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Increased blood-oxygen binding affinity in Tibetan and Han Chinese residents at 4200 m

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Abstract

High-altitude natives are challenged by hypoxia and a potential compensation could be reduced blood P_{50}, as seen in several high-altitude mammalian species. In 21 Qinghai Tibetan males and 9 Han Chinese, all resident at 4200 m, standard P_{50} was calculated from measurements of arterial PO_2 and forehead oximeter oxygen saturation (SpO_2), which was validated in a separate examination of 13 healthy sea-level subjects. In both Tibetans and Han Chinese, standard P_{50} was 24.5 (± 1.4 and 2.0 mmHg, respectively) and was lower than in the sea-level subjects (26.2 ± 0.6 mm Hg, $p < 0.01$). There was no relationship between P_{50} and [Hb] (the latter ranging from 15.2 and 22.9 g/dl in Tibetans). During peak exercise, P_{50} was not associated with alveolar-arterial PO_2 difference or VO_2/kg. There appears to be no apparent benefit of a lower P_{50} in this adult high-altitude Tibetan population.

Keywords

P_{50}; hypoxia; altitude

1. Introduction

Hemoglobin (Hb) P_{50} is the PO_2 at which Hb is 50% saturated with O_2, and is commonly used to describe the position of the Hb-O_2 dissociation curve. For a given hemoglobin type, blood P_{50} depends on pH, PCO_2, temperature and the concentration of 2,3-diphosphoglycerate (DPG). This has led to definition of the standard P_{50} (about 27 mmHg in healthy subjects at sea level), as the P_{50} when pH is 7.40, the PCO_2 is 40 mmHg, and temperature is 37 degrees centigrade (Lenfant *et al.*, 1968).

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Acute hypoxic exposure, as occurs during rapid ascent to altitude, causes a reduction in blood-O_2 binding affinity (i.e. an increase in P_{50}) due to elevated 2,3-DPG levels that occur over several hours and persist at least several weeks (Wagner *et al.*, 2007). However, greater blood-O_2 affinities have been documented in several vertebrate species that are native to high altitude (Monge & Leon-Velarde, 1991), and in some cases, this effect is due to genetically based increases in Hb-O_2 affinity (i.e. deer mice (Storz *et al.*, 2009; Storz *et al.*, 2010; Natarajan *et al.*, 2013) and hummingbirds (Projecto-Garcia *et al.*, 2013)).

Human highland populations exhibit a wide range of standard P_{50}. Sherpa P_{50} values (Samaja et al., 1979) have been reported as comparable to those of non-native acclimatized visitors of European descent (~28 ± mmHg) (Samaja *et al.*, 1979; Wagner *et al.*, 2007) with the exception of one report (22.6 ± 0.5 mmHg (Morpurgo *et al.*, 1976)). Tibetan subjects examined at intermediate and high altitude exhibit considerable variation in standard P_{50} (Tashi *et al.*, 2014) that is, on average, less than lowlander and Andean (Lenfant *et al.*, 1968) values at high altitude. Considering previous studies have shown that average P_{50} differs among populations from the same continental region, it is important to note that such disparities may result from differences in equipment, environmental factors, techniques used for analysis (Winslow *et al.*, 1985), or sub-population structure (Wuren *et al.*, 2014). To our knowledge, variance in P_{50} has not been compared to variance in [Hb], nor has P_{50} been examined in relation to exercise capacity, or oxygen transport, in Tibetan inhabitants residing in the northeast region of the Qinghai-Tibetan Plateau. Some Tibetans in this region have adaptive genetic factors associated with reduced [Hb] (Beall *et al.*, 2010; Simonson *et al.*, 2010; Yi *et al.*, 2010) in addition to several genetic regions yet to be examined in a physiological context. One such region contains the beta-hemoglobin gene cluster on chromosome 11, which exhibits an adaptive signal in this population (Simonson *et al.*, 2010) and other Tibetan groups (Yi *et al.*, 2010). These findings suggest adaptive genetic associations could underlie important changes related to Hb structure/function and/or expression at this locus that may affect P_{50}.

A reduction in P_{50} has usually been regarded as beneficial to the high-altitude organism by enhancing the rate of diffusive equilibration of O_2 in the pulmonary capillary with that in the alveolar gas in a hypoxic environment that is challenging to the pulmonary uptake of O_2. A left-shifted blood-O_2 dissociation curve better maintains the alveolar to capillary diffusion gradient as the red cell progress along the pulmonary capillary and capillary PO_2 increases towards the alveolar value. It is therefore possible that Tibetan blood-O_2 binding affinity has also adapted (increased) to compensate for reduced PO_2 in the Tibetan native environment.

Therefore, the purpose of this study was to determine a) whether average P_{50} in a Tibetan population resident at 4200 m above sea level is lower than values reported in native lowlanders studied both at sea level and after acclimatization at altitude; and b) if so, is P_{50} reduced more in those Tibetans who also have lower [Hb]? We hypothesized that in Tibetans who have adapted with low [Hb], standard blood P_{50} would be lower, and thus blood-O_2 affinity would indeed be higher, than in those with high [Hb].

Considering only a pulse oximeter was available for measuring O_2 saturation in the remote Tibetan site, we were limited to these measurements of arterial saturation. Therefore, in sea-

level subjects, we determined whether the same pulse oximeter was accurate in measuring arterial saturation compared to direct measurements with co-oximeter and also whether the range of arterial saturations observed in the Tibetan and Han Chinese subjects encompassed values low enough to accurately estimate P_{50}.

2. Methods

2.1 Overview

The project was partly conducted in the remote Tibetan village of Maduo (altitude 4200 m). Twenty-one Tibetan males and nine Han Chinese males resident at 4200 m for at least two years were studied at this altitude (Simonson *et al.*, 2012b) and analyzed to determine if [Hb] is related to P_{50}. As a part of this study, arterial blood samples were collected at rest and during exercise. Hemoglobin P_{50} was determined using the approach described previously (Wagner *et al.*, 2007) whereby blood gas values from all samples from any one subject were combined into one data set in which the P_{50} that best fit paired saturation and PO_2 values of all samples in the set was determined by least squares minimization. In this approach, all PO_2 values, which were measured at 37 degrees centigrade, were first corrected to pH 7.40 and PCO_2 40 mmHg using equations contained within the Kelman subroutines that describe the O_2 dissociation curve (Kelman, 1966, 1967), so that the outcome was the standard P_{50}. The usual technique for measuring PO_2, PCO_2 and pH uses a blood gas electrode system, and this was available at the study site in Maduo (iSTAT Portable Clinical Analyzer, Abbott, USA). The usual technique for measuring O_2 saturation in a blood sample, be it arterial or venous, uses a co-oximeter. Because P_{50} is often in the range of venous PO_2, it has always been customary to include venous samples in the data set. However, a co-oximeter was unavailable in Maduo, and therefore only pulse oximetry could be used for measuring saturation, limiting the data to arterial saturation (during exercise, at altitude). Saturation was measured using a forehead sensor pulse oximeter (Nellcor N-395 Pulse Oximeter).

