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Effect of altitude on brain intracellular pH and inorganic phosphate levels

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Abstract

Normal brain activity is associated with task-related pH changes. Although central nervous system syndromes associated with significant acidosis and alkalosis are well understood, the effects of less dramatic and chronic changes in brain pH are uncertain. One environmental factor known to alter brain pH is the extreme, acute change in altitude encountered by mountaineers. However, the effect of long-term exposure to moderate altitude has not been studied. The aim of this two-site study was to measure brain intracellular pH and phosphate-bearing metabolite levels at two altitudes in healthy volunteers, using phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS). Increased brain pH and reduced inorganic phosphate (Pi) levels were found in healthy subjects who were long-term residents of Salt Lake City, UT (4720 ft/1438 m), compared with residents of Belmont, MA (20 ft/6 m). Brain intracellular pH at the altitude of 4720 ft was more alkaline than that observed near sea level. In addition, the ratio of inorganic phosphate to total phosphate signal also shifted toward lower values in the Salt Lake City region compared with the Belmont area. These results suggest that long-term residence at moderate altitude is associated with brain chemical changes.

Keywords

Hypoxia; Spectroscopy; Altitude

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1. Introduction

Normal brain activity is associated with task-related pH changes (Magnotta et al., 2012). Brain pH plays a critical role in maintaining optimal function of the central nervous system. In fact, an increase or decrease in the blood concentration of free protons of as little as 0.1 μM is fatal (Richerson, 2004). An increased concentration of intracellular hydrogen ions, resulting from acidification, may bind with brain proteins, potentially altering their shape and function as enzymes, transporters, contractile elements and structural components (Gowrishankar et al., 2007). Conversely, in pathological states that induce respiratory alkalosis, hypocapnia is known to produce neuronal ischemia and injury (Laffey and Kavanagh, 2002; Curley et al., 2010). Acute experimental hyperventilation causes a washout of carbon dioxide (CO_2) in healthy volunteers, thereby increasing brain pH (Friedman et al., 2007). While the central nervous system syndromes associated with extreme degrees of acidosis and alkalosis are well understood (Snively and Becker, 1968), the clinical consequences of chronic, milder pH derangements are less clear.

It is well known that acute, short-term exposure to high altitudes increases the ventilation rate during ascent. Goldberg et al. (1992) measured brain pH using phosphorus magnetic resonance spectroscopy (^{31}P-MRS) in four men who resided for 7 days in a hypobaric chamber at 447 Torr (simulated altitude = 4267 m, pO_2 = 93 Torr). Although the subjects' brain pH increased from 6.998 ± 0.029 (pre-exposure) to 7.023 ± 0.046 (post-exposure), the change may not have reached significance due to the small sample size, and the effects of longer-term, chronic exposure to altitude on brain pH have not been studied.

As one ascends above sea level, the partial pressure of oxygen (O_2) in the inspired air falls with ambient barometric pressure (Martin et al., 2010). While the proportion of O_2 remains constant at 20.93% up to 12,000 m, barometric pressure decreases exponentially with altitude (Wilson et al., 2009). The resulting decrease in the driving gradient across the lung alveoli can compromise oxygen delivery to the tissues. To maintain O_2 supplies to tissues at increased altitude, acclimatization occurs, consisting of adaptive changes including improved tissue oxygen delivery (Bouverot, 1985), increased respiratory rate (Nishimura et al., 1989), altered acid-base balance, and renal acid excretion (Gonzalez et al., 1990). Some studies have noted that acute exposure to hypoxia causes cerebral vasodilatation (Lennox and Gibbs, 1932). However, this effect diminishes within a few days with continued exposure to hypobaric hypoxia at altitude (Roy et al., 1968). With chronic exposure to high altitude (Milledge and Sorensen, 1972; Marc-Vergnes et al., 1974; Sorensen et al., 1974), measurement of the cerebral arterial-venous oxygen difference suggests that cerebral blood flow is slightly below sea level values in highlanders. This reduction may be explained by increased blood viscosity resulting from elevated hemoglobin concentration at altitude, or may result from the decreased blood CO_2 content.

Neuroimaging has been proposed as a rational basis for studying the transient and permanent alterations in brain function associated with hypobaric hypoxia and altitude (Raichle, 1999). In this study, we used phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS), a noninvasive technique that provides information on phosphate-bearing metabolites in vivo, to measure brain intracellular pH. The aim of this study was to compare the intracellular

brain pH of healthy adults residing in Salt Lake City, UT, USA (altitude = 4720 ft/1438 m), with values obtained from healthy subjects in Belmont, MA, USA (altitude = 20 ft/6 m). We also conducted a comparative analysis of individual ^{31}P-MRS metabolites, to investigate the effect of altitude on high-energy phosphorus neurochemicals. Previous work has shown that the arterial pressure of CO_2 in healthy nonsmokers in Salt Lake City is lower than in near-sea level residents (Los Angeles, CA, USA: 233 ft/71 m; and Hartford, CT, USA 59 ft/18 m) (Crapo et al., 1999). We therefore hypothesized that brain pH would be increased in long-term residents at moderate altitude, possibly as a result of chronic hyperventilation and resulting hypocapnia.

