During hypoxia, $P_{ET}O_2$ and Sa_{O_2} averaged 55 ± 1 mmHg and 83.6 ± 0.4%, respectively. Hypoxia caused a 15% reduction in cardiac PCr/ATP (from 2.0±0.1 to 1.7±0.1, P<0.01) and reduced diastolic function (measured as E/E', rising from 6.1±0.4 to 7.5±0.7, P<0.01). Normobaric hypoxia causes a rapid decrease in high-energy phosphate metabolism in the human cardiac left ventricle, which may lead to a decline in diastolic function. These findings are important in understanding the response of normal individuals to environmental hypoxia, and to situations in which disease reduces cardiac oxygen delivery.—Holloway, C., Cochlin, L., Codreanu, I., Bloch, E., Fatemian, M., Szmigielski, C., Atherton, H., Heather, L., Francis, J., Neubauer, S., Robbins, P., Montgomery, H., Clarke, K. Normobaric hypoxia impairs human cardiac energetics. *FASEB J.* 25, 3130–3135 (2011). www.fasebj.org**

Key Words: cardiac energy metabolism · cardiac diastolic function · hypoxic chamber

INCREASING NUMBERS OF INDIVIDUALS are exposed to hypoxic environments through work, recreation, or residence at high altitude. In addition, all age groups may suffer reduced global oxygen delivery, whether through disease of the heart itself (*e.g.*, cyanotic congenital heart disease) or of the lung (*e.g.*, neonatal or adult respiratory distress, pulmonary embolus, or acute pneumonia). In others, specific organ oxygen delivery may be limited through disease of the subtending arterial vasculature (1). Our understanding of the *in vivo* human tissue molecular response to such hypoxia remains limited, although cellular metabolic reprogramming is known to occur, with hypoxia inducible factor (HIF) leading to alterations in specific cellular protein transcription and translation within hours (2).

Both cardiac contraction and relaxation are highly energy dependent, and the heart might thus be considered particularly vulnerable to restrictions in oxygen supply. Indeed, decreases in cardiomyocyte contractility, and in global ventricular systolic and diastolic function, have been observed in healthy volunteers with as little as 18 h of hypoxic exposure (3–6). The origin of this dysfunction is poorly understood, although we have recently used ^{31}P magnetic resonance spectroscopy (MRS) to show that high-energy phosphate metabolism [measured as the phosphocreatine (PCr)/ATP ratio] is impaired following 17 d of exposure to a high-altitude hypoxic environment (7, 8). This impairment is also associated with a decline in diastolic function. However, whether such changes represent a unique response to the high-altitude field environment is unknown. So, too, is the time course of onset and whether it may underlie the diastolic impairment observed in the early stages of normobaric hypoxia. Here, we used ^{31}P-MRS, magnetic resonance imaging (MRI), and echocardiography to study the changes in cardiac function and metabolism associated with 20 h of exposure to normobaric hypoxia.

METHODS AND METHODS

The research was carried out according to the principles of the declaration of Helsinki, and approval was obtained from the University of Oxford ethics committee.

Subjects

Recruited for this study were 12 male, healthy, recreationally active nonsmokers, aged 20 to 41 yr, with no baseline endo-

[1] Correspondence: Department of Physiology, Anatomy and Genetics, Sherrington Bldg., The University of Oxford, Parks Rd., Oxford OX1 3PT, UK. E-mail: cameron.holloway@dpag.ox.ac.uk

doi: 10.1096/fj.11-183426

0892-6638/11/0025-3130 © FASEB

crinological abnormalities or significant medical comorbidities. None had traveled to an altitude beyond 1500 m within the previous 12 mo or took routine or incidental medications. Subjects' weight and height were measured on arrival at the Oxford Cardiac Magnetic Resonance (OCMR) unit. Body fat percentage, water percentage, and lean weight were obtained using a bioimpedance analyzer (Bodystat, Douglas, UK).