These limitations posed two questions: First, was the pulse oximeter sufficiently accurate in measuring arterial saturation, and second, was the degree of arterial desaturation observed in each subject sufficient to allow accurate determination of P_{50}? To resolve these uncertainties, additional exercise studies were performed in San Diego (SD), California, USA, on return from Maduo using seven sea-level residents. Additionally, previously unused arterial blood gas data (unpublished data) from an exercise study conducted for other purposes in Athens, Greece, using six healthy cyclists were included with permission. Data from all sea-level subjects were collected and analyzed for the primary purpose of validating the use of arterial O_2 saturation measurements from a forehead oximeter (rather than co-oximeter venous and arterial O_2 saturation) to estimate P_{50}.

2.2 Methods in subjects at 4200 m altitude in Tibet

Native highland Tibetan and Han Chinese male subjects residing in the village at 4200 m completed a health history questionnaire and physical examination; heart/lung disease, diabetes, anemia, or hypertension were used as exclusion criteria from the study. A total of 21 healthy Tibetan and nine Han males (23 ± 6 and 26 ± 9 years of age, respectively) agreed

to volunteer and were included, and all subjects provided informed written consent per Qinghai Medical College and University of California San Diego (UCSD) ethical guidelines and studies were approved by both review boards. First, [Hb] was measured from peripheral venous blood (2 ml) with a Mindray Hematology Analyzer (BC-2300, Shenzhen, People's Republic of China). EKG leads and a Nellcor forehead oximetry sensor were attached to measure heart rate and arterial O_2 saturation. After locally anesthetizing the skin over the intended placement site, a 20-gauge catheter was placed into the radial artery of one wrist using sterile technique and secured to assure no bleeding and to minimize risk of infection.

Subjects were then seated on a cycle ergometer, a 2ml resting heparinized blood sample was drawn from the arterial catheter for blood gas analysis, and data from the forehead oximeter were recorded. Subjects were then asked to warm up by pedaling at light exercise breathing room air (at between 45 and 90 watts, according to individual subject preference). After two minutes, subjects were asked to pedal at a moderate level and when VO_2 stabilized, an arterial blood sample (2ml) was collected while forehead saturation measurements were recorded. After two minutes at this level of exercise, subjects pedaled at maximal effort. Towards the end of this period, another 2ml arterial blood sample was drawn and forehead saturation recorded. Blood gas analysis measurements, which included PO_2, PCO_2, pH, and base deficit, were therefore recorded during rest, submaximal, and maximal exercise using an i-Stat Abbott blood gas analyzer at 37C. All data collected from rest to peak VO_2 were used for analyses.

2.3 Methods in sea-level subjects

While the following technical experiments were secondary to the primary objective of assessing P_{50} in Tibetan and Han Chinese, they are now described in some detail because of the importance of method validation in Maduo.

We conducted analyses in lowlanders at sea level in order to determine: 1) whether arterial saturation measurements from the forehead oximeter used in Maduo correspond to those simultaneously collected from a radial arterial catheter and analyzed by a co-oximeter, and 2) whether arterial saturation measurements alone are low enough to accurately estimate P_{50} during exercise under hypoxic conditions, or whether both arterial and venous data are required for an accurate P_{50} estimate. Since arterial saturation was never lower than 70% in the Maduo Tibetans at maximal exercise, and previous validation of forehead oximetry fell within a comparable range (73-100%) (Yamaya *et al.*, 2002), we used only those data in the saturation range of 70-100% for the forehead pulse oximeter comparison with co-oximetry in Caucasians at sea level.

The 13 subjects were divided in two cohorts. Group A was a group of six Greek cyclists (studied for other purposes in Athens, Greece), from whom samples of arterial and peripheral venous blood were available (and used with permission) during exercise in normoxia and hypoxia. The samples from these cyclists were collected following 5 minutes of rest, then 5, 3, and 2 minutes at 30%, 80%, and 100% maximal effort, each at F_IO_2s of 0.21, 0.15, and 0.12 O_2, respectively, (with one hour rest between each F_IO_2). Using those samples, PO_2, PCO_2, pH, were determined by blood gas electrodes and saturation by co-oximetry. Group B was a group of seven American cyclists, studied in San Diego only for

the purposes of the present project, in whom similar measurements including arterial blood gases were made both at rest and during exercise in normoxia and hypoxia (F_IO_2=0.12), with the addition of the very same Nellcor forehead pulse oximeters used in Maduo. Studies conducted in both Group A and B subjects were used for comparison of P_{50} estimates based on arterial and arterial plus venous O_2 saturation measurements; cooximeter vs oximeter comparisons were conducted based on O_2 saturation data collected in Group B. All studies were approved by the University of Athens and UCSD review boards.

Prior to the study, all subjects provided written informed consent, and were then screened to determine their maximal exercise capacity, which was used to set an appropriate exercise regime for light (20-30% of max) and moderate (50%-75% of max) exercise under varying inspired O_2 conditions (12%, 15%, 21%) lasting no more than 15 minutes for the entire period of rest and exercise (1-3 minutes at each level of exercise). Catheters (20 gauge, 25 mm) were placed in one radial artery and one peripheral, superficial arm vein using local anesthetic and sterile technique as described above for Tibetan subjects. Once seated on the cycle ergometer and after four minutes of rest, 2 ml of blood was drawn from arterial and venous catheters. During each blood sample taken in group B subjects, data from two the Nellcor forehead oximeters (designated as A and B) were recorded.

