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Comparing integrative ventilatory and renal acid–base acclimatization in lowlanders and Tibetan highlanders during ascent to 4,300 m

Nicole A. Johnson[a,1], Jessica A. Dickenson[a,1], Benjamin W.L. MacKenzie[a], Rodion Isakovich[a], Anne Kalker[b], Janne Bouten[c,d], Nicholas D.J. Strzalkowski[a], Taylor S. Harman[e], Pontus Holmström[f], Ajaya J. Kunwar[g], Nilam Thakur[g], Sunil Dhungel[h,i], Nima Sherpa[j], Abigail W. Bigham[k], Tom D. Brutsaert[e,1], and Trevor A. Day[a,1,2]

Affiliations are included on p. 10.

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With over 14 million people living above 3,500 m, the study of acclimatization and adaptation to high altitude in human populations is of increasing importance, where exposure to high altitude (HA) imposes a blood oxygenation and acid–base challenge. A sustained and augmented hypoxic ventilatory response protects oxygenation through ventilatory acclimatization, but elicits hypocapnia and respiratory alkalosis. A subsequent renally mediated compensatory metabolic acidosis corrects pH toward baseline values, with a high degree of interindividual variability. Differential renal compensation between acclimatizing lowlanders (LL) and Tibetan highlanders (TH; Sherpa) with ascent was previously unknown. We assessed ventilatory and renal acclimatization between unacclimatized LL and TH during incremental ascent from 1,400 m to 4,300 m in age- and sex-matched groups of 15-LL (8F) and 14-TH (7F) of confirmed Tibetan ancestry. We compared respiratory and renally mediated blood acid–base acclimatization (PCO_2, $[HCO_3^-]$, pH) in both groups before (1,400 m) and following day 8 to 9 of incremental ascent to 4,300 m. We found that following ascent to 4,300 m, LL had significantly lower PCO_2 (P <0.0001) and $[HCO_3^-]$ (P <0.0001), and higher pH (P = 0.0037) than 1,400 m, suggesting respiratory alkalosis and only partial renal compensation. Conversely, TH had significantly lower PCO_2 (P < 0.0001) and $[HCO_3^-]$ (P < 0.0001), but unchanged pH (P = 0.1), suggesting full renal compensation, with significantly lower PCO_2 (P = 0.01), $[HCO_3^-]$ (P < 0.0001) and pH (P = 0.005) than LL at 4,300 m. This demonstration of differential integrative respiratory–renal responses between acclimatizing LL and TH may indicate selective pressure on TH, and highlights the important role of the kidneys in acclimatization.

high Altitude | ventilatory acclimatization | respiratory alkalosis | renal compensation | Tibetan highlander

Significance

Understanding integrated acclimatization and adaptation to high altitude (HA) in human populations is of increasing importance, particularly comparing lowlander (LL) to highlander populations. HA exposure imposes a blood oxygenation and acid-base challenge, compensated by respiratory and renal acclimatization. We assessed acclimatization between unacclimatized age- and sex-matched groups of LL and Tibetan highlander (TH; Sherpa) during incremental ascent to 4,300 m. TH had a larger magnitude and/or more rapid time-course of respiratory and renal acclimatization, resulting in fully-compensated pH at 4,300 m, whereas LL were still alkalemic. This study provides insight into the interplay of ancestry and physiological mechanisms contributing to acclimatization to HA, which may indicate selective pressure on TH populations related to renal function with acclimatization.

Over 14 million people live at altitude above 3,500 m, with over five million of these residing in China (1). The Qinghai-Tibetan plateau, located at the intersection of south, central, and east Asia, is the highest and largest plateau in the world. This plateau spans approximately 2.5 million square km and has an average elevation that exceeds 4,000 m. The plateau may have been occupied by nomadic populations as long as 40,000 to 30,000 y ago (2), and although debated, was permanently colonized as early as 6,500 y ago (e.g., 3, 4). Emerging research demonstrates that long-term residence of populations at or above 4,000 m for millennia presents a selective pressure for adaptation to high altitude in Tibetan highlanders (TH) (TH; e.g., Sherpa; e.g., 5). These adaptations likely involve several distinct but integrated physiological systems involved in oxygen sensing and metabolism (e.g., 6), and may affect both acclimatization processes, as well as long-term adaption while living, working, and reproducing at high altitude (e.g., 7). Indigenous highlanders of Tibetan ancestry are known for their unique adaptations to sustained HA exposure, which have been widely explored (e.g., (6–11). Less explored is the renal acclimatization to high altitude ventilation-associated respiratory alkalosis in Tibetan highlander populations (e.g., 12).

During ascent to high altitude (HA; ≥2,500 m) in unacclimatized individuals, respiratory and renal acclimatization protect blood oxygenation and acid–base homeostasis, respectively (12–16). Ascent to HA exposes individuals to a lower atmospheric partial pressure of oxygen, which decreases arterial partial pressure of oxygen (PaO_2; hypoxemia) and triggers a cascade of important integrated physiological responses. First, the sustained hypoxic ventilatory response (HVR) and ventilatory acclimatization, which protect

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[1]N.A.J., J.A.D., T.D.B., and T.A.D. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: tday@mtroyal.ca.

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oxygenation but decrease P_aCO_2 (i.e., hypocapnia), elicit a respiratory alkalosis (11–20). Second, a subsequent renal compensation decreases bicarbonate ion (HCO_3^-) reabsorption and increases hydrogen ion (H^+) retention, resulting in a renally mediated compensatory metabolic acidosis, which corrects arterial pH toward baseline values (12, 13, 15, 16, 19, 20).

From a ventilatory perspective, the hypoxemia that results from inspired hypoxia during HA ascent causes increases in carotid body activation and afferent input from the carotid bodies to brainstem respiratory centers (14, 18, 21). This signaling causes an increase in alveolar ventilation (i.e., HVR), which protects oxygenation by increasing PaO_2 and SaO_2 (14, 18, 21). With sustained exposure to HA (i.e., days), carotid body hypertrophy and hyperplasia increase sensitivity to hypoxia, inducing ventilatory acclimatization (22, 23).

From a renal compensation perspective, sustained increases in ventilation during exposure to hypoxia elicits hypocapnia and, acutely, respiratory alkalosis. The HCO_3^-/CO_2 buffer pair is the most important buffer system in blood and interstitial fluids, with pH mediation described by the Henderson–Hasselbalch equation (13):

$$pH = pK + log\frac{[HCO_3^-]}{s \times P_{CO_2}},$$

$$\text{Where } pK_a = 6.1 \text{ at } 37\,^{\circ}\text{C}; s \text{ (}\textit{solubility coefficient for } CO_2\text{)} = 0.03 \text{ (mmol/l/mmHg)}$$

In response to respiratory alkalosis, a subsequent renally mediated metabolic acidosis partially returns pH toward baseline values, with a high degree of interindividual variability (e.g., (12, 15, 16, 19). Specifically, the renal tubules reduce the normal bicarbonate ion reabsorption and hydrogen ion secretion, developing a compensatory metabolic acidosis, partly correcting arterial pH toward baseline levels (13). Full renal compensation in indigenous lowlanders (LL) appears to be limited to below ~4,000 to 4,500 m in LL (e.g., cf., 16 vs. 24 and 19). At higher altitudes, where $PaCO_2$ is lower, blood pH appears to remain alkalotic, regardless of the duration of exposure (e.g., 19, 24–26). However, the magnitude, time-course, and altitude threshold, including the differential ventilatory and renal acclimatization, in TH compared to LL requires further experimental testing but may be enhanced in TH given their evolutionary history.

Although reports vary in conclusions, previous studies suggest that LL and TH have similar ventilatory acclimatization during ascent to and residence at HA (reviewed in (6) and 27). From a renal compensation perspective, although data are limited, TH had more complete renal compensation than acclimatized LL, residing at the same altitude (e.g., 28, 12). For example, a previous study showed that a group of acclimatized LL were significantly more alkalemic than a group of TH at both 4,300 m and 5,000 m (12). However, this study included convenience samples of participant data included from different groups and expeditions following differential time courses of ascent and durations of exposure and acclimatization, nor were participants compared to their own baseline variables at low altitude.