Blood and urine tests

Fasting venous blood samples were taken on the morning before entering the hypoxic chamber, after hypoxic exposure, and on arrival at the OCMR unit. In addition, 4 subjects had 18-gauge venous cannulas placed in an anticubital vein with blood samples taken every 4 h for plasma glucose and free fatty acid (FFA) assays. Samples were immediately centrifuged, and the plasma supernatant was frozen for determination of glucose and cholesterol. Plasma metabolites were measured using an ABX Pentra Clinical Chemistry bench-top analyzer (Horiba ABX, Montpellier, France). Urine dipstick analysis was performed for ketones, glucose, and specific gravity.

Hypoxia protocol

After 2 h of rest following the prescans, subjects were exposed to 20 h of normobaric hypoxia in custom-built chambers measuring 2.3 × 1.6 × 2.2 m, being kept awake during daylight hours and allowed to sleep overnight, as described previously (9). Subjects did not spend >15 min in total outside the hypoxic chamber during the 20 h. Minute ventilation and heart rates were continuously assessed using Lifeshirts (Vivometrics, Ventura, CA, USA). End-tidal partial pressure of oxygen ($P_{ET}O_2$, an estimate of arterial P_aO_2) was analyzed by mass spectroscopy of samples drawn from nasal catheters every 20 ms. Chamber oxygen/nitrogen composition was computer adjusted every 5 min such that $P_{ET}O_2$ was maintained between 50 and 60 mmHg, and peripheral oxygen saturation (Sao_2) at ≥80%. We did not control for environmental changes in carbon dioxide. To allow heart rates to return to baseline levels after the hypoxic exposure, subjects rested in room air for up to 1 h prior to assessment at the nearby OCMR unit.

Measurement of blood pressure, cardiac volume, mass, and function

Resting heart rates were assessed by electrocardiogram. Systolic and diastolic blood pressure were measured. Left and right ventricular cardiac volume, mass, and function were assessed using cardiac MRI in a 1.5-T Siemens Sonata clinical scanner (Siemens Medical Solutions,, Erlangen, Germany). With the subjects in a supine position, pilot images were acquired, followed by horizontal and vertical long-axis cine images. A stack of steady-state, free precession (SSFP), short-axis cine images were subsequently obtained using breath holding and cardiac gating (10). The short-axis images were obtained in a prospective manner, from the base to the apex, in 1-cm-thick slices. Left and right ventricular endocardium and epicardium were manually contoured from the short-axis cine images. Using Argus processing (Siemens Medical Solutions), left and right ventricular volume, ejection fractions, and left ventricular mass were obtained (11). On average, 19 phases over each cardiac cycle were acquired.

Echocardiography

Left ventricular diastolic function was assessed by transthoracic echocardiographic evaluation of mitral inflow (*E*/*A*) and an average tissue Doppler (*E*′; iE33 echocardiograph, Philips, Amsterdam, The Netherlands). Subjects were scanned in a left lateral position with pulse wave velocity obtained at the mitral valve tips and tissue Doppler at the basal interventricular septum, respectively. Peak tricuspid regurgitant jet velocity was measured as an estimate of pulmonary artery pressure.

Cardiac MRS

^{31}P-MRS was used to measure the cardiac PCr/ATP ratio on a Siemens 3-T Tim Trio MR system, as described previously (12). Subjects lay prone with the left ventricle positioned over the center of a modified Siemens heart/liver ^{31}P coil in the magnet isocenter. ^{31}P-MR spectroscopy was performed using 3-dimensional acquisition-weighted chemical shift imaging (AW-CSI), utilizing the ultrashort echo time (UTE)-CSI technique (TE=0.3 ms) in conjunction with an optimized radiofrequency (RF) pulse centered between γ and α ATP resonances to maximize the signal-to-noise ratio (SNR), improve baseline artifacts, and ensure uniform excitation of all spectral peaks (13). The acquisition matrix size was 16 × 16 × 8 voxels, and the field of view was 240 × 240 × 200 mm, with 10 averages at *k*-space center. All acquisitions were prospectively cardiac gated with data acquisition during diastole. Nuclear Overhauser Enhancement (NOE) was used to increase the SNR (14).

Proton localization images were used to obtain short-axis left ventricular planes. To reduce potential signal contamination, 2 saturation bands were placed over the anterior chest wall skeletal muscle (12). The CSI grid was positioned on a slice designated as the first short-axis slice, in which the papillary muscle became visible, and rotated to obtain 3 voxels containing midventricular septal myocardium (**Fig. 1** and ref. 12).