2.4 Data analysis in sea-level subjects and estimated P_{50} in all groups

Correspondence between O_2 saturation measurements from the two Nellcor forehead oximeters (labeled A and B) and from the paired blood samples analyzed by co-oximeter (IL model 682) was examined using Bland-Altman plots (Bland & Altman 1986) for the seven SD subjects (Figure 1). Adequacy of using only arterial saturation for P_{50} determination was determined in the 13 sea-level subjects by testing whether P_{50} derived from arterial saturation measurements alone (reflecting only saturations greater than 70%) differed from estimates derived from data sets containing both arterial and venous blood, which provided information both about the upper and lower regions of the curve (Figure 2).

In order to determine the standard P_{50}, we first computed “virtual PO_2” from actual (measured) PO_2 values, using computer algorithms published by Kelman (Kelman, 1966, 1967). Virtual PO_2 is the PO_2 that would exist after correcting for temperature, PCO_2, and pH when any of these variables differed from standard conditions (37° C, 40 mmHg, and 7.4, respectively), as previously reported (Wagner *et al.*, 2007). Temperature required no correction since the samples were measured in an analyzer at 37 degrees. Using a range of trial P_{50} values from 10 to 40 mmHg in 0.1 mmHg increments, each sample’s O_2 saturation was then calculated from the virtual PO_2 for each trial P_{50}. The squared difference between calculated and measured saturations (from the co-oximeter for sea-level and forehead oximeter for highland resident subjects, respectively) was summed for each subject, and the trial P_{50} that resulted in the lowest sum of squares was determined as the standard P_{50} for that subject.

In vivo P_{50} during peak exercise was also estimated in Tibetans and Han Chinese by using Kelman routines with correction for PCO_2 and pH from arterial blood gases; core temperature could not be measured, and was estimated at 37° C for all subjects as the short duration and limited power output during peak exercise (<2 minutes) would not be expected

to affect core temperature beyond an average of 0.6° C (data from (Wagner *et al.*, 1986; Wagner *et al.*, 1987)).

Using all data points from the set of arterial and venous samples spanning from rest to maximal exercise and $F_IO_2$values of 0.21, 0.15 and 0.12 resulted in a set of data for each subject in which some points lay on the flat part of the blood-O_2 dissociation curve (i.e., resting arterial data at F_IO_2=0.21) while others lay on the steep portion of the dissociation curve (venous data during hypoxic exercise). The non-linear nature of the blood-O_2 dissociation curve means that a given error in measured saturation for a point on the flat part of the curve would result in a large error in estimated P_{50}, while the same error in saturation for a point on the steep portion of the curve would give rise to a much smaller error in P_{50}. We therefore excluded saturation limits greater than 95% when estimating P_{50}.

2.5 Data analysis of subjects in Tibet

We also performed regression analysis of data from the 21 Tibetan subjects at maximal exercise to determine whether a relationship was present between a) P_{50} and [Hb], b) P_{50} and the alveolar-arterial PO_2 difference ($AaPO_2$), c) P_{50} and systemic O_2 extraction, and d) P_{50} and peak VO_2/kg in the 21 Tibetan subjects at maximal exercise. AaP O_2 and systemic O_2 extraction were determined using computer algorithms that model diffusive O_2 uptake in the lungs and tissues, respectively, using a forward integration procedure (Wagner & West, 1972; Wagner, 1996). Mixed venous O_2 content was determined by solving for this unknown variable in the Fick equation: VO_2, cardiac output (PhysioFlow Enduro™, Paris, France, based on Signal-Morphology Impedance Cardiology), and arterial oxygen saturation/[Hb] were measured; the only remaining variables, venous SO_2 and CvO_2, were calculated, and PvO_2 was determined from the dissociation curve for that SO_2/CvO_2 (base excess and O_2-CO_2 interactions taken into account by the same Kelman computer routines used to compute P_{50}). The first comparison tests the hypothesis that as [Hb] is reduced in adapted Tibetans, P_{50} is also lower, as seen in many high-altitude native mammals. The second comparison tests the hypothesis that, if all other factors affecting pulmonary gas exchange were equal, $AaPO_2$ should be less when P_{50} is lower, as explained in the introduction. The third comparison tests the hypothesis that, if all other factors affecting systemic O_2 extraction were equal, extraction should be less when P_{50} is lower.

3. Results

The results will be presented in the following order: First, correspondence between the arterial saturation measured by pulse oximeter and that measured directly in arterial blood by co-oximeter will be shown for the seven sea-level subjects studied in San Diego. Second, the P_{50} values estimated from arterial data alone will be compared to those determined from both arterial and venous samples for the 13 sea-level subjects providing such data. Next, the values of the standard P_{50} will be shown for all three-subject groups: 13 sea-level subjects; 21 Tibetan natives at 4200 m; nine Han residents at 4200 m. Finally, in the cohort of 21 Tibetan and nine Han Chinese males at 4200 m, the relationships between P_{50} and [Hb], SaO_2, $AaPO_2$, systemic O_2 extraction, and peak VO_2/kg will be shown.

3.1 Results in sea-level subjects and estimated P_{50} in all groups

Overall, agreement is excellent for heart rate, which indicates appropriate acquisition of pulse-rate signal, and generally excellent for saturation as measurements below 70% were excluded from analysis (low saturations are commonly known to be less accurate) (Table 1). The two pulse oximeters agreed with each other more than either agreed with the co-oximeter, suggesting that pulse oximeter values were most different from those of the co-oximeter. Table 1 gives the mean values for saturation, heart rate, and P_{50} estimated from O_2 saturation measurements for the three devices. Mean saturation values are significantly if slightly (< 1 %) higher for two pulse oximeters compared to the co-oximeter ($p < 0.03$, 0.59), and average heart rate measured by the two pulse oximeters was slightly lower (one beat/minute) than that indicated by EKG. Estimates of P_{50} using O_2 saturation measurements from the cooximeter and each pulse oximeter were not different ($p > 0.8$ and 0.5 for co-oximeter versus oximeters A and B, respectively; Table 1) and fall within the range of acceptable limits based on Bland-Altman analysis (Figure 1). The standard P_{50} in sea-level (26.6 ± 2.2; n=13), Tibetan (24.5 ± 1.4; n=21), and Han Chinese (24.5 ± 2.0; n=9) subjects, the latter two groups both living and studied at 4200 m, were all estimates based on saturation measurements between 70% and 95%. Both Han and Tibetan values were significantly lower than those of the sea-level subjects, whose values correspond well with generally accepted sea-level norms.