2. Methods

2.1. Subject selection

The Institutional Review Boards of the University of Utah and McLean Hospital approved study's protocol. Written informed consent was obtained from all study subjects. Healthy volunteers were recruited in Salt Lake City, UT (n = 26; 13 males and 13 females; mean age = 27.2 ± 5.6 years), and in Belmont, MA (n = 13; 6 males and 7 females; 29.3 ± 9.0 years), respectively. Eligible subjects had no history of psychiatric or neurological disease and had lived in Salt Lake City or in the Belmont area for a minimum of 1 year, with no travel in the 3 months before scanning. Other inclusion criteria were (1) age range from 18 to 55 years and (2) no medical illness identified by history and physical examination. Exclusion criteria were (1) clinically significant neurological, medical, or psychiatric illness, including substance use disorders; (2) contraindication to magnetic resonance imaging; (3) positive urine drug screen; and (4) current prescription or over-the-counter medications. Pregnant females and nursing mothers were also excluded.

2.2. Data acquisition

Subjects recruited independently at each site were scanned using similar Siemens 3 T MRI scanners (Tim-Trio, Siemens Medical Solutions, Erlangen, Germany) and identical ^{31}P/^{1}H dual-tuned volume head coils (Clinical MR Solutions, LLC, Brookfield, WI, USA). All ^{31}P-MRS spectra were acquired using an identical ISIS localized pulse sequence (Jeong et al., 2011), a voxel size of 8 × 11 × 3 cm^3 along RL × AP × SI directions (RL: Right-Left; AP: Anterior-Posterior; SI: Superior-Inferior), repetition time (TR) 20.5 s, echo time (TE) 0.05 ms, scan number 16, acquisition time 5 min 28 s, receiver bandwidth 2.5 kHz, and vector size 1024. The ISIS sequence was implemented without proton decoupling, and the center frequency of the RF pulse was set on phosphocreatine (PCr). A hyperbolic secant RF pulse with a bandwidth of 4 kHz and duration of 5.12 ms was used for ISIS localization. To facilitate voxel placement, high resolution T_1 weighted images were acquired using a three-dimensional magnetization-prepared rapid gradient echo acquisition (MPRAGE) pulse sequence with the following parameters: TR/TE/TI = 2000/3.37/1100 ms; flip angle = 8°; field of view = 256 × 192 × 144 mm^3; 256 × 192 × 144 matrix size; 1 × 1 × 1 mm^3 spatial resolution; Bandwidth = 300 Hz/pixel. To effectively saturate unnecessary fat signal from the scalp, six outer volume saturation bands were placed in the scalp and skull regions. Fig. 1 depicts the region of interest (ROI) in three planes, and displays a representative ^{31}P-MRS

spectrum. The Anterior–Posterior (AC–PC) line was identified on midsagittal images acquired from the MPRAGE pulse sequence.

2.3. Data processing

One spectroscopist (X.F.S.) analyzed the MRS data from both study sites, without knowledge of subjects' demographic and clinical information. All spectra were pre-processed using locally written MATLAB (The Mathworks, Inc., Natick, MA, USA) programs. Each spectrum was apodized with 10 Hz of exponential decay line broadening before zero-filling and fast Fourier transform. Zero- and first-order phase corrections were performed on all spectra. The signal intensity of each metabolite was obtained using the Advanced Magnetic Resonance (AMARES) fitting algorithm within jMRUI (Naressi et al., 2001). The AMARES routine incorporates the use of prior spectral knowledge such as J-coupling constants, chemical shifts and line width. In our time domain-free induction decay data-fitting template, we modeled PCr, Pi, dinucleotide, the metabolic precursors of phospholipid synthesis (PE, phosphoethanolamine; and PC, phosphocholine) and the α-, β-, and γ-NTP as exponentially damped sinusoids. During data preprocessing, zero- and first-order phase correction was applied manually. Metabolite signals were calculated as a percentage of the total phosphorus (TP) signal acquired from the ROI. The whole brain intracellular pH values were computed using the modified Henderson–Hasselbalch equation (Iotti et al., 2000). The formula ($pH = 6.77 + \log_{10}[(\Delta_{\mathbf{Pi-PCr}} - 3.29)/(5.68 - \Delta_{\mathbf{Pi-PCr}})]$) uses the fitted chemical shift of Pi and PCr as described by Petroff et al. (1985). In the present study, all the chemical shifts were determined from experimental data fitting. Our hypothesis regarding altitude-sensitive pH group differences was tested using analysis of covariance (ANCOVA), controlling for subjects' age and gender. All statistical calculations were performed using R, version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria) (R Development Core Team, 2010).