Understanding potential differences in active acclimatization processes between LL and TH may elucidate the capacity of TH to live, work, and reproduce at HA. To our knowledge, no studies have investigated the integrated respiratory and renal acclimatization in LL vs. TH during incremental ascent to HA in the same groups following the same ascent profile. Comparing respiratory and blood-gas variables in LL and TH following an identical ascent profile up to 9 d of HA exposure provides a unique model of systematically assessing the integrated respiratory and renal acclimatization responses in healthy age- and sex-matched LL vs. TH groups. Differences between LL and TH groups during active acclimatization may reveal intrinsic differences in renal function between groups, which could provide advantages in protecting blood pH during incremental ascent and/or high altitude residence in TH. Thus, we aimed to assess potential differences in ventilatory and renal acclimatization between LL and TH during incremental ascent from 1,400 m to 4,300 m over an 8 to 9 d period. We hypothesized that ventilatory acclimatization would not be different between groups, but acclimatizing TH would have larger magnitude renal compensation compared to acclimatizing LL.

Results

Participants. The data included in this investigation were collected during a single HA research expedition in the Everest region in the Nepal Himalaya. We recruited 15 unacclimatized self-reported LL (8 females, 7 males; Age = 22.4 ± 3.0 yrs; BMI = 25.0 ± 3.2 kg/m^2) who were born and resided below 1,400 m, and were not of indigenous highlander descent. Two of these LL female participants indicated they were on hormonal birth control, with four indicating the use of an IUD. In addition, 14 unacclimatized self-reported indigenous TH (7 females, 7 males; Age = 22.9 ± 4.5 yrs; BMI = 22.3 ± 3.9 kg/m^2) were recruited and included in the final analysis. None of these TH female participants were on hormonal birth control.

We did not control for ovarian cycle in female participants, in part due to logistical constraints of this fieldwork expedition, where some baseline measures at 1,400 m were obtained up to 8 d before the ascent profile began.

All LL participants were nonsmokers. However, from our participant interviews, it appears that social smoking is cultural and gendered in the Sherpa population, with all male TH participants admitting to some social smoking at least once/week, whereas all female TH participants denied any smoking.

Self-reported TH ancestry was confirmed via interview through surname analysis of the individual, their two parents, and their four grandparents. Of our TH participants recruited at 1,400 m, only two of them were confirmed to be born at low altitude (in Kathmandu). The rest indicated they were born above 2,500 m in the Khumbu region. One TH participant was living at low altitude for only six months prior to the study, having visited Khumjung (~3,800 m) prior to descending to 1,400 m. All other TH participants reported they were residing at 1,400 m between 1 to 3 y prior to recruitment for this study. Thus, although these participants were fully unacclimatized before the ascent protocol, a key strength of this study, we cannot rule out a role for developmental adaptation (e.g., 29) contributing to the observed differences between groups in this study, as with most other studies investigating indigenous highlanders.

Although Sherpa have a unique ethnic identity, indigenous oral histories and genetic evidence both point to Sherpa being a Tibetan highlander-derived population (30). Thus, although they have a distinct cultural identity, it is common in the adaptation literature to treat Sherpa and TH as part of the same evolutionary population. Thus, for the remainder of the manuscript, the Sherpa participants recruited herein will be referred to as TH, given their genetic ancestry, and the potential broader implications of this study.

Ventilatory and Blood Gas and Acid–Base Variables. All relevant ventilatory, blood gas, and acid–base data before and after ascent for both LL and TH are reported in Table 1, with both main effects (altitude and group), as well as interaction effects, reported there.

Of note, LL had significantly lower PCO_2 ($P < 0.0001$), and higher V_A ($P < 0.0001$), and steady-state chemoreflex drive (SSCD)

Table 1. Comparison of ventilatory and blood gas and electrolyte data between LL and TH before (Day 0; 1,400 m) and after ascent to 4,300 m (Days 8 to 9)

		Altitude		*P*-values		
Variable	Population	1,400 m	4,300 m	Altitude (*)	Population (†)	Interaction
$\dot{V}_A$ (l/min)	LL	4.5 ± 0.3$^{\dagger *}$	5.5 ± 0.4$^{\dagger *}$	<0.0001	0.0057	0.5745
	TH	4.8 ± 0.4$^{\dagger *}$	5.9 ± 0.3$^{\dagger *}$			
SSCD (a.u.)	LL	11.7 ± 1.5*	16.0 ± 2.8$^{\dagger *}$	<0.0001	0.0061	0.2642
	TH	13.1 ± 2.1*	18.5 ± 2.0$^{\dagger *}$			
SpO_2 (%)	LL	97.7 ± 1.2*	90.4 ± 4.2$^{\dagger *}$	<0.0001	0.3081	0.0601
	TH	97.3 ± 0.9*	92.4 ± 2.9$^{\dagger *}$			
[Hb] (g/L)	LL	14.2 ± 1.3*	15.6 ± 1.8*	<0.0001	0.5636	0.8736
	TH	13.8 ± 1.7*	15.3 ± 1.5*			
CaO_2 (mL/dL)	LL	18.6 ± 1.8	19.0 ± 2.5	0.0185	0.7029	0.3194
	TH	17.9 ± 2.2*	19.0 ± 1.6*			
PCO_2 (mmHg)	LL	38.2 ± 2.4$^{\dagger *}$	31.5 ± 2.5$^{\dagger *}$	<0.0001	0.0097	0.9236
	TH	36.1 ± 2.9$^{\dagger *}$	29.5 ± 1.5$^{\dagger *}$			
$[HCO_3^-]$ (mM)	LL	23.2 ± 1.3$^{\dagger *}$	20.6 ± 1.1$^{\dagger *}$	<0.0001	<0.0001	0.1372
	TH	21.9 ± 1.7$^{\dagger *}$	18.4 ± 1.0$^{\dagger *}$			
$[H^+]$ (nM)	LL	40.6 ± 4.1*	37.0 ± 2.0$^{\dagger *}$	<0.0001	0.0564	0.0231
	TH	39.7 ± 1.6	38.8 ± 1.0†			
pH	LL	7.40 ± 0.04*	7.43 ± 0.02$^{\dagger *}$	<0.0001	0.0489	0.02
	TH	7.40 ± 0.02	7.41 ± 0.01†			
TCO_2 (mM)	LL	24.4 ± 1.3$^{\dagger *}$	21.5 ± 1.2$^{\dagger *}$	<0.0001	<0.0001	0.3722
	TH	22.6 ± 1.5$^{\dagger *}$	19.2 ± 1.0$^{\dagger *}$			
Base Excess (mM)	LL	−0.74 ± 1.16$^{\dagger *}$	−2.44 ± 0.71$^{\dagger *}$	<0.0001	<0.0001	0.0861
	TH	−1.88 ± 1.48$^{\dagger *}$	−4.52 ± 0.89$^{\dagger *}$			
Osmolality (mOsm/kg)	LL	283.5 ± 3.9*	285.8 ± 3.1*	0.0029	0.676	0.5931
	TH	284.3 ± 3.1	285.9 ± 3.00			

$\dot{V}_A$, alveolar ventilation (calculated from PCO_2); SSCD, steady-state chemoreflex drive (Materials and methods); SpO_2, oxygen saturation from pulse oximeter; [Hb], hemoglobin concentration; CaO_2, oxygen content; PCO_2, partial pressure of capillary carbon dioxide; $[HCO_3^-]$, concentration of capillary bicarbonate; $[H^+]$, hydrogen concentration; pH, capillary pH; TCO_2, total capillary carbon dioxide. *P*-value <0.05 indicates a significant difference between altitude (*) or population (†), calculated using a mixed-model, two-factor ANOVA. LL n = 15, TH n = 14.

($P < 0.0001$) at 4,300 m compared to 1,400 m (Fig. 1 *A*, *C*, and *E*). LL also had significantly lower $[HCO_3^-]$ ($P < 0.0001$), BE ($P < 0.0001$), and higher pH ($P = 0.0006$) at 4,300 m compared to 1,400 m as illustrated in Fig. 2 *A*, *C*, and *E*. Conversely, TH had significantly lower PCO_2 ($P < 0.0001$), and higher $\dot{V}_A$ ($P < 0.0001$) and SSCD ($P < 0.0001$) at 4,300 m compared to 1,400 m (Fig. 1 *B*, *D*, and *F*). TH also had significantly lower $[HCO_3^-]$ ($P < 0.0001$) and BE ($P = 0.0001$), but an unchanged pH ($P = 0.0946$) at 4,300 m (Fig. 2 *B*, *D* and *F*).