Figure 2 shows how different methods (with and without inclusion of venous saturation data) are reflected in P_{50} values obtained. As explained in the methods section, we compared estimates obtained from arterial (A) samples alone with those from both arterial and venous (AV) samples. The motivation for comparing AV and A data in Caucasian subjects was to determine whether just arterial saturation measurements during exercise under hypoxic conditions could be used to estimate P_{50} in contrast to needing both arterial and venous data, as the latter provide points at the lower end of the O_2 dissociation curve. Figure 2 shows that either approach falls within acceptable limits based on Bland-Altman analysis. While the arterial values appear systematically greater than the arterial and venous combined values, the insignificant difference is less than 0.32 mmHg.

3.2 Results in subjects at high altitude

Figure 3 shows, over a wide range of Hb concentration, the lack of relationship between P_{50} and [Hb] (A) but a significant relationship between P_{50} and SaO_2 (B) in the 21 Tibetans and nine Han Chinese. Figure 4 shows the relationships between in vivo P_{50} and gas exchange in the lungs as reflected by the alveolar-arterial PO_2 difference (panel A), in the tissues as reflected by % O_2 extraction (panel B), and in overall peak VO_2/kg (panel C). There was no significant relationship between P_{50} and these variables at maximal exercise.

4. Discussion

4.1 Summary of main findings

There were four main observations made in the present study: First, standard P_{50} in native Qinghai Tibetans at 4200 m was 2 mmHg lower than normal sea level values, at 24.5 ± 1.4 mmHg; also, standard P_{50} in sea-level Han Chinese relocated to this altitude (resident

approximately two years) was not different from Tibetan values at 24.5 ± 2.0 mmHg, while P_{50} in sea- level subjects, using the same technique applied in the altitude studies, was found to be 26.6 ± 2.2 mm Hg, well within the normal range. Second, arterial O_2 saturation in Tibetans was higher when P_{50} was lower, but P_{50} was unrelated to [Hb], $AaPO_2$, O_2 extraction or exercise capacity. Third, determining P_{50} from arterial saturation values alone measured at rest and during exercise at altitude, spanning the saturation range down to 70% was found to yield a P_{50} value not different from that measured using both arterial and venous data (which bracket P_{50}). Fourth, the very same pulse oximeters used in Maduo yielded arterial saturation values in San Diego that were very close to those measured simultaneously in radial arterial blood by co-oximetry.

4.2 Findings in Tibetans and Han Chinese

P_{50} in Han Chinese and Tibetans at 4200 m were similar and significantly lower than those in sea-level subjects. No difference in peak VO_2/kg was observed between groups, consistent with some (Brutsaert 2008; Faoro *et al.*, 2014) but not other studies (Ge *et al.*, 1995; Sun *et al.*, 1990) regarding aerobic capacity in different ethnic groups. The former findings do not exclude the possibility that individual P_{50} and VO_2 values may be correlated. Additionally, while no group signal was detected in our data, this may be limited by a modest sample size.

At least two of the following criteria for peak VO_2 were met for participants of the study: 1) heart rate ≥ age predicted (220 minus age) maximum (this is conservative, as maximal heart rate is generally reduced in chronic hypoxia); 2) respiratory exchange ratio > 1.10; 3) with increasing workload, there was no further increase (or decrease) in VO_2; and 4) despite an increase in workload, there was no further increase in heart rate; 5) rest to peak exercise increase in base deficit of 6 mmol/liter or more. Overall, at exhaustion, average heart rate was 172/min; RER was 1.19; and base deficit was 13 mmol/l (rising from 4 mmol/l at rest). This was similar in both Tibetans and Han Chinese groups.

It is recognized that P_{50} increases to values in excess of 30 mmHg as sea-level residents acclimatize to high altitude, due to elevated 2,3-DPG levels (Wagner *et al.*, 2007). The Han and Tibet data we report are thus not just considerably different (by 6 mmHg or more), but actually opposite in direction, from the acute and sub-acute responses seen in acclimatizing lowlanders (Wagner *et al.*, 2007). Interestingly, it appears that native high-altitude Andeans at or above 3700m have increased P_{50} values, with values similar to those in acclimatized lowlanders (30.6 ± 0.7 mmHg) (Lenfant *et al.*, 1968); Andeans also have not evolved a sea-level [Hb] phenotype like many Tibetans. This further underscores likely different evolutionary hypoxia-response pathways between Andeans and Tibetans.

The possible effect(s) of 2,3-DPG, ATP, intracellular Cl and Hb isoforms were not examined in this study, but warrant future investigation based on the findings presented here. In addition, the calculated SO_2 values at higher PO_2 may be slightly underestimated based on the O_2 dissociation curve (Severinghaus, 1979) used in the Kelman subroutines thereby overestimating P_{50}; in such case, the estimates of P_{50} shown here might be even lower than reported. That the sea-level subjects had normal standard P_{50} values of 26.6 mm Hg suggests this may be of minor significance in the present study.

These observations raise a number of questions. First, if Han and Tibetans have similar, lower P_{50} values and yet only Tibetans have adapted by reduced [Hb], does this suggest that the reduced P_{50} in Tibetans is not genetically related to the [Hb] adaptation? Second, why do Han and Tibetans exhibit a reduced P_{50} (compared to sea- level subjects) while high-altitude natives in South America have a P_{50} higher than that of sea-level subjects? Third, what is the functional importance of the P_{50} changes in any of these three groups compared to sea-level subjects? The latter is discussed in some detail in the next section.

Similarly reduced P_{50} in Tibetan and Han could be a result of convergent alteration(s) to this physiological outcome or shared common ancestry, although the reliability of P_{50} in nine Han Chinese will need to be explored in future studies with more subjects of this ethnicity. The human beta-globin gene cluster on chromosome 11 is a candidate gene identified in more than one genomic study of adaptation in Tibetans (reviewed in (Simonson *et al.*, 2012a)). It is therefore plausible that genetic changes in protein-coding variants, such as those reported in high-altitude deer mice (Natarajan *et al.*, 2013), and/or alterations in regulatory regions, which control Hb isoform levels that naturally vary among humans (Thein *et al.*, 2009), may be associated with P_{50} in Tibetans. Whether this will account for the reduced P_{50}, and whether similar genetic changes will be found in Han Chinese, both remain to be determined.