All analyses of cardiac spectra were performed by 2 independent spectroscopists, after data blinding. Nonlocalized inversion recovery spectra were acquired to measure the flip angle at a reference vial containing phenylphosphonic acid (PPA) located inside the RF coil housing. This information was used in conjunction with a calculated RF field profile to determine and correct for the flip angle at each of the spectral voxels for every acquisition, using in-house-developed software in Matlab 6.5 (Mathworks, Natick, MA, USA). The selected spectra were summed, preprocessed (DC and baseline correction), and fitted using the automated processing algorithm AMARES within the jMRUI software packages (jMRUI, Leipzig, Germany). NOE, RF saturation correction

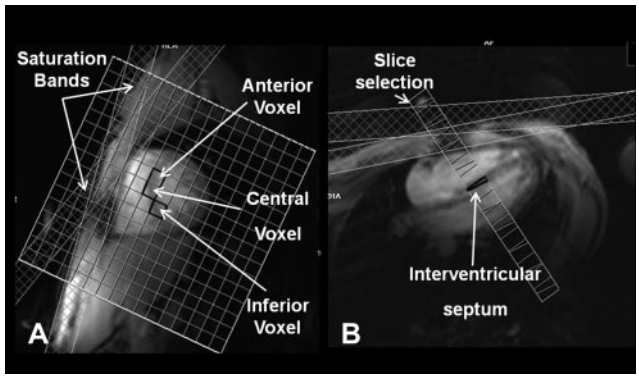

Figure 1. Voxel selection for ^{31}P magnetic resonance spectroscopy. *A*) Short-axis image with 3 voxels in the interventricular septum of a midventricular slice. *B*) Four-chamber image showing slice selection.

TABLE 1. *Body weight and composition measurements in subjects before and after hypoxic exposure*

Parameter	Prehypoxia	Posthypoxia	*P*
Hemodynamics			
Heart rate (bpm)	57 ± 3	57 ± 3	0.98
Systolic BP (mmHg)	119 ± 3	122 ± 3	0.32
Diastolic BP (mmHg)	79 ± 1	78 ± 2	0.64
Body weight and composition			
Total body weight (kg)	81.0 ± 3.4	80.5 ± 3.3	0.02
Body mass index (kg/m^2)	24.6 ± 1.0	24.4 ± 1.0	0.04
Fat (% body weight)	17 ± 2	14 ± 2	0.57
Water (% body weight)	56 ± 1	56 ± 1	0.62
Water (L)	45 ± 1	45 ± 1	0.74

Data are expressed as means ± SE; n =12. Bpm, beats per minute, BP, blood pressure.

factors (identified in previous experiments), and blood correction factors were applied (12).

Statistics

Values are presented as means ± SE. Differences were tested using a paired Student's t test and considered significant at values of $P < 0.05$. A sample size of 12 was chosen, so as to be able to detect a difference in PCr/ATP of 0.2 with 90% power, and with an α significance of 0.05 (12).

RESULTS

All subjects tolerated the hypoxia without significant illness. None had to leave the chambers for >5 min for rest breaks.

Heart rates rapidly increased from 57 beats per minute (bpm) prechamber, to 80 bpm when in the hypoxic environment ($P<0.01$). The relative tachycardia was maintained over the study, never falling below the preassessment awake heart rates. Minute ventilation doubled after exposure to hypoxia and remained elevated during the day, with a significant reduction overnight compared with awake values ($P<0.01$). Heart rates and blood pressure recordings had returned to baseline within 30 min following removal from hypoxia (**Table 1**). There was a 0.5-kg loss of total body weight after hypoxia, with no change in fat or percentage water.

After hypoxic exposure, there were increases in blood hemoglobin (16.2±0.3 to 17.0±0.3 g/dl, $P<0.001$), platelet count (238±17 to 249±17×10^9/L, $P=0.02$) and white cell count (5.9±0.6 to 6.8±0.7×10^9/L, $P=0.02$, **Table 2**). During hypoxia, there was a 3-fold elevation in circulating FFAs, from 0.19 ± 0.07 to 0.66 ± 0.08 mM (**Fig. 2**, $P<0.05$), with no change in plasma cholesterol or glucose. There was no change in plasma urea, but there was a significant increase in plasma creatinine from 96 ± 2 to 102 ± 2 mM ($P<0.001$). There was no change in urine pH, metabolites, or specific gravity (Table 2).