3. Results

We enrolled 26 subjects at the Salt Lake City site (13 female; 13 male; mean age 27.2 (SD 5.6) years) and 13 subjects in Belmont, MA (7 female; 6 male; mean age 29.3 (SD 9.0) years). There were no significant differences in the gender composition ($p = 1$) or mean age ($p = 0.38$) of the two samples. Group comparison of brain pH and ^{31}P-MRS metabolites was performed between sites. Fig. 2a depicts the brain pH values of subjects residing at moderate altitude, and near sea level. We observed a significantly higher mean intracellular brain pH in long-term residents of Salt Lake City compared with long-term residents of Belmont (mean pH of subjects from SLC/Belmont = 7.022 ± 0.021/6.997 ± 0.017; $[H^+]_{SLC}/[H^+]_{Belmont}$ = 0.093 μmol/l/0.1 μmol/l; p-value = 0.001). This observation is consistent with our hypothesis, and is similar to the mean pH values reported by Goldberg et al. (1992). To increase our confidence in this finding, we also determined brain pH using an independent method published by Pettegrew et al., 1988. Using the Pettegrew method, we similarly found that subjects' mean brain pH was higher in Salt Lake City compared with Belmont ($p = 0.004$).

An additional finding was that Pi levels in Salt Lake City subjects (mean Pi ± SD = 0.058 ± 0.015) were significantly lower than those observed in the Belmont subjects (mean Pi ± SD = 0.075 ± 0.021), as shown in Fig. 2b. Inorganic phosphate plays an important role in regulating energy metabolism (Chance et al., 1988). No significant differences were observed in the other measured ^{31}P-MRS metabolite levels, as shown in Table 1.

To assess the potential influence of signal-to-noise ratios (SNRs) on intracellular pH measurements and the stability of our fitting method, simulations with a variety of spectral SNRs were undertaken by adding random noise to a noise-free ^{31}P spectrum. This noise-free spectrum was generated using the AMARES fitting result from in vivo brain data. SNR is defined as the ratio of the PCr peak amplitude divided by the noise standard deviation. For each SNR value, $n = 50$ spectra with the addition of random noise of fixed standard deviation were generated and fitted, following the same post-processing steps used to process this study's in vivo data. The mean pH values and standard deviations were computed from the $n = 50$ fitted pH results using the AMARES routine in jMRUI. Fig. 3a demonstrates that the simulated pH value approaches pH0 with increased SNR. pH0 is a true pH value which is used for simulation of the variance around pH0 with respect to various signal to noise ratios. However, there is a pH bias that stems from the non-linear transform of the Henderson–Hasselbalch equation (Thompson and MacDonald, 1991; Newman, 1993). Therefore, the expected value of mean pH noise is not zero, but a negative value. In addition, the pH value standard deviation decreases as shown in Fig. 3b. When the SNR is greater than 9, the variation in pH values introduced by the fitting method is less than 0.020 units. In the present study, the mean SNR of subject's spectra from Salt Lake City, UT, and Belmont, MA, are computed as 18.6 and 14.6, respectively. For the simulated multiple averaged pH, the standard errors of the mean pH = 0.0055 and 0.0039 for $n = 13$ and $n = 26$ averages, respectively. These standard errors are significantly smaller than the difference (0.025) in the mean in vivo pH observed between the sites in Salt Lake City, UT, and Belmont, MA.

To investigate the comparability of spectral data across sites, a single cylinder-shaped phantom was produced and scanned at the University of Utah, then shipped to McLean Hospital and scanned using an identical pulse sequence. This 2 L phantom contained a solution consisting of methylphosphonic acid (MPA, 10 mM), phosphorylcholine (PC, 20 mM), and phosphate-buffered solution (Pi, 19.1 mM). Before scanning, the phantom was placed in the scanner room for 2 days to equilibrate. The scanner room temperatures in Salt Lake City and Belmont were measured as 22 °C and 21.7 °C, respectively. Both sites implemented the same ^{31}P –MRS pulse sequence used for in vivo data acquisition, with voxel size ($4 \times 4 \times 4$ cm^2) and spectral bandwidth (2 kHz). First order shimming on the water resonance within the voxel was performed. A similar degree of field inhomogeneity correction was achieved at both sites. As pH is computed from the relative chemical shift differences between Pi and PCr, the comparison of chemical shift differences among these metabolites was investigated. Fig. 4 provides a comparison of the spectra acquired from the phantom at Salt Lake City and Belmont. Columns A, B, and C represent spectral overlap from SLC (green)/Belmont (red), zoom-in MPA, and zoom-in PC/Pi. Table 2 shows the chemical shift differences among the three phosphorus metabolites. The maximum

uncertainty of site-dependent chemical shift change is 0.039, resulting in a unit uncertainty of 0.008 for a single pH measurement. The standard error of pH due to site differences for 13 subjects is 0.0022 pH units, which is less than the observed between-site pH difference (0.025). The results of this phantom experiment suggest that site-related uncertainty in pH measurement is unlikely to account for the observed pH difference in our in vivo experiment.