4.3 Significance: A) Relationship between P_{50} and SaO_2 but not between P_{50} and [Hb]

Greater blood-O_2 binding affinity is a very common, putatively adaptive mechanism well documented in native high-altitude species with genetic variants that underlie Hb-O_2 binding affinity such as deer mice (Storz *et al.*, 2009; Storz *et al.*, 2010; Natarajan *et al.*, 2013) and hummingbird (Projecto-Garcia *et al.*, 2013), in addition to reports of increased affinity in Andean llama, vicuña (Hall *et al.*, 1936), chinchillas and guinea pigs (Velarde *et al.*, 1991), yak and pika on the Tibetan Plateau (Adams *et al.*, 1975; Ge *et al.*, 1998). Previous work indicates how low P_{50} at altitude may be beneficial at high altitude (Eaton *et al.*, 1974; Hebbel *et al.*, 1978). Considering P_{50} is, on average, lower in the Tibetan subjects examined here, it is conceivable that a reduction in P_{50} would be associated with reduced [Hb], a trait shown to be previously associated with adaptive genetic factors in this population. There was, however, no correlation between P_{50} and [Hb] in our Tibetan subjects or when Tibetan and Han Chinese data are analyzed together as a single group (Figure 3A). Since lower [Hb] was previously found to be associated with improved exercise capacity in the Tibetan subjects examined here (Simonson *et al.*, 2012b), it is possible that in vivo P_{50}, which is not associated with [Hb] nor peak VO_2/kg as shown (Figure 4), has minor if any influence in the adult Tibetan males examined here; however, whether lower P_{50} results from genetically based changes in Tibetans remains undetermined.

P_{50} in Tibetan and Han Chinese subjects is, however, associated with SaO_2 (Figure 3B). We considered three possible physiological explanations for the SaO_2-P_{50} relationship. One is that subjects with low P_{50} ventilate more, but this was not the case, as ventilation did not correlate with P_{50} (data not shown). A second is that gas exchange is more efficient in subjects with a low P_{50}, as assessed by the Alveolar-arterial (A-a) PO_2 difference. This too

was not found to be the case. The higher saturation at lower P_{50} is therefore likely the direct result of a left shift in the blood-O_2 dissociation curve with similar PO_2 values.

4.4 Significance: B) P_{50} and pulmonary gas exchange

Increased blood-O_2 affinity has long been considered to enhance diffusive O_2 loading in the pulmonary capillary, which is challenged in normal subjects at altitude because gas exchange takes place entirely on the steep part of the Hb-O_2 curve (West, 2012). A left-shifted Hb-O_2 dissociation curve allows more O_2 molecules to move from alveolar gas to pulmonary capillary blood without as much increase in capillary PO_2 as would occur with a right-shifted curve. This preserves the alveolar to capillary diffusion gradient for O_2, and explains enhanced diffusive equilibration. Thus, a left shift should be of value in augmenting O_2 transport at the reduced PIO_2 at altitude, increasing arterial PO_2 and reducing the alveolar-arterial PO_2 difference ($AaPO_2$).

However, in the present study, no relationship was found between either arterial PO_2 or $AaPO_2$ and P_{50} in the Tibetans, which was unexpected given the preceding logic. Prior findings in healthy humans during exercise at altitude have shown that the majority of the $AaPO_2$ is due to failure of diffusion equilibration (Wagner *et al.*, 1986; Wagner *et al.*, 1987). The results of this study show that $AaPO_2$ is unrelated to in vivo P_{50} (Figure 4A), implying that reduced P_{50} in Tibetans is of little significance in pulmonary gas exchange, whether in the context of VA/Q inequality or diffusion limitation.

We considered whether the benefit of a reduced P_{50} might not be seen over the relatively narrow range of the standard P_{50} (21.9-27.9 mmHg) and in vivo (22.3-30.6 mmHg) P_{50} values encountered. Note that in vivo P_{50} was greater than standard P_{50} because, during exercise, the effect of acidosis outweighs the influence of lowered PCO_2, resulting in an overall right-shift of the blood-O_2 dissociation curve. To evaluate the possibility of reduced P_{50} in either case, we used algorithms for computing the rise in PO_2 along the pulmonary capillary (Wagner & West, 1972; West & Wagner, 1980). Using the average blood gas data measured at peak exercise in the Tibetans (Simonson *et al.*, 2012b; Simonson *et al.*, 2012c), we calculated the alveolar-arterial PO_2 difference that would be expected as a function of P_{50} over a wide range, from 15 to 32 mmHg. Figure 5, upper panel, shows the outcome. In essence, the benefit of a reduced P_{50} at the intermediate altitude of the Tibetan subjects (4200 m), as reflected by a reduction in alveolar-arterial PO_2 difference, is not seen across the range of both standard and in vivo P_{50} we encountered. It is only when P_{50} falls below about 20 mmHg that conventional logic applies and the alveolar-arterial PO_2 difference starts to fall. In sum, the lower P_{50} appears to have little or no functional significance for pulmonary gas exchange during exercise in this adult population at this altitude.

4.5 Significance: C) P_{50} and systemic O_2 extraction

Variation in in vivo P_{50} among Tibetan subjects appears not to be associated with systemic O_2 extraction. However, reduction in P_{50} should interfere with O_2 extraction, the standard explanation being as for the lungs (described above), but opposite in direction: Just as a lower P_{50} should enhance diffusive equilibration in the lungs, a higher P_{50} should enhance diffusive unloading of O_2 in the muscles. This is because with a right-shifted blood-O_2

dissociation curve, there can be greater movement of O_2 across the muscle capillaries while maintaining a higher capillary PO_2 and thus capillary to mitochondrial O_2 diffusion gradient (compared to a left-shifted curve). Because of the findings in the upper panel of Figure 5, showing that P_{50} would not affect pulmonary gas exchange in the actual range encountered, we performed similar modeling of peripheral O_2 extraction by diffusion again using the same algorithms and actual average blood gas data from the Tibetans at peak exercise. In this case (Figure 5, lower panel) the relationship between % O_2 extraction and P_{50} was essentially linear, and similar to the findings shown in Figure 4B. Over the range of standard and in vivo P_{50} values (21.9-27.9 and 22.3-30.6 mmHg, respectively), the calculations in Figure 5 suggest extraction increasing by 10 percentage points from about 70% to about 80%.

