Point: Positive effects of intermittent hypoxia (live high:train low) on exercise performance are mediated primarily by augmented red cell volume

For nearly half a century, athletes have used “altitude training” to enhance sea level performance. Both altitude acclimatization and hypoxic exercise have been proposed as mediating this enhancement. However, hypoxic exercise impairs training quality (18, 19) and, in the absence of acclimatization, does not augment performance (30). The “living high-training low” model was therefore developed (19) and demonstrated to be effective for athletes of all abilities (4, 18, 26).

So which of the myriad aspects of altitude acclimatization (16) might be responsible for improving performance of athletes at sea level? Rigorous use of accepted scientific principles must be applied to determine cause and effect. We would like to propose “Levine and Stray-Gundersen’s Postulates” (a modification of Nobel laureate Robert Koch “Koch’s Postulates”) to determine the etiology of performance enhancement with altitude exposure, as follows.

First, the response (improvement in VO₂ max and performance) must be present when the mechanism (increase in erythrocyte volume) is present. Corollary: when no increase in erythrocyte volume is present, there is no increase in VO₂ max and no improvement in aerobic performance.

Second, the mechanism must be isolatable and demonstrated to have an unequivocal relationship to altitude exposure and improved performance.

Third, when the mechanism is manipulated independently (without altitude exposure), then the same improvement in physiological parameters and performance must occur. Corollary: in the presence of altitude exposure, when the specific mechanism is inhibited, then the outcome is prevented.

In the original publication of the “live high-train low” model, we demonstrated clearly that exposure for >20 h/day to 2,500 m altitude for 4 wk led to an increase in erythrocyte volume, an increase in VO₂ max, and improved performance in an event (5,000 m time trial) that is dependent on high rates of oxygen transport (18). In contrast, a control group exposed to an event (5,000 m time trial) that is dependent on high rates of oxygen transport (18, 19) did not improve their race time by more than the group mean ("responders") and those that got worse (“nonresponders”; Ref. 4). There were no differences between these groups with respect to numerous physiological variables that might influence acclimatization to altitude (4).

Rather, the key distinguishing feature was that the responders had a greater increase in erythropoietin concentration with acute altitude exposure, which remained elevated for a more prolonged period of time. Indeed, the erythropoietin increase in the responders after 2 wk at altitude was equivalent to the peak response in the nonresponders, in whom erythropoietin had returned to baseline. This difference in erythropoietin response patterns was clearly physiologically significant and not a chance occurrence; the responders had an increase in erythrocyte volume and increased VO₂ max, whereas the nonresponders did not. Furthermore, the increase in VO₂ max was exactly what would be predicted from change in blood volume and hemoglobin concentration (31): predicted increase 248 ml/min – actual increase 245 ml/min(4). This derivation model was confirmed prospectively in another population (4, 26).

Although our results have led us to focus on the erythropoietic pathway, this was not an exclusive hypothesis at the beginning of our experiments. For example, in large numbers of runners (n > 100), running economy never changed (18, 19); anaerobic capacity never changed (16, 18, 19); muscle biopsies did not increase in buffer capacity or oxidative enzymes (27). Thus the weight of evidence has led us inexorably toward the primary effect of altitude acclimatization, given an adequate exposure, on sea level performance in competitive athletes being on the erythropoietic pathways.

But is there other evidence that altitude exposure is erythropoietic? Indeed, this evidence is extensive and quite compelling, particularly when the exposure is high enough and sustained for a long enough period of time. For example, cross-sectional studies in North (33) and South America (12, 20, 23) have demonstrated that there is an elevated red cell mass in natives of high altitude that is proportional to the altitude of residence and oxyhemoglobin saturation (12, 33). When sea level natives ascend acutely to altitude, there is a large increase in iron turnover that begins immediately on exposure (6, 11, 20). Most convincingly, direct examination of the bone marrow during acute high-altitude exposure has documented a dramatic increase in erythroid cell lines, from 20.0% at sea level to 40.5% after 1 mo at 4,300 m (11, 20). Accelerated erythropoiesis has also been confirmed in elite athletes at more moderate altitudes (7, 10, 24, 32). Thus despite rare exceptions (8), the evidence from multiple research groups has confirmed that moderate altitude exposure for nearly 24 h/day increases the red cell mass even in elite athletes.