Fig. 3 compares absolute ventilatory, blood gas, and acid–base data for LL and TH at 4,300 m on Days 8 to 9. TH had significantly higher $\dot{V}_A$ ($P = 0.0130$) and SSCD ($P = 0.0103$), and significantly lower PCO_2 ($P = 0.0149$), $[HCO_3^-]$ ($P < 0.0001$), BE ($P < 0.0001$) and pH ($P = 0.0059$), than LL at 4,300 m (Fig. 3 *A*–*F*).

Renal Reactivity. Fig. 4 depicts the weak correlation between renal reactivity (RR) and Δ pH in LL and TH after ascent to 4,300 m. Fig. 4*A* demonstrates that most LL had a RR below 0.5, while there was an increase in pH in most participants with ascent. Fig. 4*B* depicts the moderate correlation between RR and Δ pH of the TH. Participant data are clustered closely around an RR index of 0.5, with a range of increases and decreases in pH across individuals. Fig. 4*C* illustrates the significant difference of RR between groups, with TH having a higher RR than LL ($P = 0.0229$, ES = 0.35). We also calculated an additional index of renal responsiveness with ascent, namely %-pH compensation (31, 32). Although highly variable, both groups mounted large renal compensatory responses with ascent (LL = 62.5 ± 43.2 %; TH = 88.3 ± 27.7 %). When compared between groups, TH had significantly higher compensatory responses than LL ($P = 0.0079$, ES=0.6; Fig. 4*D*).

Davenport Diagrams. Fig. 5 consists of Davenport diagrams that illustrate the integrated acid–base variables and metabolic compensation for LL and TH at 1,400 m and 4,300 m. Fig. 5*A* presents baseline values for LL and TH at 1,400 m. Fig. 5*B* shows the rightward and downward shift from baseline after the LL ascended to 4,300 m. Fig. 5*B* also demonstrates the significant downward shift for TH from baseline, demonstrating a complete metabolic compensation. Of note, the rightward shift (i.e., alkalosis) observed in the LL (Fig. 5 *A* and *B*) was significantly different compared to the insignificant rightward shift observed in the TH (Fig. 5 *A* and *B*), indicating only a partial compensation in pH was observed for the LL, but a complete compensation was observed for TH.

Discussion

We aimed to compare ventilatory and renal acclimatization in age- and sex-matched groups of acclimatizing participants of LL and TH ancestry following the same ascent profile from 1,400 m to 4,300 m over 8 to 9 d. The principal findings were a) TH had significantly lower PCO_2 ($P = 0.01$), $[HCO_3^-]$ ($P < 0.0001$) and pH ($P = 0.005$) than LL at 4300 m and b) TH had an unchanged pH ($P = 0.1$), while LL had a significant increase in pH (i.e., alkalemia; $P = 0.0001$) from 1,400 m to 4,300 m. These findings suggest that TH have larger magnitude and/or a more rapid

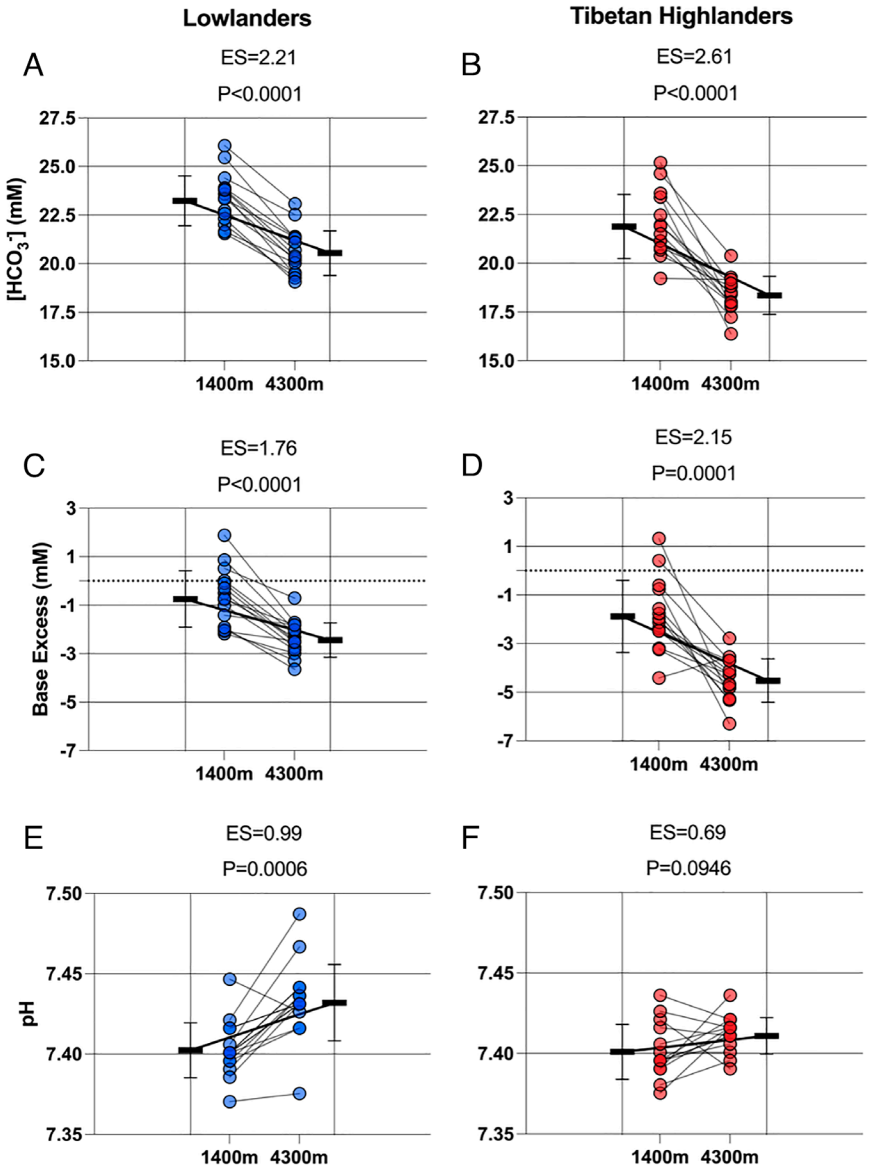

Fig. 1. Group capillary blood gas and ventilatory data in LL and TH at baseline (1,400 m) and high altitude (4,300 m). Two-tailed paired *t*-test *P*-values illustrate group trend of partial pressure of carbon dioxide (PCO_2; *A* and *B*), alveolar ventilation response (V_A; *C* and *D*), and SSCD (SSCD; *E* and *F*) before and after ascent from baseline (1,400 m) to high altitude (4,300 m). ES calculated using Cohen's d. LL n = 15, TH n = 14.

time-course of ventilatory and renal mechanisms compared to LL, resulting in fully compensated pH in TH after ascent to high altitude (HA), at least at an altitude of 4,300 m over a time course of 8 to 9 d. We compare acclimatization responses in both LL and TH as they are actively acclimatizing together during incremental ascent to 4,300 m, an altitude previously shown to be at the threshold of renal capacity in LL (e.g., (15, 16, 19, 24). We compare integrated ventilatory and renal acid–base acclimatization in LL and TH following the same ascent profile over an 8 to 9 d period, with a clear demonstration of enhanced early ventilatory acclimatization and renal compensation in the TH group with ascent. This work extends on previous work demonstrating differential acid–base regulation in acclimatized LL and TH at high altitudes. The emerging story is that TH have larger magnitude renal compensatory responses, which develop early with ascent (present study), and likely persist at higher altitudes (e.g., 4,000 to 6,000 m; 12, 28), suggesting that selective pressure acted on renal function at high altitude in populations with ancestors from the Tibetan plateau.

Ventilatory Acclimatization. Previous studies explored ventilatory acclimatization in LL and TH groups with acclimatization to HA (e.g., 6, 27). Ventilatory acclimatization can be assessed in a number of different ways following ascent. First, transient tests of the HVR are routinely performed in the laboratory contexts, whereby the transient respiratory response to an acute inspired hypoxic exposure is quantified (e.g., 33–35). However, these methods lack feasibility and portability in many high-altitude fieldwork contexts. Second, steady-state resting ventilation and/or arterial or end-tidal CO_2 can also be used to assess the steady-state ventilatory response to high-altitude exposure (e.g., 5, 11, 17, 36–42). In addition, a recently developed metric from our group called SSCD quantifies the steady-state ventilatory response to prevailing chemostimuli (35), and appears to capture ventilatory acclimatization with ascent to high altitude (43–45).