The high variance and lack of relationship between P_{50} and extraction in Figure 4B are likely due to influences of additional factors important to extraction, such as blood flow rate and muscle O_2 diffusional conductance. Feedback mechanisms, including tissue-specific changes in metabolism and pH, are also plausible contributors based on findings in other species (Storz & Moriyama, 2008) but have not been examined here. Other research in this population, focused specifically on high and low [Hb] in Tibetans and relationships to O_2 transport ((Simonson *et al.*, 2012b), unpublished data), indicates no significant difference in ventilation or blood gas measurements. The consequent effects, if any, on the overall transport and utilization of O_2 would require analysis of several additional variables, which is beyond the scope of the work reported here. However, we determined there is no significant association between peak VO_2/kg and in vivo P_{50} in high-altitude subjects examined (Figure 4C).

The wide range of P_{50} observed in Han Chinese and Tibetans may be attributed to various experimental and/or biological factors. We suspect that the amount of time participants spent at altitude did not contribute to this variation as Tibetan subjects were permanent residents and Han Chinese had been resident above 4000m for at least two years. Considering many adaptive factors have been reported in Tibetans, it is likely that different genetic loci and/or variants within the Hb genetic cluster may, at least in part, underlie this variation, perhaps "pulling" P_{50} across the wide range observed. Furthermore, [Hb] varies across a wide range, even though P_{50} and [Hb] are not correlated.

While the lower P_{50} does not play a major role during exercise at altitude, the context in which these adult subjects were examined, we speculate that it may be important at other stages such as fetal development, when O_2-binding affinity is greatest (~19mmHg at sea level) to ensure in utero survival, or pertinent in neonatal development. In such cases, increased affinity may persist without rigid constraint into adulthood. It therefore remains to be determined if adaptive variants underlie the lower P_{50} observed in this population, whether such changes may be beneficial under different conditions (e.g. development) and/or are related to other adaptive changes that serve to compensate or coordinate hypoxia tolerance in these populations.

4.6 Methodological issues

Taken together, the above findings indicate that valid measurements of blood-P_{50} can be obtained at altitude in the absence of standard co-oximetry and in the absence of data at a low (<70%) saturation, using only arterial blood gases and pulse oximetry. We used a pulse oximeter that employs optodes laid against the forehead rather than the more common digital pulse oximeters. During peak exercise, our experience has been that fingertip oximeters may not always be reliable when subjects grip cycle handlebars tightly. This risk is compounded by exercise in a cold environment (the rooms in which we performed the studies in Maduo were unheated and at relatively low temperatures), potentially comprising digital perfusion and thus oximeter signals. Whether the methods we used would have produced similar results using more common fingertip oximeters was not studied and is thus unclear.

Because we did not have blood temperature, we modeled the effects of a 0.6° C increase in blood temperature, which is the average increase reported in previous studies under comparable conditions (Wagner *et al.*, 1986; Wagner *et al.*, 1987) (i.e., several minutes of exercise at 150-180 watts, which was the range observed in high-altitude subjects examined here). We found that a) $AaPO_2$ was estimated to fall by just 0.1 mm Hg (from 18.6° C to 18.5° C as temperature was raised from 37° C to 37.6° C), and b) that O_2 extraction was estimated to increase from 75.4 to 77.0% for the same 0.6 degree temperature increase. These are small effects, and indicate that the uncertainty in core temperature, which would affect in vivo but not standard P_{50} numbers, would not have changed the overall outcome or interpretation of the study.

4.7 Conclusions

High-altitude native Tibetans living at 4200 m have a standard P_{50} that is about 2 mmHg lower than that of sea-level natives, and perhaps as much as 6 mmHg lower than that of both Andean high-altitude natives and Caucasian sea-level natives acclimatized to high altitude. The reduction in P_{50} was found to be unrelated to [Hb], suggesting the absence of a link between [Hb] itself and blood P_{50}. To our surprise, P_{50} in Han Chinese living at the same altitude was similarly reduced, further casting doubt that genetic adaptations associated with [Hb] are also related to P_{50}. During maximal exercise at 4200 m, reduction in in vivo P_{50} does not appear to enhance pulmonary gas exchange, impair systemic O_2 extraction, or influence peak VO_2/kg. Thus, the low P_{50} was not found to confer any physiological advantage. Why Tibetans appear to be adapting to altitude by reducing [Hb] and not improving pulmonary gas exchange while Andeans appear to have evolved with distinct pulmonary adaptations (increased lung volumes and diffusing capacity) but remain polycythemic remains to be elucidated.

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New Findings

What is the central question of this study?

Is Tibetan and Chinese highlanders' blood oxygen-binding affinity (P_{50}) different from that of other populations (at altitude or sea level) and does Tibetan P_{50} relate to hemoglobin concentration and/or exercise capacity at altitude?

What is the main finding and its importance?

- Tibetans and Chinese at 4200m have slightly lower P_{50} than sea-level residents.
- During maximal exercise at 4200m, reduced P_{50} does not enhance pulmonary gas exchange, impair systemic oxygen extraction, or affect peak exercise capacity.
- Oxygen saturation measurements based upon forehead oximetry are sufficiently reduced during exercise at altitude (and accurate compared to those obtained from arterial blood by co-oximetry) to reliably determine P_{50}.