TABLE 2. *Blood and urine measurements in subjects before and after hypoxic exposure*

Parameter	Prehypoxia	Posthypoxia	*P*
Blood			
Hemoglobin (g/L)	16.2 ± 0.3	17.0 ± 0.3	<0.001*
Platelets (10^9/L)	238 ± 17	249 ± 17	0.02*
WCC (10^9/L)	5.1 ± 0.6	5.7 ± 0.7	0.02*
Urea (mM)	4.5 ± 0.2	4.0 ± 2	0.13
Creatinine μM	96 ± 2	102 ± 2	<0.001*
Cholesterol (mM)	4.6 ± 0.3	4.7 ± 0.6	0.12
Glucose (mM)	4.9 ± 1.0	4.9 ± 1.0	0.91
Urine metabolites			
pH	6.3 ± 1	6.3 ± 1	1
Ketones g/L	0 ± 0	0 ± 0	1
Glucose g/L	0 ± 0	0 ± 0	1
Density (specific gravity)	1.02 ± 0.0	1.02 ± 0.0	0.32

Data are expressed as means ± SE; n = 12. WCC, white cell count. *$P < 0.05$ *vs.* corresponding preassessment.

Cardiac high-energy phosphate metabolism and cardiac function

Cardiac PCr/ATP fell by 15%, from 2.0 ± 0.1 to 1.7 ± 0.1 ($P<0.01$) after 20 h in the hypoxic chamber (**Fig. 3**). There were no differences in cardiac ejection fractions or left or right ventricular volume or mass,

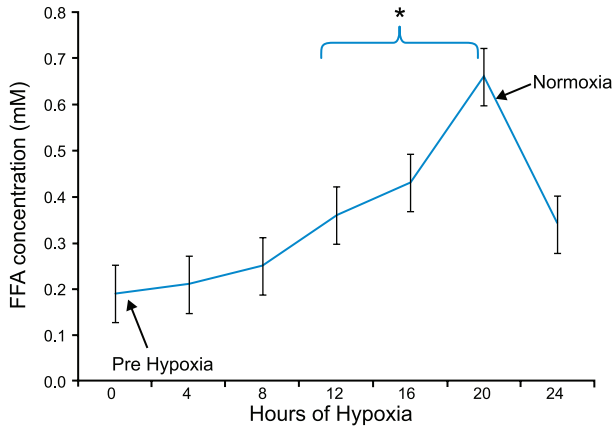

Figure 2. Changes in plasma FFA concentrations (n=4) during and after hypoxic exposure. *$P < 0.05$ *vs.* prehypoxia.

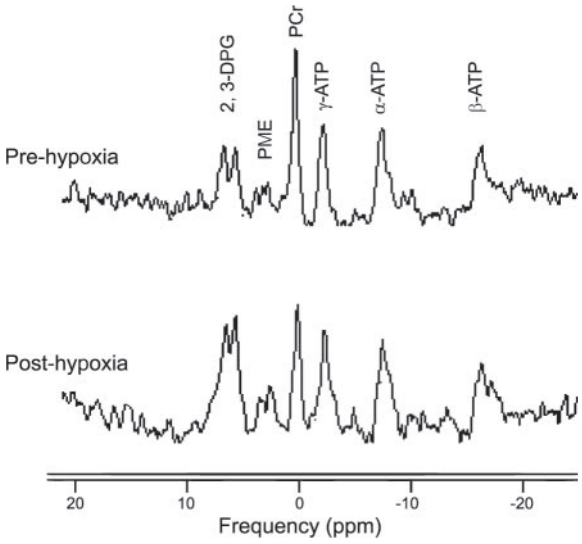

Figure 3. Example of cardiac ^{31}P MR spectra pre- and posthypoxia, showing a lower PCr/ATP after 20 h of hypoxia.

measured using cardiac cine MRI (**Table 3**). Echocardiographically assessed E/E' ratio rose from 6.1 ± 0.4 to 7.5 ± 0.7, ($P<0.01$), owing to a decrease in E' of 2.3 cm/s ($P<0.01$), with no change in E/A ratio. There was no change in peak tricuspid regurgitant velocity (0.81±0.05 *vs.* 0.84±0.08 m/s, $P>0.05$, $n=7$), as a measure of right ventricular systolic pressure (Table 3).