4. Discussion

The present study used ^{31}P-MRS to measure intracellular brain pH in healthy subjects at moderate altitude and near sea level. Our results confirmed our hypothesis by demonstrating a significantly increased whole brain pH in adult volunteers with no history of neurological or psychiatric illness who were long-term (i.e., >1 year) residents of the Salt Lake City (4720 ft/1438 m) area, compared with the Belmont (20 ft/6 m) area. An additional, secondary finding was a significantly lower mean cerebral Pi level in the moderate altitude subjects. To the best of our knowledge, this is the first ^{31}P-MRS study of alterations in brain pH and Pi in healthy subjects residing at moderate altitude versus near sea level.

Increasing altitude is associated with a logarithmic decrease in ambient barometric pressure, and a consequent decrease in the partial pressure of inspired oxygen. At the altitude of Salt Lake City (4720 ft/1438 m), the partial pressure of inspired oxygen is reduced by approximately 15% compared with sea level (Baillie, 2010; Hackett and Roach, 2011). This decline in the oxygen pressure gradient in the lung alveoli is known as hypobaric hypoxia.

In animal models, hypobaric hypoxia induces oxidative stress on the brain (Maiti et al., 2006), rapidly altering the expression of $n = 296$ genes in the oxidative stress pathway (Sethy et al., 2011). Similarly, human studies show that markers of oxidative stress and damage are detectable within 1 h of exposure to hypobaric hypoxia (Magalhaes et al., 2004). One consequence of hypobaric hypoxia is hyperventilation, which leads to a decreased partial pressure of CO_2 in the bloodstream. This reduction in the CO_2 substrate of the carbonic acid equation $[\mathrm{CO_2 + H_2O <->H_2CO_3 <->H^+ + HCO_3^-}]$ drives the equation to the left, resulting in decreased serum concentrations of hydrogen ion and bicarbonate, or respiratory alkalosis. In addition to this shift in the acid-base system, hypocapnia that is associated with hyperventilation reduces cerebral blood flow (Raichle and Plum, 1972; Nishimura et al., 2010). This occurs because CO_2 in the bloodstream is a vasodilator, and thus hypocapnia leads to cerebral vasoconstriction (Brian, 1998). Interestingly, not only does hypocapnia reduce cerebral blood flow, but cerebral autoregulation in humans does not adapt to sustained hypoxia (Krasney et al., 1990). This may be due to the fact that CO_2 is a critical signaling molecule involved in chemosensory control of breathing within the central nervous system (Gourine et al., 2005). In fact, the human respiratory response to hypoxia is attenuated by mild hypocapnia, and under conditions of moderate stable hypocapnia, it is not significantly different from zero (Corne et al., 2003).

Investigators have consistently found that exposure to a hypobaric hypoxic environment impairs human cognitive and physical performance in domains such as memory, computation, decision-making and muscle fatigue (Wilson et al., 2009). For example,

construction of a tunnel in Colorado required 25% more time to complete at 11,000 ft/3352 m than comparable work performed at sea level (Dryzek, 2002), and scientists working at the Mauna Kea Observatory at an altitude of 13,779 ft/4200 m in Hawaii committed more errors than at sea level and reported their thinking was slowed (Morrison et al., 1973). Studies conducted in military settings have found that these physical and cognitive decrements are present even in young, well-conditioned individuals. For example, lowlanders (defined as those cadets from hometowns at an altitude < 1500 m) at the U.S. Air Force Academy (7250 ft/2210 m) continue to have lower fitness scores, aerobic capacity (VO_2 max) and red cell indices than those from higher altitude – even after 46 weeks, or nearly 1 full year on the Academy campus (Brothers et al., 2010). This would appear to contradict the view that complete cardiovascular acclimatization occurs within days or weeks. Altitude also affects cognitive measures in military personnel. Marines training at the Mountain Warfare Training Center (6463 ft/1970 m) for 30 days reported mental fatigue and anger scores comparable to those of psychiatric outpatients, and reductions in mood rating scale scores that persisted 90 days after completion of their altitude training (Bardwell et al., 2005). Whether altitudes lower than 10,000 ft/3048 m exert an influence on the brain and cognition remains controversial, but recent work has shown that healthy subjects desaturate at rest at 6843 ft/2086 m (Wiseman et al., 2013), and that physical activity at 7001 ft/2134 m produces hypoxic symptoms that would be expected at 15,000 ft/4572 m (Smith, 2007).