Although reports are equivocal, the consensus in the literature appears to be that acclimatized TH have similar ventilatory acclimatization to acclimatized LL, at least when assessing the HVR (see 6 for summary). The variability and discrepancy in results may be due in part to differential exposure times, maximum

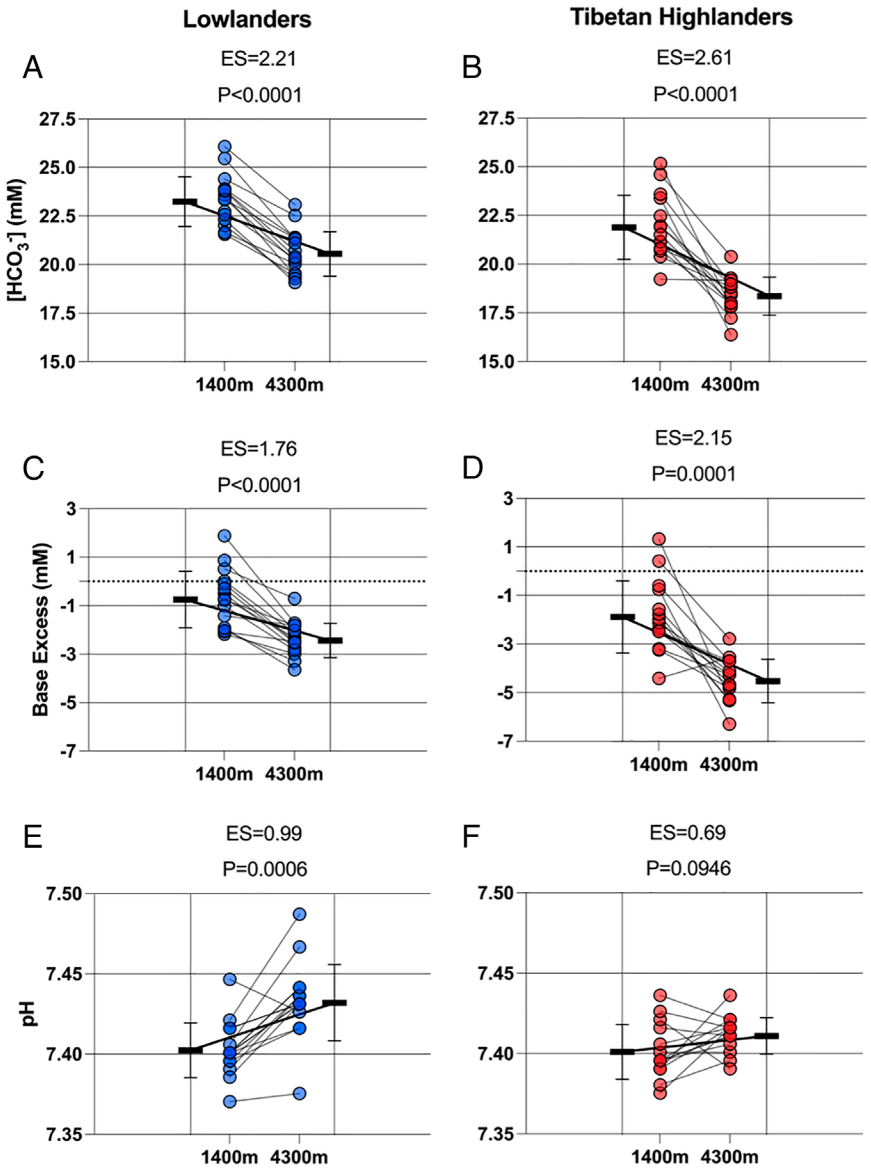

Fig. 2. Group capillary acid–base data in LL and TH at baseline (1,400 m) and high altitude (4,300 m). Two-tailed paired *t*-test *P*-values illustrate each group trend of bicarbonate concentration ($[HCO_3^-]$; *A* and *B*), base excess (BE; *C* and *D*), and pH (*E* and *F*) before and after ascent from baseline (1,400 m) to high altitude (4,300 m) ES calculated using Cohen's d. pH. LL n =15, TH n = 14.

altitudes, and protocol design. Recently, a systematic review and meta-analysis was published of all known studies that quantified the HVR in humans using acute tests (e.g., transient, rebreathing, step-down) in humans (34). They found that regardless of the test or metric utilized, the distribution of HVR values was highly variable and positively skewed (i.e., low in magnitude). These values were only higher in acclimatized participants, but no differences were apparent between acclimatized populations (e.g., LL vs. TH; 34).

However, results from steady-state perspectives appear to betray a different conclusion, whereby ventilatory acclimatization can be also assessed via resting ventilation (14). One study compared HVR (using rebreathing) and resting ventilation in LL and TH at low altitude and HA. At ~1,400 m, resting ventilation was significantly higher in TH compared to LL (7.37 ± 0.34 L/min/m^2 vs. 5.94 ± 0.37 L/min/m^2 respectively; 36). Similarly, after ascent and residence (4 to 12 d) at ~4,200 m, although the HVR values and oxygen saturation were not significantly different between groups, resting ventilation was higher in TH compared to LL (9.8 ± 1 L/min/m^2 vs. 7.77±0.47 L/min/m^2 respectively; 36). These findings are consistent with our study, where we calculated resting alveolar ventilation from PCO_2 in both groups and both locations, and TH had lower PCO_2 ($P < 0.0001$; Fig. 1*A*) and higher resting alveolar ventilation ($P < 0.0001$; Fig. 1*C*) at both low and high altitude following an identical ascent profile. A number of other studies are also in agreement, demonstrating that TH have higher resting ventilation after acclimatization at various HA locations (e.g., 17, 37, 40). However, it should be noted that not all studies are consistent with these findings (e.g., 9, 12), illustrating the high variability noted in the meta-analysis (34), and the importance of making meaningful comparisons between groups using similar ascent profiles.

Many studies compare participant groups selected by convenience at various altitudes with differential exposure durations, and to our knowledge, none assessed and compared acclimatizing participants using the same ascent profile and time course of exposure. In the present study, while both groups had a significant decrease in PCO_2 following ascent to HA ($P < 0.0001$, $P < 0.0001$; Fig. 1 *A* and *B*), TH had significantly lower PCO_2 than LL at 4300 m ($P = 0.0149$; Fig. 3*A*). In addition,