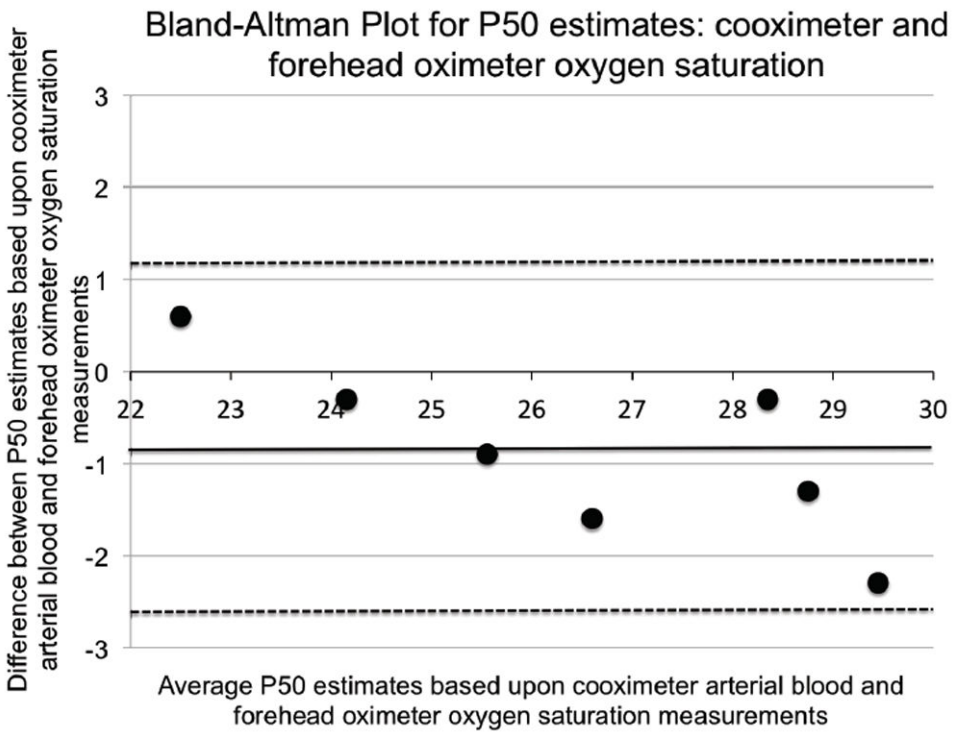

Figure 1.

Bland-Altman plot comparing P_{50} estimates based on arterial blood cooximeter to forehead oximeter saturation measurements in seven sea level subjects (70-100% values, which are within the range examined in Tibetan and Han Chinese subjects examined at 4200 m, are included).Mean difference, standard deviation: (-0.87, 0.96); 95% confidence limits (-2.76, 1.01) are shown as dashed lines; a solid line indicates identity.

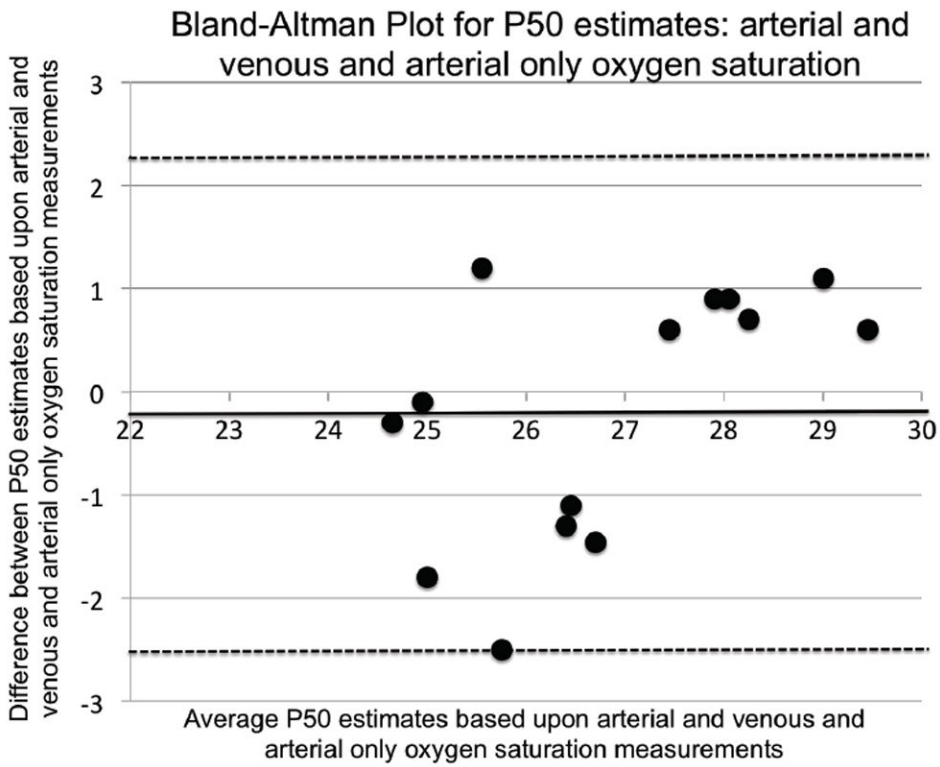

Figure 2.

Bland-Altman plot comparing standard P_{50} calculated in 13 sea-level subjects based upon 1) the use of arterial plus venous (AV) saturation data to arterial (A) saturation data alone. Mean difference, standard deviation: (-0.2, 1.23). 95% confidence limits (-2.59, 2.23) are shown as dashed lines; a solid line indicates identity.

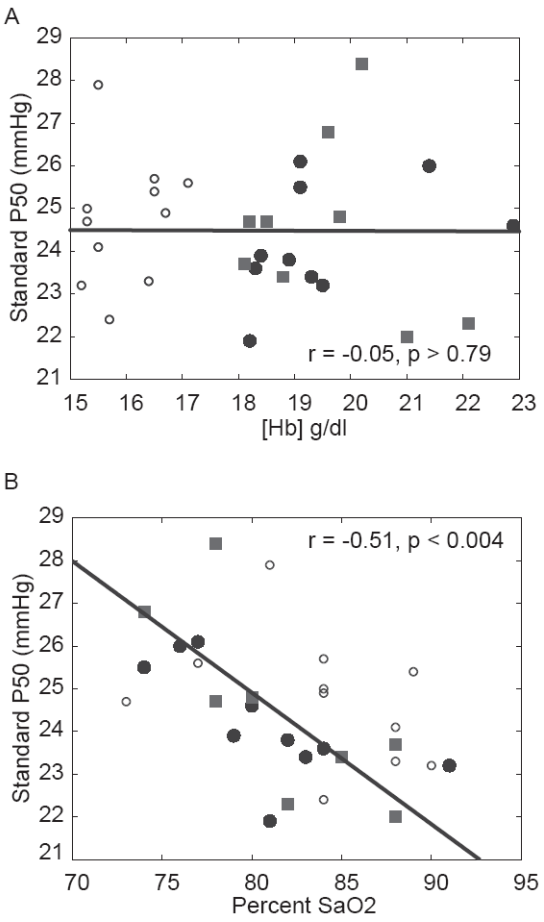

Figure 3.

Data showing the relationship between P_{50} and [Hb] (A) and SaO_2 during peak exercise (B). A) No relationship was found between [Hb] and P_{50}. (B) Exercise SaO_2 and P_{50} in Tibetan and Han Chinese subjects are significantly correlated. Han data are indicated by squares; low and high [Hb] in Tibetans indicated by open and closed circles, respectively.