The threshold altitude at which hypobaric hypoxia begins to impair human cognitive performance has yet to be defined. In addition to the aforementioned studies, a suggestion comes from the field of cardiopulmonary medicine. Synthesizing the available aerobic capacity data collected in several studies of the effects of altitude, Grover et al. (1986) calculated that beginning at an altitude of 700 m/2296 ft, human VO_2 max declines by 8.0% for each 1000-m increase in altitude. Our finding of increased brain pH in long-term residents at moderate altitude is supported by a previous study that reported similar pH elevations in a small sample (n = 4) of subjects exposed to hypobaric hypoxia for 7 days (Goldberg et al., 1992), and by arterial blood gas data in healthy adults showing that arterial PCO_2 was decreased by approximately 10% in n = 243 residents of Salt Lake City compared with a sample of n = 96 subjects recruited near sea level in Los Angeles, CA, and Hartford, CT (Crapo et al., 1999). Moreover, we have measured plasma CO_2 levels in earlier studies of healthy subjects residing in Salt Lake City (Shi et al., 2012a). We found an abnormally low mean CO_2 content of 20.7 ± 2.4 mEq/l, relative to the laboratory reference range of 23–29 mEq/l (Marques and Huang, 2001). Under experimental conditions, hypocapnia and reduced PCO_2 in blood are associated with increased brain pH (Nioka et al., 1987).

With respect to our additional finding of decreased brain Pi in moderate altitude dwellers, Kuno et al. (1994) conducted a study in which n = 4 well-trained skiers performed sub-maximal exercise for 4 consecutive days at a simulated altitude of 6562 ft/2000 m. The investigators noted higher muscle pH and PCr/(PCr + Pi) during exercise and faster recovery of PCr/(PCr + Pi) during recovery, in the post-training period compared with pre-training. Considering that brain and skeletal muscle bioenergetics are both reliant on the creatine kinase system (Wallimann et al., 2011), these observations are consistent with our finding that brain Pi levels in subjects in Salt Lake City were significantly lower than those in

subjects in Belmont, MA. Moreover the increased post-training pH value in the study of Kuno et al. (1994) also suggests lower levels of glycolysis following altitude training than during the pre-training period. Enhanced oxidation after endurance training can be expected to supply more energy. Therefore, one possibility is that brain oxidative capacity is improved in long-term altitude residents, as an adaptation to hypobaric hypoxia. Patients with panic disorder demonstrate chronic hyperventilation (Grassi et al., 2013), a trait shared with subjects exposed to hypobaric hypoxia. One previous ^{31}P-MRS study reported lower Pi levels in subjects with panic disorder compared with healthy controls (Shioiri et al., 1996), although the difference did not reach statistical significance. A recent study of anodal transcranial direct current stimulation, applied to 13 healthy subjects, reported the same combination of increased brain pH and decreased Pi that we found in our study (Rae et al., 2013). The authors speculated that PCr hydrolysis drove the increase in pH, while mitochondrial synthesis of ATP and PCr caused the concomitant decline in Pi (Rae et al., 2013). Finally, two independent studies of pediatric bipolar disorder (BD) recently reported decreased cerebral Pi in BD subjects (Shi et al., 2012b; Sikoglu et al., 2013), with one group of investigators speculating that the finding may be related to altered mitochondrial phosphorylation (Sikoglu et al., 2013).

The accuracy of brain pH measurement is affected by many factors, such as B_0 inhomogeneity-induced line broadening, peak position estimates of Pi and PCr from fitting routines, baseline fluctuation, SNR, and pH calibration curves relating to chemical shift (Madden et al., 1991). In this study, we reduced magnetic field-induced line broadening site differences through the use of similar MRI systems, and identical ^{1}H/^{31}P RF receiver coils manufactured by the same company. Subject-based line broadening for water during the shimming from the whole brain was similar at both sites. Moreover, all spectra were observed with flat baseline using the current data-acquisition method, and this did not appear to be a contributor to brain pH uncertainty in this experiment. The measurement uncertainty in ^{31}P-MRS studies of brain pH was reviewed by Madden et al. (1991). The authors reported the accuracy of pH measurements is 0.04 pH units, when the chemical shift separation between Pi and PCr is used to calculate pH values. Our simulation results (see Fig. 3a) demonstrate a pH variation of < 0.04 pH units, if the SNR is larger than 9. Moreover, the accuracy of pH determination is improved as the sample size becomes larger. For the Salt Lake City group (n = 26), the standard error of the mean pH = 0.0078, and in the Belmont group (n = 13), the standard error of the mean pH = 0.0111 assuming the 0.04 pH unit inaccuracy for a single measurement. The standard error of the mean pH is calculated by dividing 0.04 by the square root of the sample size. Both theoretical pH errors are smaller than the mean pH difference (0.025) observed in the current study. Therefore, the significant difference in mean intracellular brain pH between the Salt Lake City and Belmont samples in this study cannot be accounted for by measurement inaccuracy.