But do lesser durations of exposure also increase the red cell mass? Clearly very short duration exposures of even extreme altitude are not sufficient to accomplish this goal (14). However, 12-16 h/day of normobaric hypoxia for 3 (3) or 4 wk (15, 21, 22) closely replicates the results observed in the field studies with an increase in both hemoglobin mass and VO₂ max. In contrast, our opponents, using only 8–10 h/day of normobaric hypoxia (2,500–3,000 m) for 10–21 days failed to demonstrate an increase in hemoglobin mass or VO₂ max (1). We would submit that this exposure is insufficient to stimulate a sustained accelerated erythropoiesis—the “dose” of altitude is simply too low (17).
Recent advances in the basic science of hypoxia response pathways may explain this apparent “threshold” phenomenon (25). For example, the principal transcriptional activator of gene expression in hypoxic cells is hypoxia-inducible factor-1α (HIF-1α). Under well-oxygenated conditions, HIF-1α is hydroxylated and binds to the Von Hippel-Lindau factor, which targets the entire complex for ubiquitin degradation. This process is so rapid, that in the presence of oxygen, HIF-1α has one of the shortest half-lives of any known protein (13). Moreover, when altitude natives or sojourners return to sea level, there is a suppression of erythropoietin (4, 6, 18, 20), a reduction in iron turnover and erythroid cell lines (11, 20), and a marked decrease in red cell survival time (20), termed “neocytolysis.” Both the rapid destruction of HIF-1α and neocytolysis may compromise the ability of short duration (<12–16 h/day) hypoxia to increase the red cell mass.

So what about the last of “Koch’s postulates”? Can the red cell mass be manipulated independently from altitude exposure and obtain the same effect? Clearly this is so. For example, increasing the red cell mass directly by blood doping or indirectly by injecting erythropoietin improves V\textsubscript{O2}\text{max}, laboratory-based performance (2, 5), and success in international competition (29). Furthermore, low-dose erythropoietin injection increases the erythrocyte volume to a degree that is virtually identical to that acquired by 4 wk of altitude exposure to 2,500 m, data that have been obtained in collaboration with our opponents (17). Finally, when the increase in erythrocyte volume at altitude is inhibited in athletes by iron deficiency (9, 19) or infection (27), V\textsubscript{O2}\text{max} does not increase and performance is not augmented.

In summary, the evidence demonstrates that given adequate exposure (living high enough, long enough, for enough hours per day), altitude is clearly erythropoietic even in elite athletes and leads to an increase in erythrocyte volume/red cell mass, V\textsubscript{O2}\text{max}, and performance in endurance sport. To our knowledge, there are no other effects of altitude acclimatization (including all the alternatives proposed by our opponents) that can be manipulated independently and demonstrated to improve athletic performance over a sustained period of time. The magnitude of the response at altitude is quantitatively and qualitatively similar to that induced by isolated manipulation of the red cell mass (low-dose Epo injection), and the outcome is prevented if the erythropoietic process is impaired by iron deficiency or infection. Thus we would contend that all of Koch’s Postulates have been fulfilled for determining a cause-and-effect relationship between erythropoiesis and success of altitude training.

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Counterpoint: Positive effects of intermittent hypoxia (live high:train low) on exercise performance are not mediated primarily by augmented red cell volume

To engage in this debate we will address the following questions: what is the change in performance after adaptation to living high and training low (LHTL); what physiological mechanisms could be responsible; what is the evidence that a change in red cell volume (RCV) is one such mechanism; and what is the evidence for other mechanisms?

**Change in endurance performance after LHTL.** The smallest worthwhile change in performance time for elite middle-distance runners is ~0.5% (15, 16). Controlled trials of LHTL via either terrestrial or artificial hypoxia have consistently revealed greater increases in endurance performance, typically ~1% (10-12, 19, 22, 27). However, none of the studies was performed blind, and the placebo effect may be of similar magnitude (4). Substantial correlations between individual responses in performance and changes in physiology would provide assurance that the performance change is not entirely a placebo effect (14), but the evidence for such correlations is still unclear (see below). Meanwhile we assume that LHTL produces a physiologically mediated enhancement in endurance performance averaging approximately ±1%.

