Pulmonary nitric oxide in mountain dwellers

Populations living at high altitudes have an adaptive mechanism to offset hypoxia.

Nitric oxide is synthesized in the lungs to help regulate blood flow, and its levels have been found to drop in species native to low altitudes, including humans, upon acute exposure to reduced oxygen concentration[1–3]. But we show here that exhalation of nitric oxide by chronically hypoxic populations of Tibetans living at 4,200 m and of Bolivian Aymara at 3,900 m is unexpectedly increased compared with a low-altitude reference sample from the United States. This consistent response in two far-removed, high-altitude locales indicates that increasing the concentration of nitric oxide in the lungs may represent a means of offsetting hypoxia.

We measured the geometric mean concentration of nitric oxide (NO) exhaled by individuals in a group of healthy non-smokers from Tibet (Fig. 1) as 18.6 p.p.b. ($n = 105$; range, 5.5–55.7 p.p.b.; coefficient of variation (c.v.), 1.5%) — more than twice the value for the low-altitude reference sample (7.4 p.p.b.; $n = 33$; range, 4.5–14.6; c.v., 2.4%). The geometric mean NO concentration exhaled by the Aymara was 9.5 p.p.b. ($n = 144$; range, 2.7–30.3; c.v., 1.9%) — over 25% greater than that for the low-altitude reference (Fig. 2). Artificial relief from hypoxia (inspiration of oxygen at 42–50% *v*/*v* concentration) resulted in an increase of 2.5 p.p.b. in NO exhaled by Tibetans ($n = 26$, $P < 0.05$), but caused no change among the Aymara ($n = 25$, $P > 0.05$), suggesting that the mechanism for sustaining high NO levels may differ between the two populations.

Measurements of NO exhaled at the mouth reflect accurately and qualitatively the dynamics of NO production and consumption in the lungs[1]. Reduced consumption is unlikely to explain the higher NO values of high-altitude natives, because haemoglobin rapidly scavenges NO and both samples showed high haemoglobin concentrations compared with those taken from sea-level dwellers (see supplementary information).

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Figure 1 The high altitude at which Tibetans live means that they are constantly exposed to reduced atmospheric oxygen concentrations.

Reduced consumption is also unlikely to explain the higher NO levels found in Tibetans compared with the Aymara.

Controlling for the significantly higher haemoglobin concentrations in Aymara samples by comparing subsamples with the same range of haemoglobin (153–158 g l^{-1}) revealed mean NO concentrations of 19.9 p.p.b. and 8.8 p.p.b. for the 16 Tibetans and the 25 Aymara, respectively. NO levels did not correlate with haemoglobin concentration or resting oxygen saturation in either sample. An increase in NO synthesis is therefore a more likely explanation for the high NO concentrations in these two samples (such an increase would therefore be smaller in the Aymara).

Nitric oxide is synthesized by NO-synthase enzymes and its synthesis depends on the availability of molecular oxygen[1,4,5], so a drop in oxygen concentration would be expected to result in reduced NO production by synthases. Possible adaptations to maintain high-output NO synthesis under hypoxia include variant forms of the enzyme that show altered kinetics for oxygen dependence, modification of co-factor availability through post-translational effects, and/or increased expression of the synthase enzymes themselves.

To assess the potential benefits of increased levels of endogenous NO to high-altitude populations, we investigated whether increasing NO at sea level improves oxygen uptake by the lungs and thereby offsets hypoxia. Oxygen uptake improved in a dose-dependent manner ($P < 0.05$) in the presence of exogenous NO at concentrations of 1.3–31,600 p.p.b. during hypoxic ventilation (10.4% oxygen; measured by the progressive reduction in oxygen concentration at the end of a 10-s exhalation, when values reflect exhalate concentration from the alveoli where gas exchange occurs). End-exhalation oxygen concentration decreased from 8.5% to 7.8% as exogenous NO concentration was increased; over 70% of the effect on oxygen uptake occurred in the physiological range at 290 p.p.b NO. An unchanged end-exhalation CO_2 measurement confirmed that increased oxygen uptake was not due to a momentary increase in oxygen consumption or to metabolic demands.

The human model of high-altitude adaptation should also incorporate a functional, adaptive benefit of high NO levels in the lungs. NO produced in the lungs dilates pulmonary blood vessels, increases pulmonary blood flow and reduces pulmonary hypertension. By reacting with haemoglobin in red blood cells, NO increases haemoglobin oxygenation and may improve the delivery of oxygen to tissues by enhancing systemic vasodilation and blood flow[6].

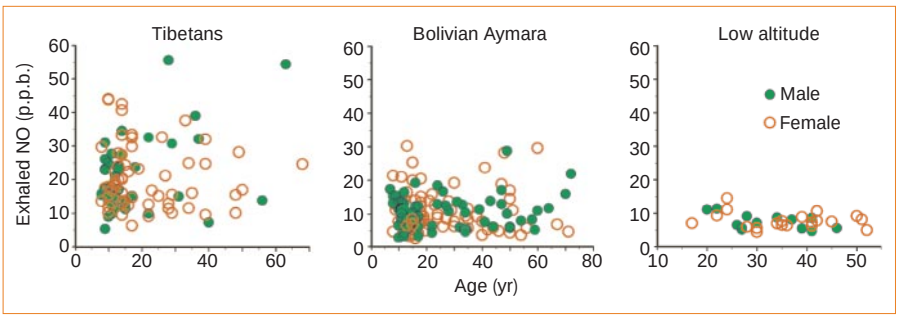

Figure 2 A Tibetan population living at 4,200 m, a Bolivian Aymara population at 3,900 m and a low-altitude population in the United States differ significantly in their mean concentrations of exhaled nitric oxide (NO; ANOVA, $F = 77.9$, d.f. $= 2$, $P < 0.05$); no sex or age differences are evident in the results. Details of methods are available from the authors.