To improve the SNR of the ^{31}P-MRS measurements, a large voxel size was selected for this study. If the hypothesis about intracellular brain pH correlations with altitude of residence is true, an additional effect on pH resulting from altitude probably occurs across the whole brain, although local brain regions may show pH variation depending on neuronal activity. The whole brain pH values would be expected to demonstrate trends similar to the data

acquired from local brain regions. However, to confirm this, additional experiments will need to be performed to interrogate specific brain regions.

A limitation of our study is the small sample size, and our findings will require replication with larger samples at diverse scanning sites. Moreover, the addition of proton-1 MRS to future studies will permit measurement of brain lactate and glutamate levels, thus providing a more comprehensive picture of brain bioenergetics. Another limitation is that we cannot rule out factors such as subjects' diet, mood state, and exercise regimen as contributors to the observed brain pH difference between the two sites; the influence of these potential confounders will be reduced by increased sample sizes. Based upon the similar direction and magnitude of the brain pH differences reported by Goldberg et al. (1992), we believe that altitude of residence is likely to be at least one contributor to this study's results.

The findings reported here suggest a number of directions for further study. Foremost among these would be multi-site, altitude-based studies of medical conditions in which alterations of brain pH and/or cerebral Pi have been reported, and therefore patients' altitude of residence could potentially impact their disorder's incidence, severity and prognosis. These include bipolar disorder (Kato et al., 1993; Hamakawa et al., 2004; Jensen et al., 2008; Shi et al., 2012b; Sikoglu et al., 2013), seizure disorder (Laxer et al., 1992), ischemic stroke (Hugg et al., 1992; Levine et al., 1992; Sappey-Marinier et al., 1992), brain tumors (Cadoux-Hudson et al., 1989; Segebarth et al., 1989; Hubesch et al., 1990; Arnold et al., 1991; Maintz et al., 2002), mitochondrial disorders (Barbiroli et al., 1993; Moller et al., 2002), congenital malformations (Barbiroli et al., 1993), neurodegenerative diseases (Martinelli et al., 2000; Moller et al., 2002; Mochel et al., 2012) and traumatic brain injury (Pettegrew et al., 1988; Lee et al., 2012). Changes in brain pH have also been found in normative processes such as healthy aging (Forester et al., 2010), and pregnancy and childbirth (Holdcroft et al., 2005).

It is estimated that between 140 million and 200 million people worldwide live at or above an altitude of 2500 m (Moore, 2001; Zubieta-Calleja et al., 2011). Much remains to be learned regarding what, if any, effect hypobaric hypoxia exerts on their health and functional status, and the gene-environment interactions that mediate the prevalence and natural history of human brain disease processes in populations residing at altitude.

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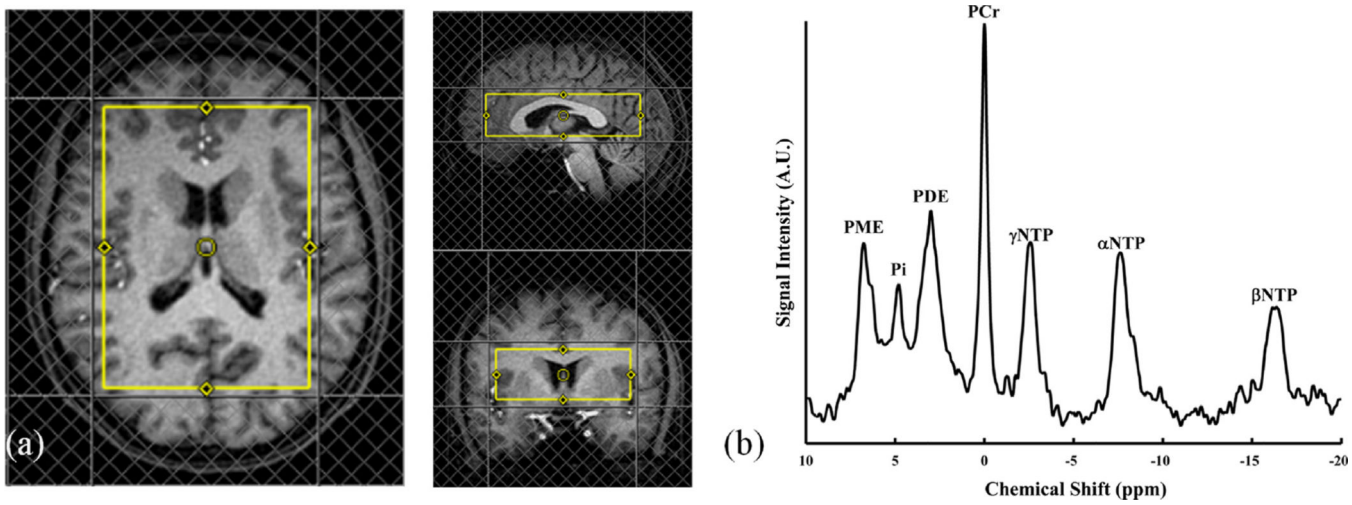

Fig. 1.