DISCUSSION

In healthy subjects, left ventricular high-phosphate energy metabolism was impaired following 20 h exposure to normobaric hypoxia. We propose that the impaired high-energy phosphate metabolism may be causally associated with the observed changes in cardiac diastolic function.

We cannot attribute functional changes to substantial differences in preload. Following hypoxic exposure, a small decrease in total body weight and an increase in hemoglobin, platelet, and white cell counts was found to have occurred, consistent with previously observed diuresis and bone marrow activation (15). The increased plasma creatinine is unlikely to be due to such a modest level of dehydration (especially given the lack of change in urine specific gravity or plasma urea), and thus most likely reflects muscle breakdown. Nor, given the return of hemodynamic parameters to normal, can we attribute such changes to alterations in cardiac rate or left ventricular afterload.

Rather, such changes may reflect fundamental metabolic changes in response to hypoxia (16). Indeed, major metabolic adaptation in cellular energy production is required to reduce oxygen consumption in hypoxia (17, 18). The heart normally produces 6 kg ATP/d (20 times its own weight) for contractile function (19). This huge cardiac energy requirement may rise in hypoxia, with greater cardiac work required to drive increased cardiac output, yet mitochondrial respiration and oxygen consumption are reduced (16, 20). To achieve this adaptive state of oxygen conservation, acute hypoxia initiates a complex cascade of molecular events, including stabilization of HIF and activation of HIF-independent pathways (21), to alter metabolism and decrease mitochondrial respiration, while increasing ATP production *via* anaerobic metabolism (16). Oxygen-sensitive enzymes, including HIF and prolyl hydroxlases, have gained recognition as modulators of acute energy production pathways (22).

TABLE 3. *Measurement of cardiac ejection fraction, left or right ventricular volume, or mass using cardiac cine MRI*

Parameter	Prehypoxia	Posthypoxia	*P*
High-energy phosphates (PCr/ATP)	2.0 ± 0.1	1.7 ± 0.1	<0.01*
Left ventricle			
Ejection fraction (%)	67 ± 1	66 ± 2	0.09
End-diastolic volume (ml)	167 ± 9	164 ± 7	0.50
End-systolic volume (ml)	57 ± 3	59 ± 4	0.30
Stroke volume (ml)	118 ± 6	112 ± 5	0.08
Left ventricular mass (g)	152 ± 6	154 ± 6	0.32
Right ventricle			
Ejection fraction (%)	58 ± 2	59 ± 1	0.18
End-diastolic volume (ml)	192 ± 7	189 ± 7	0.16
End-systolic volume (ml)	80 ± 5	79 ± 4	0.78
Stroke volume (ml)	114 ± 6	110 ± 4	0.16
Maximal TR (m/s)	0.81 ± 0.05	0.84 ± 0.08	0.21
Left ventricular diastolic function			
E/E'	6.0 ± 0.4	7.5 ± 0.7	<0.01*
E' (cm/s)	12.3 ± 1.0	10.0 ± 0.9	<0.01*
E/A	1.6 ± 0.2	1.6 ± 0.2	0.70

Data are expressed as means ± SE. Cardiac high-energy phosphates were measured using cardiac 31P spectroscopy and left and right ventricular volumes and function using cardiac magnetic resonance imaging. Diastolic function was measured using transthoracic echocardiography.*$P < 0.05$ *vs.* corresponding preassessment.