Several distinctive features of Tibetan and Andean high-altitude natives are related to their increased vasodilation and blood flow compared with acclimatized lowlanders. For example, Tibetans are better than Han Chinese at increasing their cerebral blood flow during exercise[7], as well as their utero–placental blood flow[8]; the Andean Aymara show a large capacity for pulmonary diffusion[9] and the Andean Quechua have better circulation to cold extremities than Europeans[10]; and Tibetan and Andean natives show higher oxygen saturation during exercise than do acclimatized Han Chinese and Europeans[11,12].

The similar responses of these two geographically separate high-altitude populations underlines the importance of NO for life under hypoxic stress. The functional advantage of high NO concentrations in the lungs seems to be to offset ambient hypoxia by enhancing the uptake of oxygen from the lungs, which presumably improves delivery of oxygen to peripheral tissues.

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Longevity

Extending the lifespan of long-lived mice

Ames dwarf mice are mutant mice that live about 50% longer than their normal siblings[1–3] because they carry a 'longevity' gene, *Prop1*^{df}, and in some phenotypic respects they resemble normal mice whose lifespan has been extended by restricted food intake[2,4,5]. Here we investigate whether these factors influence lifespan by similar or independent mechanisms, by deliberately reducing the number of calories consumed by Ames dwarf mice. We show that calorie restriction confers a further lifespan increase in the dwarfs, indicating that the two factors may act through different pathways.

To investigate the effects of calorie restriction on the already extended lifespan of Ames dwarf mice, we divided 45 2-month-old Ames dwarf mice and 53 of their normal siblings into two groups, which were subjected either to calorie restriction (CR) or to continued feeding *ad libitum* (AL). We fed CR mice daily, reducing their food intake in successive weeks to 90%, 80% and finally 70% of that consumed daily by genotype- and sex-matched AL animals[6]. Because the food consumption of AL mice declines naturally with age, the amount of food given to CR animals was kept constant after the age of 2 years.

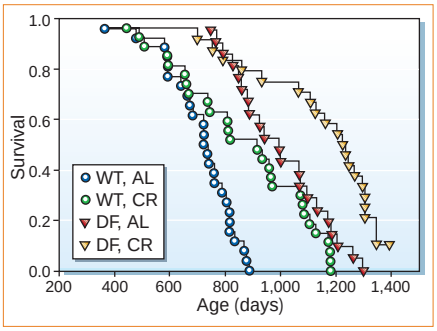

Figure 1 Survival plots of Ames dwarf (DF) and normal (wild-type, WT) mice fed *ad libitum* (AL) or restricted to 70% of normal calorie intake (calorie restriction, CR).

The survival curves shown in Fig. 1 indicate that calorie restriction causes a further significant increase in the longevity of Ames dwarf mice. When males and females are considered together, the difference between the CR and AL groups of Ames dwarf mice is significant ($P < 0.004$, log rank test). The effect of calorie restriction on lifespan in Ames dwarf mice is also significant ($P < 0.05$) when genders are considered separately. As expected, calorie restriction also extends the lifespan of normal mice ($P < 0.002$), although AL Ames dwarf mice outlive AL normal mice ($P < 0.00001$). Moreover, CR Ames dwarf mice outlive CR normal mice ($P < 0.0001$).

The survival plots (Fig. 1) reveal a further disparity: although both dwarfism and calorie restriction extend longevity, the effect of reduced food intake is associated primarily with a change in the slope of the survival curve (that is, it reduces the rate of age-related mortality), whereas the effect of dwarfism mainly reflects a shift in the age at which the age-dependent increase in mortality risk first becomes appreciable. Calorie restriction therefore seems to decelerate ageing, whereas the *Prop1*^{df} allele seems to delay it.

Our results indicate that long-lived Ames dwarf mice are not merely mimics of CR mice, and show that the pathways responsible for extending lifespan in the dwarfs and in CR animals are not identical. However, features that are shared by CR normal mice and Ames dwarf mice, and by long-lived knockout mice that lack the growth-hormone receptor[7], include reduced body size and lower plasma levels of insulin, the insulin-like growth factor IGF-1, glucose and thyroid hormone. These factors may contribute to delayed ageing and increased longevity in each of these animal models.

For example, the IGF/insulin or a similar signalling pathway is involved in lifespan determination in the fruitfly *Drosophila melanogaster*[8,9], the roundworm *Caenorhabditis elegans*[10], and yeast[11]. This supports the idea that hormonal regulation of metabolic pathways in response to altered food availability may be a way of regulating lifespan that is deeply rooted in evolutionary history.

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