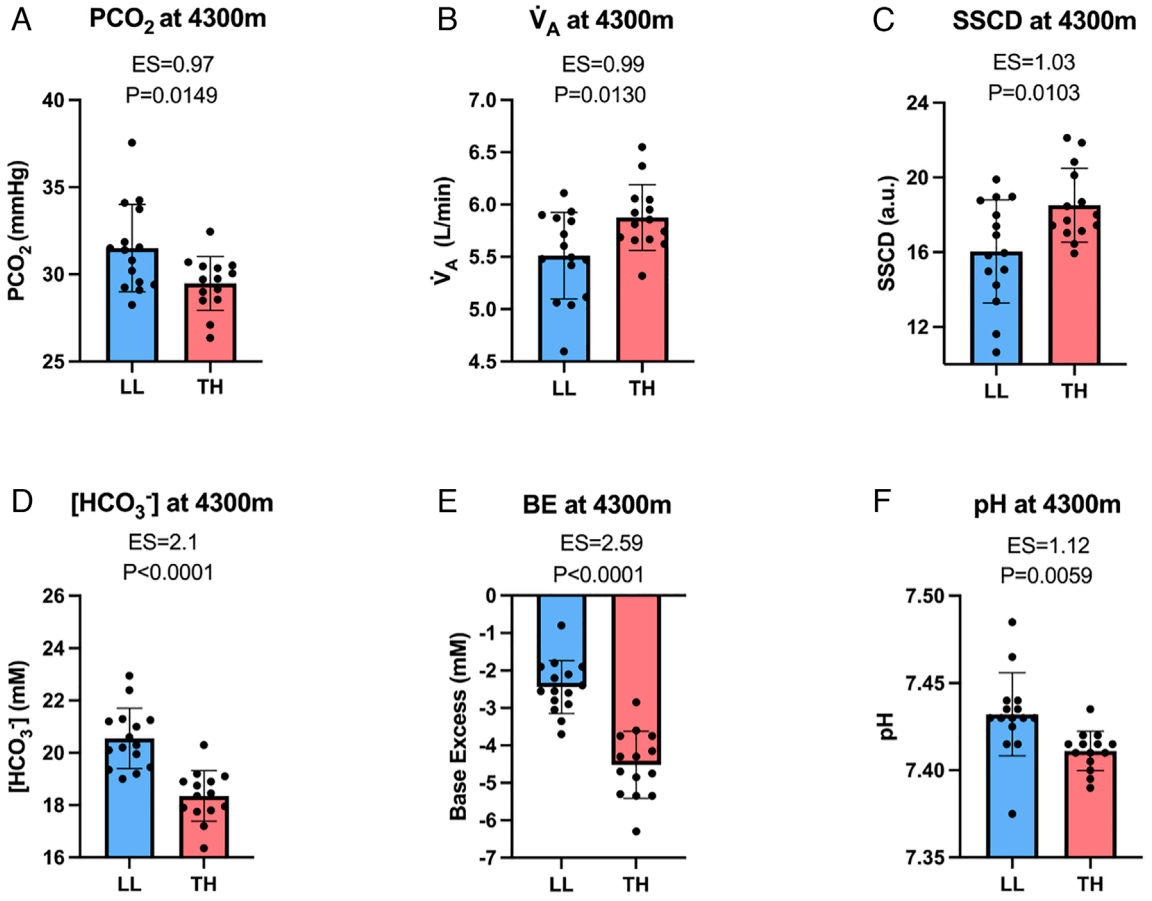

Fig. 3. Comparison of blood gas, ventilation, and acid–base capillary blood data in LL and TH at 4,300 m. Individual data in LL and TH illustrate the effect of high-altitude exposure to 4,300 m over 8 to 9 d. (*A*) Capillary partial pressure of capillary carbon dioxide (PCO_2). (*B*) Calculated alveolar ventilation (V_A). (*C*) Calculated SSCD. (*D*) Capillary bicarbonate concentration [HCO_3^-]. (*E*) Capillary base excess (BE). (*F*) Capillary pH. Two-tailed unpaired *t*-test P-values reported on each graph. ES calculated using Cohen's d. LL n =15, TH n = 14.

calculated alveolar ventilation and SSCD was also higher in TH than LL at 4,300 m ($P = 0.0130$, $P = 0.0103$, Fig. 3 *B* and *C*). Collectively, these findings suggest that TH have a larger magnitude and more rapid ventilatory acclimatization to LL after early ascent to 4,300 m, which is consistent with previous studies using resting ventilation to compare acclimatization between LL and TH (e.g., 36). This is a clear demonstration of acclimatizing TH having augmented ventilatory acclimatization compared to an age- and sex-matched group of LL following an identical ascent profile.

Renal Acclimatization and Acid–Base Regulation. In response to hypocapnia and early respiratory alkalosis elicited by the HVR, we expected to see a concomitant compensatory metabolic acidosis, marked by a decrease in [HCO_3^-] in all participants (e.g., 15). For both LL and TH, there was a significant decrease in [HCO_3^-] after ascent to 4,300 m ($P < 0.0001$, $P < 0.0001$; Fig. 2 *A* and *B*). However, while LL remained alkalotic at 4,300 m with a significantly higher pH than at baseline ($P = 0.0001$; Fig. 2*E*), TH had an unchanged pH ($P = 0.01$; Fig. 2*F*). In fact, at 4,300 m TH had significantly lower [HCO_3^-] ($P < 0.0001$; Fig. 3*D*), and pH ($P = 0.005$; Fig. 3*F*)

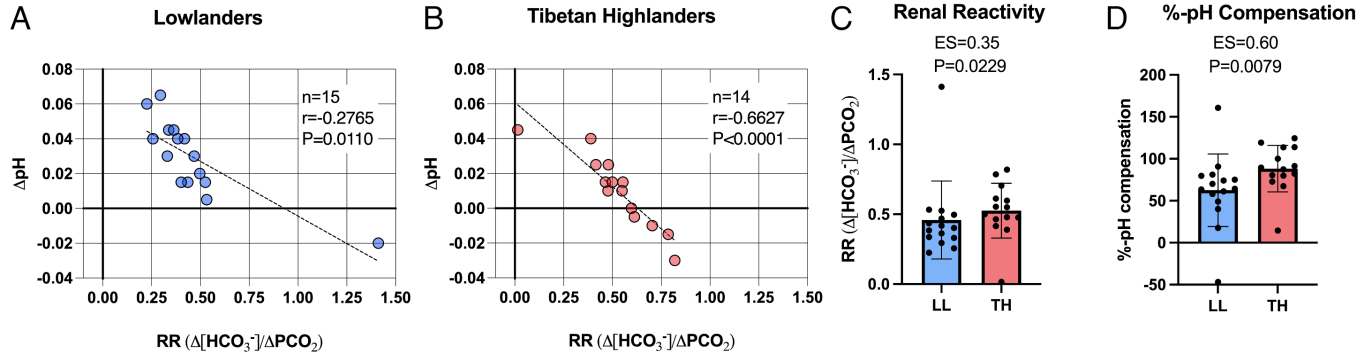

Fig. 4. RR and %-pH compensation in LL and TH after rapid ascent to 4300 m. Data were calculated from PCO_2, [HCO_3^-], and Δ pH variables reported in Fig. 2. (*A*) correlation between RR and pH at day 0 (1,400 m) and day 8 to 9 (4,300 m) for LL. (*B*) correlation between RR and pH at day 0 (1,400 m) and day 8 to 9 (4,300 m) for TH. For correlations (*A* and *B*), n, P, and r values are presented on each graph. (*C*) Mean RR and SD for both LL and TH after ascent to 4,300 m ($P = 0.0229$, ES = 0.35). (*D*) Mean %-pH compensation and SD for both LL and TH after ascent to 4,300 m ($P = 0.0079$, ES=0.60). [HCO_3^-], capillary bicarbonate concentration; PCO_2, partial pressure of capillary carbon dioxide; pH, capillary pH. Effects size (ES) calculated using Cohen's d LL n = 15, TH n = 14.

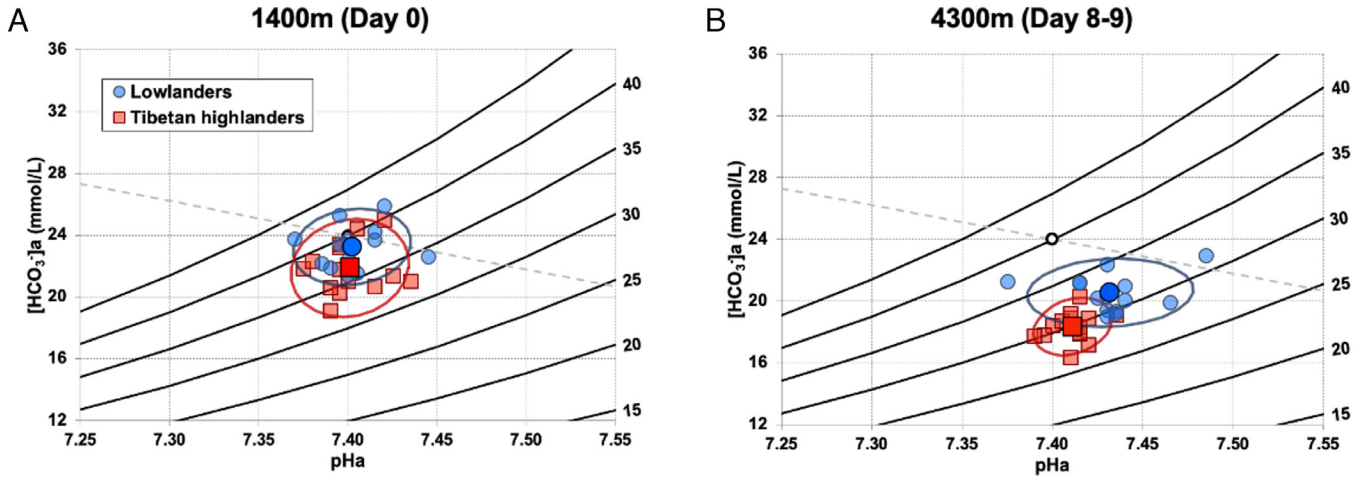

Fig. 5. Davenport diagrams at 1,400 m and after incremental ascent to 4,300 m over 8 to 9 d. Individual data of LL (LL; blue circles) and TH (TH; red squares) demonstrating relationships between PCO_2, $[HCO_3^-]$, and pH at 1,400 m on day 0 (*A*) and 4,300 m on day 8 to 9 (*B*). Individual data points and group mean (larger symbols) depicted with 95% ellipse for each group. $[HCO_3^-]$, capillary bicarbonate concentration; PCO_2, partial pressure of capillary carbon dioxide; pH, capillary pH. LL n = 15, TH n = 14.

than LL, which suggests a larger magnitude renal response, resulting in fully compensated pH, despite a larger hypocapnic stimulus.