In hypoxic conditions, HIF, *via* pyruvate dehydrogenase kinase 1 (PDK1), limits pyruvate entering the Krebs cycle, reducing mitochondrial oxygen consumption (23). Moreover, HIF-1 augments transcription of genes coding for glycolytic enzymes and glucose transporters (24), which increases conversion of glucose to lactate in anaerobic conditions. Increased glucose utilization, in preference to fat, consumes less oxygen in the generation of ATP, favoring this substrate switch in hypoxia. Despite these potential adaptive changes, we have demonstrated decreased cardiac PCr/ATP, which may indicate an inability of the myocardium to fully adapt in the early stages of hypoxia, leading to reduced mitochondrial oxidative phosphorylation. We observed sustained elevation in plasma FFAs, which increase mitochondrial uncoupling (dissipating the proton electrochemical gradient by allowing protons to reenter the mitochondrial matrix without the concomitant synthesis of ATP; ref. 25). Hence, the elevated circulating FFA concentrations may have impaired cardiac PCr/ATP in hypoxia (26).

Cardiac dysfunction in hypoxia may, therefore, result from inadequate synthesis of myocardial high-energy phosphates, especially during the period of cellular reorganization and adaptation. A reduction in PCr/ATP may reflect this altered metabolism and is commensurate with the reduced diastolic function we observed. The implications of the mildly altered E/E' observed in our subjects are uncertain, especially as we did not observe clinical evidence of heart failure, but they are consistent with previous findings of early diastolic impairment after hypoxic exposure (4) and may indicate early functional change along with the reduced PCr/ATP. This hypothesis is substantiated by evidence that reduced PCr/ATP precedes myocardial dysfunction (19, 27). Cardiac energy requirements (including those related to calcium reuptake in the sarcoplasmic reticulum) are high in diastole. Thus, it is conceivable that initial changes in cardiac function will be observed in diastole. Moreover, impaired cardiac diastolic function has consistently been observed in the early stages of hypoxic adaptation (3, 4, 6). It seems very unlikely that 24 h of being sedentary (without hypoxia) would alter cardiac PCr/ATP and diastolic function, although this remains a limitation of the present study.

The changes we have observed are consistent with studies showing impaired myocardial high-energy phosphate metabolism and function after weeks and months of hypobaric hypoxia at Mount Everest base camp (7, 8). Together, they suggest that such changes may be initiated early, may be sustained, and are related to the hypoxia itself, rather than some other aspect of the hypobaric environment. Whether changes in the cardiac energy metabolism and function occur in $<$20 h hypoxia is unknown.

The metabolic pathways of adaptation to hypoxia are important to our understanding of fetal survival, hypoxia resistance in cardiac disease, and the contribution of hypoxia to heart failure. In fetal life, the heart thrives and develops in severe hypoxia (28). Changes in energy metabolism seen in human adaptation to hypoxia, including increased glucose utilization and down-regulation of flux through the Krebs cycle, share many similarities with altered mitochondrial metabolism in the fetal heart and in heart failure (28, 29). Further investigation is required to determine the time course if activation of hypoxic adaptive pathways play a role driving in the impaired cardiac energetics seen in heart failure. A greater understanding of such adaptation might allow the identification of new molecular therapeutic targets.

CONCLUSIONS

The left ventricle exhibits a rapid energetic response to normobaric hypoxia, similar to that seen after sustained exposure to hypobaric hypoxia over longer time periods. Despite cardiac adaptation to hypoxia, reduced cardiac PCr/ATP and dysfunction still occur. We have shown that 20 h of normobaric hypoxia led to sustained elevation of plasma FFAs, decreased myocardial PCr/ATP, and changes that may represent early diastolic dysfunction in normal human heart. FJ

The authors thank Emma Carter for plasma metabolite analysis and Dr Robert Olszewski for assistance with the echocardiography. The authors are also very thankful to Federico Formenti and David O'Connor for their assistance with running the hypoxic chamber. The British Heart Foundation (BHF) supported this work. S.N. acknowledges support from the BHF Centre of Research Excellence, Oxford, UK.