(a) Region of interest (ROI) indicated by yellow boxes along three planes. Six outer saturation band were added to remove any signal from outside of ROI. (b) Representative ^{31}P magnetic resonance spectrum. PCr, phosphocreatine; NTP, Nucleoside triphosphate; PME, phosphomonoester; PDE, phosphodiester; Pi, inorganic phosphate.

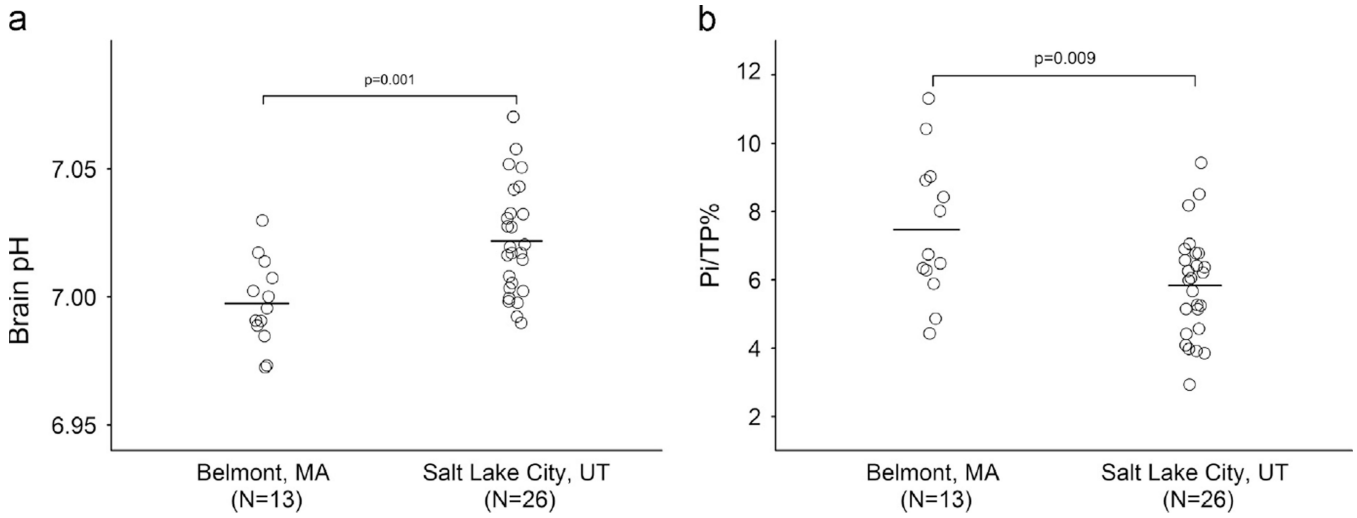

Fig. 2.

(a) Scatter plot of brain intracellular pH values measured in Salt Lake City, UT, and Belmont, MA. The horizontal line represents the mean pH value in each group. (b) Scatter plot of the brain Pi levels at both sites.

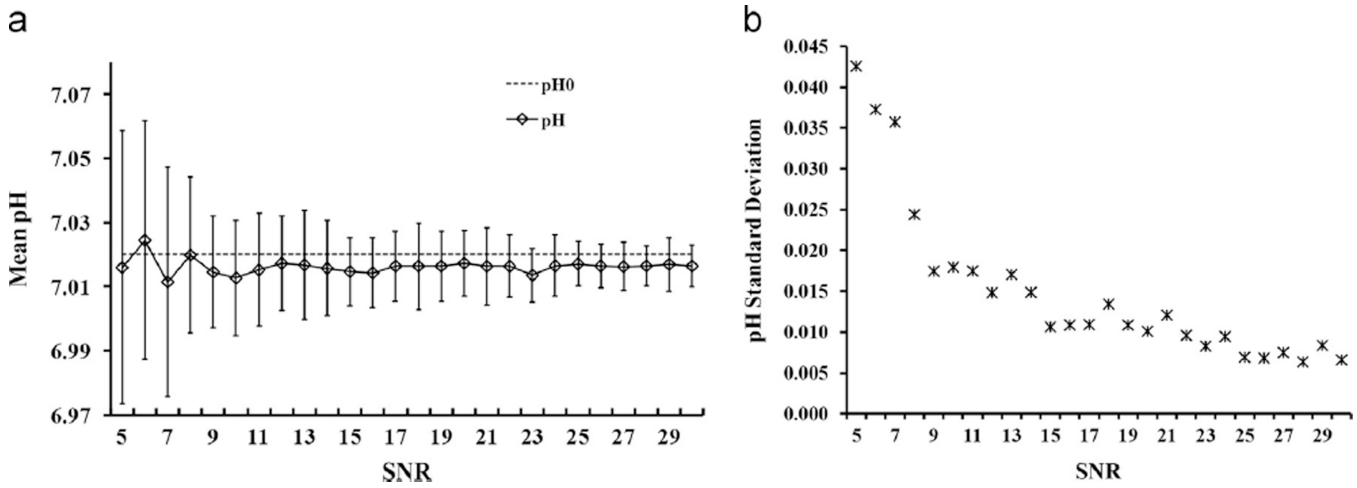

Fig. 3.