**Mechanisms for LHTL-mediated enhancement of endurance performance.** Exercise tests performed at intensities greater than VO2 max last <10 min and are powered partly by anaerobic mechanisms, whereas longer exercise is powered essentially by the aerobic system (7). If LHTL enhances only anaerobic mechanisms, the enhancement of performance would decline to zero for tests lasting >10 min. The observed effects of LHTL are as follows: 45 s, 0.8% (22); 4 min, 1.0% (10); 9 min, 1.1% (27), 1.5% (12) and 1.8% (11); and 17 min, 1.3% (19). Although uncertainty in these estimates precludes firm conclusions, it would appear that LHTL affects mainly the aerobic system.

Di Prampero (5) realized that aerobic power at intensities below VO2 max is the product of three components: VO2 max, the fraction of VO2 max representing exercise intensity (VO2 fractional), and exercise economy (power per unit of VO2). It follows that percent changes in these components add up to the percent change in endurance performance, apart from measurement error. Furthermore, changes in performance could be due to changes in any component and to more fundamental physiological effects underlying it.

**Evidence for the role of RCV.** An increased RCV would enhance performance by increasing VO2 max via increases in maximum cardiac output (from increased total blood volume) or oxygen-carrying capacity (from increased hemoglobin concentration). Levine and colleagues (3, 19) reported increased RCV of 5–8% after terrestrial LHTL, but we believe that such large changes are more likely to be artifacts of measurement error than physiological adaptations to moderate altitude (9). Indeed, RCV change in the various studies appears to be directly proportional to measurement error! In studies of artificial LHTL at the Australian Institute of Sport, where the carbon-monoxide method is used to measure hemoglobin mass rather than RCV, the measurement error is ~2% and observed changes in hemoglobin mass are consistent with little or no real change (1, 2, 24). Moreover, artificial LHTL results in little evidence of an increase in reticulocytes, despite transient increases in erythropoietin concentration (1). Nevertheless, the real changes in hemoglobin mass or RCV in some studies may be sufficient to account for the changes in performance.

A correlation between changes in individuals’ RCV and performance after LHTL would represent additional circumstantial evidence for the role of RCV. There are no reports of such correlations, presumably because the correlations were statistically nonsignificant. Lack of significance could be due to masking of a substantial correlation by large measurement error, but in our view it is more likely the correlations are truly small or trivial.

In the absence of a clear direct relationship between RCV and performance after LHTL, Levine and coauthors focused on the role of VO2 max. Again, changes in mean VO2 max and correlations between individuals’ changes in VO2 max and performance are less than compelling. There is a wide range in the mean change in VO2 max after LHTL (23), and uncertainty in
the estimates makes the range even wider. A change in \( \text{VO}_2\max \) sufficient to explain the performance change after LHTL (\( -1\% \)) is therefore possible in many studies, and we accept that these studies represent supporting but not sufficient evidence that a change in RCV is the mechanism. However, in most studies, the true change in \( \text{VO}_2\max \) could have been trivial or negative, which would necessitate some other mechanism. In the only report of a correlation between changes in \( \text{VO}_2\max \) and performance after LHTL, Levine and Stray-Gundersen (19) stated that “...the close correlation between the increase in \( \text{VO}_2\max \) and improvement in 5000-m time...argues strongly that this is the key adaptation during altitude training.” The correlation was indeed strong (0.63) for the pooled data of subjects in all three groups (LHTL, live high-train high, and control). However, performance did not improve posttreatment for the latter two groups, and the correlation was smaller for the LHTL subjects (0.51, recalculated from their Fig. 6). Most of this correlation was due to one subject, who ran 12% slower in the posttest. In any case, \( \text{VO}_2\max \) in an incremental test often does not show a plateau with endurance athletes (6); \( \text{VO}_2\max \) itself may therefore be modified by voluntary effort, so at least part of the correlation between changes in \( \text{VO}_2\max \) and time-trial time could be due to the placebo effect.

Researchers have also investigated the relationship between changes in RCV and \( \text{VO}_2\max \) for evidence of the role of RCV in performance enhancement. Levine and Stray-Gundersen (19) reported a correlation of 0.37. There have been reports of correlations of 0.70 after 24 days of LHTL (28) but only 0.04 after 46 days of artificial LHTL, with measurement of hemoglobin mass rather than RCV (25). We conclude that in some studies there is evidence consistent with an increase in \( \text{VO}_2\max \) due to increases in RCV, but the extent to which these changes contribute to performance enhancement is unclear.