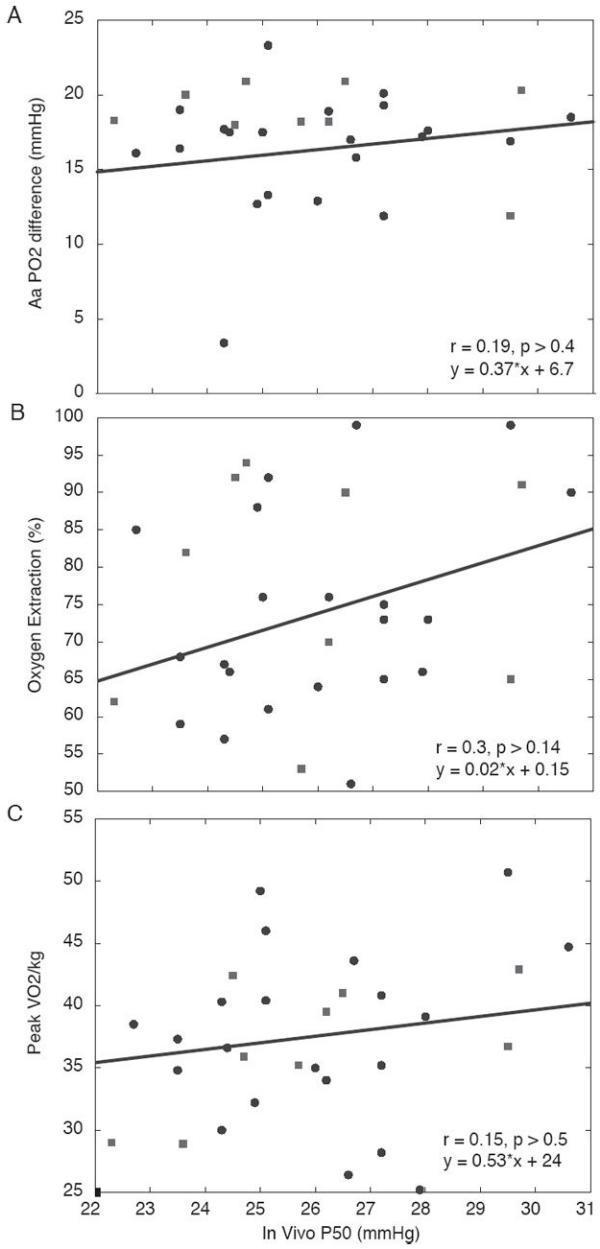

Figure 4.

Data showing the relationship between P_{50} and Alveolar-arterial PO_2 difference (A), O_2 extraction (B), and peak VO_2/kg (C). Han data are indicated by squares; Tibetan data are indicated by circles. A low in vivo P_{50} did not affect these variables over the P_{50} range encountered.

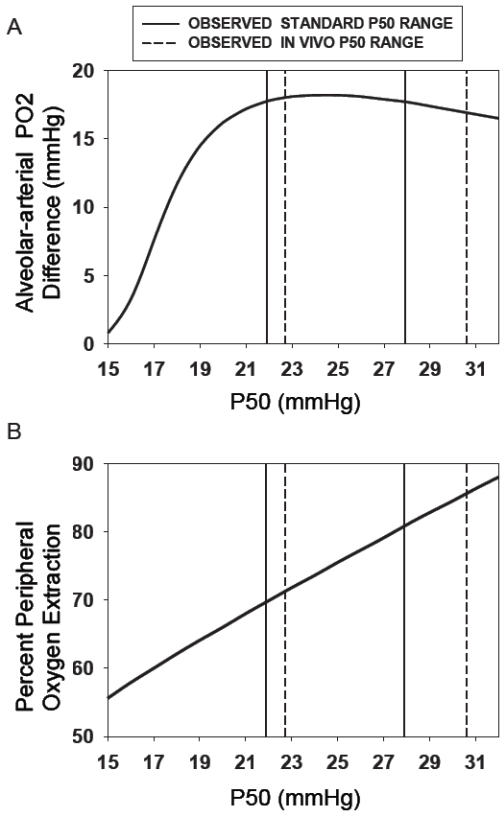

Figure 5.

Theoretical calculations of the Alveolar-arterial PO_2 difference ($AaPO_2$) (upper panel) and peripheral O_2 extraction (lower panel) during peak exercise as a function of standard and in vivo P_{50} (ranges indicated by solid and dashed lines, respectively). While extraction increases essentially linearly with P_{50} over a wide range, $AaPO_2$ falls with P_{50} only when the latter is lower than about 20 mmHg. These outcomes help explain the observations in Figure 4.

Table 1

Means and standard deviations of pulse oximeter and co-oximeter saturation measurements of percent SaO_2, standard P_{50} based on each method for measuring O_2 saturation, and pulse oximeter and EKG heart rate (HR) measurements in Caucasian subjects.

	Pulse Oximeter A	Pulse Oximeter B	Co-oximeter/EKG
SaO_2 (percent)	91.5±8.5	91.0±9.1	90.8±8.2
P_{50} (standard, mmHg)	26.0±2.2	26.9±3.0	26.2±0.6
Heart Rate (beats/minute)	125.9±38.5	125.9±38.4	127.0±37.7

Table 2

Hemoglobin concentration, percent oxygen saturation, blood gas variables (PaO_2, $PaCO_2$, and pH), and standard and in vivo P_{50} during peak exercise at 4200m in Tibetan and Han Chinese males. Hemoglobin concentration is significantly different in Tibetan and Han Chinese subjects ($p < 0.05$).

Group Ethnicity	[Hb] (g/dl)	Arterial PO_2 (mmHg)	Arterial PCO_2 (mmHg)	Arterial pH	% SaO_2	Standard P_{50} (mmHg)	In vivo P_{50} (mmHg)
All subjects: Mean, Standard deviation	18.2 ±2.1	45.7 ±4.6	26.0 ±2.6	7.31 ±0.05	82.0 ±5.0	24.5 ±1.6	26.0 ±2.1
Tibetan: Mean, Standard deviation	17.7 ±2.1	46.3 ±4.9	26.0 ±2.9	7.31 ±0.06	82.3 ±5.2	24.5 ±1.4	26.0 ±2.0
Han Chinese: Mean, Standard deviation	19.6 ±1.4	44.3 ±3.7	26.0 ±1.8	7.30 ±0.04	81.2 ±4.9	24.5 ±2.0	25.9 ±2.5