Previous studies showed that for LL, pH can return to normal values within 24 h of arrival to 3,800 m (e.g., 16). In addition, a previous study found that in LL, pH was normalized back to baseline values after one night at 4,300 m (15). However, pH was alkalotic after further ascent to 5,160 m (15). A similar study found that after ascent to 4,300 m, $[HCO_3^-]$ and pH of LL stabilized after 3 d and remained elevated for 21 d (19). Another study similarly found that after 5 and 10 d of acclimatization at 4,300 m, cerebrospinal and arterial pH remained significantly elevated (i.e., alkaline; 24). On balance with earlier work, it appears that a threshold altitude for a complete metabolic compensation in LL is between 4,000 to 4,500 m (15, 16, 19, 24). Our results are congruent with these findings, as LL were not able to fully compensate their pH, despite 3 to 4 d of acclimatization at 4,300 m, whereas the TH were fully compensated from a pH perspective.

Conversely, after ascent to and residence at 4,300 m, pH was statistically unchanged from 1,400 m in TH ($P = 0.01$; Fig. 2*F*) and TH were able to maintain a significantly lower pH than LL ($P = 0.0059$; Fig. 3*F*), even though both groups followed the ascent profile with the same time course of acclimatization. Very few studies have compared LL and TH when assessing renal-acid base acclimatization to HA. Previous studies assessed acid–base regulation in TH (i.e., Sherpa) and LL by comparing their arterial pH at different altitudes (e.g.,12, 28). For example, it has been found that TH had significantly lower pH than LL at 4,300 m and 5,000 m (12). In addition, another study found that there was a steady increase in the pH of native LL (i.e., respiratory alkalosis), but an almost constant pH in Sherpa with ascent from 3,400 m - 6,450 m (28). Unfortunately, one major caveat with both studies when comparing pH of LL with TH is that the groups were not following the same ascent profile over the same time course, which makes comparing integrated responses between these groups challenging. In the present study, which includes an identical ascent profile and time-course in both groups, we demonstrate that while both LL and TH had significant decreases in PCO_2 and $[HCO_3^-]$, TH had an unchanged pH (Figs. 1*A* and *B* and 2 *A*, *B*, and *F*), suggesting that TH have augmented acid–base regulation with ascent compared to LL with early acclimatization. The finding that pH remained stable in TH with ascent to HA, while pH remained alkalemic in LL, demonstrates the differential renal compensation between LL and TH following the same time course and ascent profile, at least to 4,300 m, suggesting a genetic influence on renal function in TH related to acid–base homeostasis.

RR and %-pH Compensation. Researchers created a novel index that measures the extent to which the renal response is able to counter the acid–base challenge faced during HA ascent (15). This RR index demonstrates the relative change in bicarbonate concentration ($[HCO_3^-]$) in response to the relative change in the partial pressure of carbon dioxide (PCO_2) after ascent to HA ($\Delta [HCO_3^-]/\Delta PCO_2$) (15). They found that a) RR increased from 3,400 m to 3,800 m, but b) at altitudes 3,800 m and higher, RR plateaued, suggesting that there is plasticity in the renal response up to an asymptote (15). At all altitudes, there was a strong negative correlation between RR and the Δ pH from baseline, which increased with further ascent. Here, we found that for both LL and TH, there was a moderate negative correlation between RR and delta pH from baseline (Fig. 4 *A* and *B*). Of note, most LL had a RR below 0.5 as pH increased with ascent across most participants, while TH had an RR around 0.5 with a range of both increases and decreases in pH across individuals with ascent, indicating that TH had an overall stronger renal response to the renal stimulus (PCO_2). Of note, RR was significantly higher in TH compared to LL at 4,300 m ($P = 0.0229$; ES = 0.35; Fig. 4*C*).

A previous study introduced an index of %-pH compensation, indexing the pH after compensation to a CO_2 perturbation, to that under control conditions (32). Both measures are first expressed as a delta from a "maximum pH", calculated from the $[HCO_3^-]$ under control conditions and the PCO_2 from the response conditions (see *Materials and Methods*; 31, 32). Consistent with the findings using our RR metric, %-pH compensation was significantly larger in TH than LL after ascent to 4300 m ($P = 0.0079$; ES = 0.6; Fig. 4*D*).

Although significantly different between groups at 4,300 m, we acknowledge that the reported differences in pH and $[H^+]$ are small (Table 1 and Fig. 3; Effect size 1.12). Indeed, small differences in pH can mean large relative differences in free H^+, which exert effects on the tertiary shape and thus function of proteins. However, we also demonstrated larger magnitude renal responses to the reduced CO_2 with ascent in TH compared to LL, as measured by $[HCO_3^-]$

(Table 1 and Fig. 3; Effect size 2.1), as well as metrics of RR and %-pH compensation, which are indices that are indexed relative to individual baseline variables at low altitude (Fig. 4).

Integrated Assessment of Acid–Base Status via Davenport Diagrams. Davenport diagrams are graphical but qualitative demonstrations of the Henderson–Hasselbalch relationship before, during, and after an acid–base disturbance and compensation. Specifically, they demonstrate acid–base disturbances, in this case a respiratory alkalosis, and the corresponding metabolic acidosis, by depicting PCO_2 isopleths, [HCO_3^-], pH, and the [non-HCO_3^- buffer] slope (46). Fig. 5 consists of two Davenport diagrams to illustrate the metabolic compensation to the respiratory alkalosis for LL and TH before (on day 0; 1,400 m) and after ascent (days 8 to 9; 4,300 m). For LL, there was a significant decrease in PCO_2 (respiratory disturbance) and [HCO_3^-] (renal compensation) and significant increase in pH after ascent to 4,300 m, demonstrating the partial metabolic compensation (Fig. 5 *A* and *B*). For TH, there was also a significant decrease in PCO_2 and [HCO_3^-], but an unchanged pH, which demonstrates a complete metabolic compensation (Fig. 5 *A* and *B*). These diagrams illustrate integrative respiratory and renal acclimatization, while also highlighting the variability between groups, with a larger renal compensation in TH to the same environmental P_IO_2 challenge, both in time-course and magnitude.

Potential Underlying Mechanisms. Acclimatization to HA includes a myriad of interrelated processes across various organ systems, including hematological, ventilatory, and renal systems (18, 47). Ventilatory acclimatization results in part from carotid body hypertrophy and hyperplasia in response to sustained hypobaric hypoxia, resulting in an augmented HVR and increases in resting ventilation (14, 18, 21–23). The resulting increase in ventilation at HA elicits hypocapnia, and acutely, a respiratory alkalosis (e.g., 13). Depending upon the time course and magnitude of exposure, the low arterial and interstitial PCO_2/[H^+] acts as a stimulus for renal tubular cells to a) reduce the normal reabsorption of filtered bicarbonate, eliciting bicarbonate excretion and b) reduce H^+ secretion into filtrate, and a full or partial compensatory metabolic acidosis results (13, 15, 16, 48, 49). Indeed, our data suggest that metrics representing acid–base compensation ([HCO_3^-], pH, [H^+], base excess, RR, and %-pH compensation) were augmented in TH after 8 to 9 d ascent to 4,300 m (Table 1 and Figs. 1 and 2), with full compensation to baseline pH values in TH, whereas LL were still alkalemic (Fig. 2). This relative acidemia in TH may protect systemic oxygenation, in part through a) stimulating respiratory chemoreceptors and improving SpO_2 (18, 50) and b) a rightward/downward shift in the oxyhemoglobin dissociation curve, facilitating unloading at the tissue for a given PO_2 (51, 52), although this latter mechanism may be antagonistic to O_2 loading in the pulmonary circulation. Our data suggest that metrics representing ventilation ($\dot{V}_A$, PCO_2, and SSCD; Table 1) were higher in TH at 4,300 m, potentially mediated by a both ventilatory acclimatization and a relative acidemia (i.e., fully compensated pH) in the TH group. However, SpO_2, [Hb], and CaO_2 were not different between LL and TH at 4,300 m, but differences in tissue oxygenation are unknown.