REFERENCES

1. Rader, D. J., and Daugherty, A. (2008) Translating molecular discoveries into new therapies for atherosclerosis. *Nature* **451,** 904–913
2. Semenza, G. L. (2004) O_2-regulated gene expression: transcriptional control of cardiorespiratory physiology by HIF-1. *J. Appl. Physiol.* **96,** 1173–1177; discussion 1170–1172
3. Balasubramanian, V., Behl, A., Das, G. S., Wadhwa, A. K., Mathew, O. P., and Hoon, R. S. (1978) Effect of digoxin and diuretics on high-altitude left ventricular dysfunction. *Circulation* **57,** 1180–1185
4. Kjaergaard, J., Snyder, E. M., Hassager, C., Olson, T. P., Oh, J. K., and Johnson, B. D. (2006) The effect of 18 h of simulated high altitude on left ventricular function. *Eur. J. Appl. Physiol.* **98,** 411–418
5. Allemann, Y., Rotter, M., Hutter, D., Lipp, E., Sartori, C., Scherrer, U., and Seiler, C. (2004) Impact of acute hypoxic pulmonary hypertension on LV diastolic function in healthy mountaineers at high altitude. *Am. J. Physiol. Heart Circ. Physiol.* **286,** H856–H862
6. Boussuges, A., Molenat, F., Burnet, H., Cauchy, E., Gardette, B., Sainty, J. M., Jammes, Y., and Richalet, J. P. (2000) Operation Everest III (Comex '97): modifications of cardiac function secondary to altitude-induced hypoxia. An echocardiographic and Doppler study. *Am. J. Respir. Crit. Care Med.* **161,** 264–270
7. Holloway, C. J., Montgomery, H. E., Murray, A. J., Cochlin, L. E., Codreanu, I., Hopwood, N., Johnson, A. W., Rider, O. J., Levett, D. Z., Tyler, D. J., Francis, J. M., Neubauer, S., Grocott, M. P., and Clarke, K. Cardiac response to hypobaric hypoxia: persistent changes in cardiac mass, function, and energy metabolism after a trek to Mt. Everest Base Camp. *FASEB J.* **25,** 792–796