(a) Simulated pH versus signal-to-noise ratio (SNR). pH0 is computed from a noise-free spectrum, which is plotted as a dotted line. Simulated pH values with standard deviations are represented with open-diamond shaped markers. (b) pH standard deviation with respect to SNR in simulation.

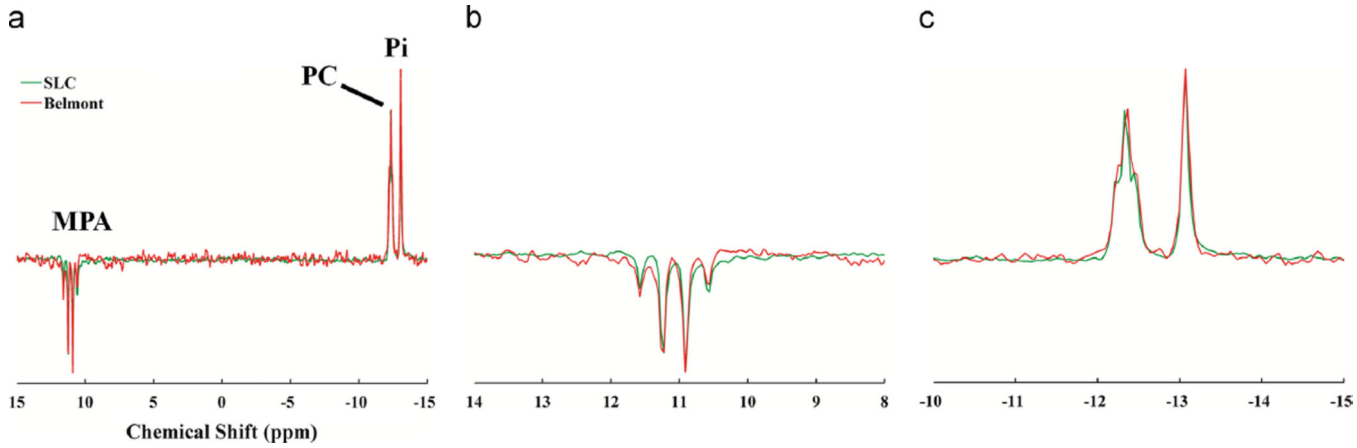

Fig. 4.

(a) Spectral overlap in data acquired from scanners in Salt Lake City and Belmont. (b) Zoom-in overlapping spectrum at methylphosphonic acid peak position in (a). (c) Zoom-in overlapping spectrum at phosphocholine and inorganic phosphate. MPA, methylphosphonic acid; PC, phosphocholine; Pi, inorganic phosphate.

Table 1

Mean pH values, phosphorus-31 metabolite concentrations, and *p*-values of long-term residents of Salt Lake City, Utah and Belmont, Massachusetts (i.e. the Boston, MA metropolitan area).

	Subject#	**SLC. Mean**	**SD**	**Subject#**	**Belmont mean**	**SD**	***p*-value (ANCOVA)**
Age (yrs)	26	27.2	5.6	13	29.3	9.0	0.38
Gender	F13/M13			F7/M6			
pH		7.022 (0.021)			6.997 (0.017)		0.001
PCr/TP		0.194 (0.023)			0.200 (0.022)		0.414
αNTP/TP		0.164 (0.023)			0.164 (0.014)		0.954
βNTP/TP		0.132 (0.019)			0.137 (0.026)		0.493
γNTP/TP		0.181 (0.015)			0.175 (0.017)		0.322
Pi/TP		0.058 (0.015)			0.075 (0.021)		0.009
PME/TP		0.228 (0.060)			0.199 (0.016)		0.095
$[Mg^{2+}]$ (mM)		0.148 (0.019)			0.155 (0.035)		0.397

PCr, phosphocreatine; NTP, Nucleoside triphosphate; Pi, inorganic phosphate; PME, phosphomonoesters; F, Female; M, Male.

Table 2

Measurement of chemical shift difference among MPA, PC, and Pi.

	Salt Lake City	Belmont
PC-Pi[a] (ppm)	0.745	0.706
MPA-PC[b] (ppm)	23.241	23.280
MPA-Pi[c] (ppm)	23.985	23.985

MPA, methylphosphonic acid; PC, phosphocholine; Pi, inorganic phosphate.

[a]The chemical shift difference between PC central peak and Pi.

[b]The chemical shift difference between the third peak of MPA from left side in Fig. 4 and PC central peak.

[c]The chemical shift difference between the third peak of MPA from left side in Fig. 4 and Pi peak.