The renal tubules operate on a rapid stimulus–response time-course, whereby increases in bicarbonate diuresis are apparent within 3 to 6 h of simulated high altitude (2,800 m) in a hypobaric chamber, in an altitude- (or rather, CO_2)-dependent fashion (e.g., 49, 53). The intriguing possibility exists that there is plasticity in renal tubular function, whereby oxygen status may affect the responses of the renal tubular network to sustained CO_2 challenges, with persistent hypoxia increasing the renal compensatory mechanisms (e.g., 49; cf., 16 vs. 15). Indeed, there may be direct or indirect hypoxia-dependent effects on renal responses given a) hypoxia sensing mechanisms exist in renal parenchyma (e.g., erythropoietin release; e.g., 54), and b) hypoxia increases sympathetic outflow through carotid body stimulation (e.g., 55, 56), with renal sympathetic nerves innervating the renal tubular network (e.g., 57). Potential differences in sympathetic activation with ascent between TH and LL (e.g., 58) may affect differential reabsorption or excretion of solutes in renal tubules.

Differential oxygen-dependent renal function may be mediated by genes showing evidence of natural selection in TH related to the hypoxia-inducible factor (HIF) oxygen signaling pathway (e.g., EGLN1, EPAS1; e.g., 8, 59, 60–63), or other non-HIF genes (e.g., genes related to the expression of carbonic anhydrase isoforms). Although speculative, genetic variation may play a role in the differential expression of membrane transporters and/or carbonic anhydrase expression in renal tubular cells in sustained hypoxia and/or hypocapnia (e.g., 20, 64). Although there are limited data on renal function in TH with ascent, our study suggests differential renal compensation of blood acid–base homeostasis with early acclimatization to HA between groups, highlighting the important role of the kidney (i.e., renal tubules) in contributing to superior acclimatization to and performance at HA in TH compared to LL, at least during early ascent. These differences appear to persist longitudinally, and with higher ascent (e.g., 12, 28), but more work is needed to better characterize this phenotype, and the underlying genetic, molecular, and cellular mechanisms.

Methodological Considerations. We recruited age- and sex-matched groups of unacclimatized LL and TH at 1,400 m, who subsequently followed the same ascent profile over the same time course. However, some LL participants began baseline testing on day 3 after arrival to 1,400 m from ~sea level. This group of LL may have experienced a mild acute hypoxic response during the baseline testing; however, this likely only had a mild effect on baseline variables. Indeed, TH had significantly lower PCO_2 than LL at 1,400 m (Table 1), suggesting the possibility of differential acclimatization to 1,400 m between groups. To ensure that all LL had sufficient time to acclimatize to 1,400 m, a longer acclimatization period before baseline testing may have been employed to ensure acute ventilatory or renal responses were not captured, but logistical constraints of the HA expedition precluded this. Regardless, previous reports suggest that renal acclimatization after a moderate steady-state stimulus (3,800 m) is complete within ~24 h (3,800 m; 16), and the hypoxic stimulus in the case of LL ascending acutely from sea level to 1,400 m represents a reduction in atmospheric PO_2 of ~20 mmHg, a very mild "hypoxic" stimulus indeed.

The gold standard for blood gas, electrolyte, and acid–base assessment is an arterial blood draw from an indwelling arterial catheter (e.g., 65–67); however, these samples require medical and technical expertise. Even single sample arterial draws carry some risk to participants, including pain (e.g., 68), and associated risk of hyperventilation, potentially confounding resting $PaCO_2$ and acid–base measures. Due in part to the constraints of this fieldwork study (e.g., 69), we utilized average data from a dual series of finger capillary blood draws as surrogate measures of arterial values. Capillary blood draws have been shown to be adequate surrogate measures for arterial blood PCO_2, [HCO_3^-], pH and [H^+] (68, 70–73). However, capillary draws are not adequate measurements for oxygenation (71–77). Accordingly, we used a pulse oximeter to measure peripheral oxygen saturation (SpO_2), which was subsequently utilized to calculate CaO_2 (using [Hb] from the capillary draws).

Importantly, our PCO_2, $[HCO_3^-]$, pH, $[H^+]$ and CaO_2 values are in a similar range to those reported using arterial blood draws in LL at similar altitudes and ascent profile (15).

Participants were able to eat and drink ad libitum, both at 1,400 m prior to ascent, and during the ascent profile, prior to retesting at 4,300 m. However, despite the same ascent profile and accommodations between groups with ascent, dietary and fluid intake were not controlled, due to logistical constraints of the expedition. Given the potential for differential Na^+ intake and handling within and between groups, it is possible that differential bicarbonate reabsorption and/or excretion were affected by Na^+ and/or water intake, which is an important consideration in studies of renal handling of solutes and water, and subsequent regulation of plasma osmolality. We note that blood osmolality was only slightly (but significantly) increased in both groups with ascent, suggesting mild but equivalent effects on electrolyte and fluid handling between groups.

Conclusions

We compared the differences in integrative ventilatory and renal acid–base acclimatization in LL and TH during incremental ascent from 1,400 m to 4,300 m following an identical ascent profile. We found that after ascent to 4,300 m over an 8 to 9 d period, TH had a significantly lower PCO_2, $[HCO_3^-]$, and pH than LL and while pH was increased in LL, it remained unchanged in TH. These findings suggest that TH have larger magnitude and/or more rapid time-course of ventilatory and renal acclimatization mechanisms compared to LL, resulting in fully compensated pH in TH during incremental ascent to 4,300 m. This study compares active acclimatization responses in both LL and TH during incremental ascent to 4,300 m, an altitude previously shown to be near the threshold of renal capacity in LL. Differences in renal acclimatization between LL and TH groups during active acclimatization may reveal intrinsic differences in renal function between populations, which could provide advantages in protecting blood pH during incremental ascent and/or high altitude residence in TH. This study provides insight into the interplay of ancestry and physiological mechanisms contributing to acclimatization to sustained exposure to hypobaric hypoxic environments, which may indicate selective pressure on TH populations related to renal function with acclimatization. Last, these data highlight yet another important role of the kidneys in acclimatization to high altitude (e.g., renal tubules), in this case mediating a larger magnitude compensatory metabolic acidosis, and potentially contributing to the known superior performance of people of TH ancestry at high altitude.

Materials and Methods

Ethical Approval. This study was approved in advance by the Syracuse University Institutional Research Board (Protocol 22-364) as the lead institution, and the University of California Los Angeles via a reliance agreement, as well as the Mount Royal University Human Research Ethics Board (Protocol 103354). The HA expedition was also approved by the Nepal Health Research Council, the national board that oversees research in Nepal. The recruitment and protocol were consistent with the Canadian Government Tri-Council Policy on human research participants and the Declaration of Helsinki, except for registration in a database.

Participant Recruitment. Lowlander participants were recruited via word-of-mouth from a large research expedition in the Nepal Himalaya in Kathmandu (1,400 m) after at least three nights following arrival. Tibetan highlander participants of Sherpa ancestry were recruited by a local collaborator in Kathmandu. No participants had been above 1,400 m in the last year, with the exception of one TH participant, who descended from 3,800 m to 1,400 m six months prior. Recruitment criteria included both males and females who were unacclimatized, nonsmoking, 18 to 35 y of age, with a BMI<30 kg/m^2, with no self-reported underlying cardiac, respiratory, metabolic, or neurological disorders/diseases, were not anemic (<120 g/L), and not on any medications related to these conditions. Efforts were made to recruit an approximately equal number of participants from each group, as well as approximately equal number of males and females in each group, all of a similar age and body mass index.

Participants presented to a hotel room in Kathmandu (1,400 m) for consenting and medical prescreening for eligibility by the research team. Participants provided free, informed verbal and written consent, and then filled out a prescreening questionnaire. If eligible, the protocol was explained prior to data collection. The consent form was provided in either English or Nepali as requested, and a local collaborator provided verbal translation to ensure that Nepali participants understood what was required prior to providing verbal and written consent. If they passed prescreening and provided consent, they completed the protocol in Kathmandu (1,400 m) and subsequently accompanied the research team on an incremental trek to the village of Pheriche (4,300 m) in the Nepal Himalaya. Acetazolamide or corticosteroids were not utilized as prophylaxis nor treatment of acute mountain sickness symptoms in either group.

Measurements. Participants were positioned semirecumbent on a bed with their back against the headboard and their legs elevated with a pillow for ~15-min rest to ensure uniform blood volume distribution and plasma volume stabilization. Their hands were prewarmed with warm water bottles in order to improve capillary blood flow. They were instrumented with a portable finger pulse oximeter, and a steady-state SpO_2 value was recorded just prior to the blood draws.