8. Hochachka, P. W., Clark, C. M., Holden, J. E., Stanley, C., Ugurbil, K., and Menon, R. S. (1996) 31P magnetic resonance spectroscopy of the Sherpa heart: a phosphocreatine/adenosine triphosphate signature of metabolic defense against hypobaric hypoxia. *Proc. Natl. Acad. Sci. U. S. A.* **93,** 1215–1220
9. Howard, L. S., Barson, R. A., Howse, B. P., McGill, T. R., McIntyre, M. E., O'Connor, D. F., and Robbins, P. A. (1995) Chamber for controlling end-tidal gas tensions over sustained periods in humans. *J. Appl. Physiol.* **78,** 1088–1091
10. Hudsmith, L., and Neubauer, S. (2008) Detection of myocardial disorders by magnetic resonance spectroscopy. *Nat. Clin. Pract.* **5,** s49–s56
11. Hudsmith, L. E., Petersen, S. E., Tyler, D. J., Francis, J. M., Cheng, A. S., Clarke, K., Selvanayagam, J. B., Robson, M. D., and Neubauer, S. (2006) Determination of cardiac volumes and mass with FLASH and SSFP cine sequences at 1.5 vs. 3 tesla: a validation study. *J. Magn. Reson. Imaging* **24,** 312–318
12. Tyler, D. J., Emmanuel, Y., Cochlin, L. E., Hudsmith, L. E., Holloway, C. J., Neubauer, S., Clarke, K., and Robson, M. D. (2009) Reproducibility of (31)P cardiac magnetic resonance spectroscopy at 3 T. *NMR Biomed.* **22,** 405–413
13. Robson, M. D., Tyler, D. J., and Neubauer, S. (2005) Ultrashort TE chemical shift imaging (UTE-CSI). *Magn. Reson. Med.* **53,** 267–274
14. Bottomley, P. A., and Hardy, C. J. (1992) Proton Overhauser enhancements in human cardiac phosphorus NMR spectroscopy at 1.5 T. *Magn. Reson. Med.* **24,** 384–390
15. Koller, E. A., Buhrer, A., Felder, L., Schopen, M., and Vallotton, M. B. (1991) Altitude diuresis: endocrine and renal responses to acute hypoxia of acclimatized and non-acclimatized subjects. *Eur. J. Appl. Physiol. Occup. Physiol.* **62,** 228–234
16. Kelly, D. P. (2008) Hypoxic reprogramming. *Nat. Genet.* **40,** 132–134
17. Ramirez, J. M., Folkow, L. P., and Blix, A. S. (2007) Hypoxia tolerance in mammals and birds: from the wilderness to the clinic. *Annu. Rev. Physiol.* **69,** 113–143
18. Andrews, M. T. (2004) Genes controlling the metabolic switch in hibernating mammals. *Biochem. Soc. Trans.* **32,** 1021–1024
19. Neubauer, S. (2007) The failing heart—an engine out of fuel. *N. Engl. J. Med.* **356,** 1140–1151
20. Duranteau, J., Chandel, N. S., Kulisz, A., Shao, Z., and Schumacker, P. T. (1998) Intracellular signaling by reactive oxygen species during hypoxia in cardiomyocytes. *J. Biol. Chem.* **273,** 11619–11624
21. Arany, Z., Foo, S. Y., Ma, Y., Ruas, J. L., Bommi-Reddy, A., Girnun, G., Cooper, M., Laznik, D., Chinsomboon, J., Rangwala, S. M., Baek, K. H., Rosenzweig, A., and Spiegelman, B. M. (2008) HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1alpha. *Nature* **451,** 1008–1012
22. Aragones, J., Schneider, M., Van Geyte, K., Fraisl, P., Dresselaers, T., Mazzone, M., Dirkx, R., Zacchigna, S., Lemieux, H., Jeoung, N. H., Lambrechts, D., Bishop, T., Lafuste, P., Diez-Juan, A., Harten, S. K., Van Noten, P., De Bock, K., Willam, C., Tjwa, M., Grosfeld, A., Navet, R., Moons, L., Vandendriessche, T., Deroose, C., Wijeyekoon, B., Nuyts, J., Jordan, B., Silasi-Mansat, R., Lupu, F., Dewerchin, M., Pugh, C., Salmon, P., Mortelmans, L., Gallez, B., Gorus, F., Buyse, J., Sluse, F., Harris, R. A., Gnaiger, E., Hespel, P., Van Hecke, P., Schuit, F., Van Veldhoven, P., Ratcliffe, P., Baes, M., Maxwell, P., and Carmeliet, P. (2008) Deficiency or inhibition of oxygen sensor Phd1 induces hypoxia tolerance by reprogramming basal metabolism. *Nat. Genet.* **40,** 170–180
23. Papandreou, I., Cairns, R. A., Fontana, L., Lim, A. L., and Denko, N. C. (2006) HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab.* **3,** 187–197
24. Semenza, G. L., Roth, P. H., Fang, H. M., and Wang, G. L. (1994) Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. *J. Biol. Chem.* **269,** 23757–23763
25. Murray, A. J., Anderson, R. E., Watson, G. C., Radda, G. K., and Clarke, K. (2004) Uncoupling proteins in human heart. *Lancet* **364,** 1786–1788
26. Holloway, C. J., Cochlin, L. E., Emmanuel, Y., Murray, A., Codreanu, I., Edwards, L. M., Szmigielski, C., Tyler, D. J., Knight, N. S., Saxby, B. K., Lambert, B., Thompson, C., Neubauer, S., and Clarke, K. A high-fat diet impairs cardiac high-energy phosphate metabolism and cognitive function in healthy human subjects. *Am. J. Clin. Nutr.* **93,** 748–755
27. Jung, W. I., Hoess, T., Bunse, M., Widmaier, S., Sieverding, L., Breuer, J., Apitz, J., Schmidt, O., van Erckelens, F., Dietze, G. J., and Lutz, O. (2000) Differences in cardiac energetics between patients with familial and nonfamilial hypertrophic cardiomyopathy. *Circulation* **101,** E121
28. Rajabi, M., Kassiotis, C., Razeghi, P., and Taegtmeyer, H. (2007) Return to the fetal gene program protects the stressed heart: a strong hypothesis. *Heart Fail. Rev.* **12,** 331–343
29. Shen, W., Asai, K., Uechi, M., Mathier, M. A., Shannon, R. P., Vatner, S. F., and Ingwall, J. S. (1999) Progressive loss of myocardial ATP due to a loss of total purines during the development of heart failure in dogs: a compensatory role for the parallel loss of creatine. *Circulation* **100,** 2113–2118

Received for publication March 16, 2011.
Accepted for publication May 26, 2011.