Venous Blood Draws. Venous blood samples were obtained using universal precautions. Each venous blood sample was obtained via single venipuncture from the antecubital fossa vein with the use of a tourniquet up to a maximum of 60-sec, as required to access the vein, using a disposable 18-gauge straight needle into a 3 mL lithium heparin vacutainer tube. Subsequently, ~1 mL of blood was drawn into a syringe with an 18-gauge needle, such that samples could be immediately introduced into an i-STAT cartridge. Blood samples were then immediately processed using a portable blood gas/electrolyte machine (i-STAT, Abbott, Mississauga, Ontario, Canada) and a single use cartridge (EC8+) for analysis of venous blood electrolyte values, allowing the subsequent calculation of blood osmolality. All samples were subject to thermal correction (37 °C) and local atmospheric pressure calibration. To increase standardization and consistency, and reduce variability, the same member of the research team was responsible for all venous blood draws (AK) and handling and processing of all venous blood samples (TAD).

Capillary Blood Draws. Following the venous sample, finger capillary blood samples were obtained in duplicate by an experienced researcher from a fingertip using universal precautions and new lancet into new 125uL heparinized capillary tubes (safeCLINITUBES, Radiometer, Mississauga, Ontario, Canada). The capillary blood samples were immediately processed using a bench-top blood gas/electrolyte machine (Radiometer ABL80, Mississauga, Ontario, Canada) using full panel oximetry cartridges and solution packs (SC80 CO-OX, Radiometer, Mississauga, Ontario, Canada) to measure or calculate Hct, [Hb], CaO_2, PCO_2, pH, $[HCO_3^-]$, $[H^+]$, base excess (BE), and total CO_2 (TCO_2). All samples were subject to thermal correction (37 °C) and local atmospheric pressure calibration. Previous studies demonstrated that fingertip capillary blood draws are adequate surrogate measures for arterial blood CO_2 and acid–base variables (e.g., 70–73), but without the associated technical expertise or participant risk and discomfort associated with single sample arterial blood draws (e.g., 68), and are thus ideal for fieldwork studies (e.g., 69). All measured and derived variables from dual samples were averaged within-individual to obtain a single representative value, with the exception of three LL participants at 4,300 m and one TH participant at 1,400 m, where a single sample was utilized, due to measurement error.

Ascent Profile. Participants were tested in Kathmandu (1,400 m), and subsequently participated in an incremental HA ascent profile as a group. Participants were first transported by helicopter to ~2,800 m, and immediately began a trekking profile over 5 d to 4,300 m, including a rest day on day 3 at 3,400 m. Upon arrival at 4,300 m on day 5, participants stayed in a local lodge for 3 to 4 consecutive days to allow time for renal acclimatization, and were subsequently retested with the same measurement protocol as 1,400 m on days 8 to 9, equally balanced on each day between LL and TH.

Data Analysis. To assess ventilatory acclimatization, we calculated alveolar ventilation ($\dot{V}_A$) from PCO_2 from capillary blood draws in both locations, using the standard equation:

$$Alveolar\ ventilation\ \left(\frac{l}{min}\right) = \left(\frac{200 \times 0.863}{PCO_2}\right).$$

In addition, we calculated an index of SSCD. First, a stimulus index (SI; PCO_2/SpO_2) was first calculated, whereby ventilation is known to be linearly and directly proportional to changes in the pressure CO_2 (e.g., 78) and linearly and inversely proportional to changes in SpO_2 (e.g., 79). Alveolar ventilation was then indexed to SI to calculate SSCD (35, 43–45) to assess the combined contributions of chemostimuli to resting ventilation in the steady-state.

To assess hydration status and renal function, we calculated osmolality from the venous blood draws in both locations, using the following equation (15, 80):

$$Osmolality = 1.86\left(\left[Na^+\right] + \left[K^+\right]\right) + 1.15\left(\frac{[Glucose]}{18}\right) + \left(\frac{[Urea]}{6}\right) + 14.$$

To assess oxygenation, we calculated arterial oxygen content (CaO_2) using [Hb] from the capillary blood draws and SpO_2, in both locations, using the following equation (81):

$$CaO_2 = 1.34 \times [Hb] \times \left(\frac{SpO_2}{100}\right).$$

To assess a measure of renal compensation, we calculated an index of RR using the following equation (15):

$$RR = \frac{\Delta[HCO_3^-]}{\Delta PCO_2}.$$

Where delta refers to the change in variable between 4,300 m and 1,400 m.

To assess the relationship between RR and pH, we plotted RR against a change (Δ) pH from 1,400 m to 4,300 m (15, 16).

Total %-pH compensation achieved in capillary blood at 4,300 m between groups as a result of renal compensation with ascent was calculated (31, 32). %-pH compensation was calculated using:

$$\%-pH\ compensation = \frac{\Delta(pH\ at\ 4{,}300\ m\ -\ maximum\ pH)}{\Delta(control\ pH\ at\ 1{,}400\ m\ -\ maximum\ pH)} \times 100.$$

Maximum pH was calculated via the Henderson–Hasselbalch equation using the [HCO_3^-] under control conditions at 1,400 m and the PCO_2 at 4,300 m.

For qualitative assessment of renal compensation with ascent in both groups, Davenport diagrams were plotted comparing CO_2, [HCO_3^-], and pH in both groups and both locations, with the nonbicarb buffer slope set at the following (46):

$$\left[HCO_3^-\right] = 24 + 22 \times (7.4 - pH).$$

Statistical Analysis. To compare all measured and derived values with ascent between altitudes (1,400 m with 4,300 m; main effect) and groups (LL and TH; main effect), we utilized a mixed-model two-factor (2F) ANOVA, which was repeated-measures (RM) with altitude (two levels) and non-RM with groups (two levels). Where significant F-ratios were detected, a Fisher's LSD post hoc test was performed for pairwise comparisons (Table 1).

For more specific comparisons of ventilatory and acid–base variables within groups with ascent, we utilized a two-tailed, paired *t*-test, comparing 1,400 m with 4,300 m in both groups (Figs. 1 and 2). To assess differences in ventilatory and acid–base variables between LL and TH groups 4,300 m, we utilized two-tailed unpaired *t*-tests (Fig. 3). Where tests of normality failed using the Shapiro–Wilk test, a Wilcoxon signed rank test (for paired data) or Mann–Whitney test (for unpaired data) was utilized instead. Effect sizes were calculated using Cohen's d.

To assess the relationship between RR and ΔpH, we performed a Pearson product-moment correlation.

In all cases, statistically significant differences were assumed at $P < 0.05$ (GraphPad Prism v10.0, GraphPad Software, La Jolla, CA).

Data, Materials, and Software Availability. All study data are included in the main text.

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Author affiliations: [a]Department of Biology, Faculty of Science and Technology, Mount Royal University, Calgary, AB T3E 6K6, Canada; [b]Radboud University Medical Center, Nijmegen 6525 XZ, Netherlands; [c]Department of Movement and Sports Sciences, Ghent University, Ghent 29000, Belgium; [d]Laboratory of Sport, Expertise and Performance, French Institute of Sport (INSEP), Paris 75012, France; [e]Department of Exercise Science, Syracuse University, Syracuse, NY 13210; [f]Department of Health Sciences, Mid Sweden University, Östersund 831 25, Sweden; [g]Kathmandu Center for Genomics and Research Laboratory, Global Hospital, Lalitpur 44700, Nepal; [h]College of Medicine, Nepalese Army Institute of Health Sciences, Kathmandu 44600, Nepal; [i]Medical University of the Americas, Charlestown, Nevis, Saint Kitts and Nevis, West Indies, KN0802; [j]Glory of Nepal Travels and Tours, Kathmandu 44600, Nepal; and [k]Department of Anthropology, University of Los Angeles, Los Angeles, CA 90095

Author contributions: A.W.B., T.D.B., and T.A.D. conceived of or designed the study and organized the expedition; A.W.B., T.D.B., and T.A.D. obtained funding and ethical clearance; A.J.K., N.T., S.D., and N.S. assisted with local ethical clearance and study coordination; J.A.D., B.W.L.M., R.I., A.K., J.B., N.D.J.S., T.S.H., P.H., A.W.B., T.D.B., and T.A.D. recruited participants and collected data; all authors contributed to editing the manuscript; all authors approved the final manuscript; and N.A.J., J.A.D., N.D.J.S., A.W.B., T.D.B., and T.A.D. wrote the paper.

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