Evidence for other mechanisms. We suspect that exercise economy is the component of the di Prampero model most likely to mediate effects of LHTL. Improvements in economy of 3–6% have been observed after various hypoxic interventions with athletes (8, 17, 18, 21, 24, 25), although correlations with change in performance have not been reported. A switch to a more economic mode of oxygen utilization is a teleologically appealing adaptation to a shortage of oxygen in tissues (13), and a suitable regulatory system involving various intracellular changes mediated by hypoxia inducible factor (20, 26) exists in most cells.

LHTL researchers have neglected the \( \text{VO}_2\text{tracmax} \) component of endurance performance and its surrogate, lactate anaerobic threshold. Although it is unclear why adaptive responses to hypoxia would include changes in this component, its role needs to be clarified experimentally. Finally, even if an increase in the \( \text{VO}_2\max \) component mediates the effect of some LHTL protocols on endurance, the underlying mechanism need not be an increased RCV; teleologically and physiologically plausible alternatives could involve changes in cardiovascular regulation that result in increased muscle blood flow during intense exercise.

In conclusion, the quality and quantity of published data are insufficient to elucidate the mechanism of the effect of LHTL on performance, but improvements in economy seem more likely than increases in RCV. Future studies should attend to methodological issues, including double-blind designs, smaller errors of measurement for performance and putative physiological mechanism variables, and measurement of economy and \( \text{VO}_2\text{tracmax} \), in addition to \( \text{VO}_2\max \).

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Christopher J Gore  
*Department of Physiology*  
Australian Institute of SPORT  
Canberra, Australia  
e-mail: chris.gore@ausport.gov.au

Will G Hopkins  
*Division of Sport and Recreation*  
Auckland University of Technology  
Auckland, New Zealand

REBUTTAL FROM DRS. LEVINE AND STRAY-GUNDERSEN

The discussion of measurement error by Gore and Hopkins, although erudite, is not germane to this debate. Their own meta-analysis (3) demonstrated that the error of measurement for Hbmass by CO rebreathing (30 days) was 4%, with confidence intervals that overlapped with Evans blue dye-based erythrocyte volume. Indeed, measurements of RCV made in our studies had among the lowest error reported by any laboratory and were consistent with Hbmass errors (3). When blood volume was measured by both our laboratories simultaneously, before and after hypobaric hypoxia (4), the results were virtually identical (slope = 1.0, $r^2 = 0.85$). Finally, when all the athletes in our studies who have spent at least 1 mo living (>20 h/day) at 2,500 m are considered together ($n = 74$), the $P$ value for the increase in RCV is 0.0007—even Dr. Hopkins would consider this level of probability “very likely” real and not random measurement error (5).

In contrast, an improvement in economy has not been consistently reported, even by Gore and colleagues. Thus from the same laboratory, some studies have shown an improvement in economy (2, 7), whereas others have not (1, 10). This lack of consistency argues against changes in economy being the primary mechanism of improvement after LHTL. On the contrary, even in some of our studies in which RCV was not measured, accelerated erythropoiesis was confirmed by an increase in soluble transferrin receptor (8)—measurements that were made by Dr. Gore’s laboratory at the Australian Institute for Sport! We also presented supporting evidence that increased RCV improved O$_2$ delivery; during race pace speeds on the treadmill, cardiac output was decreased in the athletes who lived at altitude, suggesting increased oxygen transport reserve (6).

Finally, we would suggest caution against using simple linear correlations to “prove” or “disprove” cause and effect. Performance in sport is dependant on many factors, the interactions among which may be decidedly nonlinear. In fact, even a clear increase in VO$_2$max may not improve track performance, as seen in our athletes who lived and trained at altitude in whom the increase in RCV and VO$_2$max was offset by muscle deconditioning (6, 9). Moreover, contrary to the contention by our opponents, we did report the sustainable fraction of VO$_2$max, as assessed from the ventilatory/lactate threshold (6). Indeed, we found that only the LHTL athletes had an increase in the VO$_2$ at ventilatory/lactate threshold (and velocity at VO$_2$max), which contributed to the improved performance. This low-altitude training effect is critical to the success of the LHTL paradigm.

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REBUTTAL FROM DRS. GORE AND HOPKINS

Levine and Stray-Gundersen’s adaptation (4) of Koch’s postulates for a causal link between a microbe and a disease provides an amusing and hopefully logically sound approach to evidence for their Point. We will address their evidence in the order they present it.

Levine and Stray-Gundersen focused on the association between maximum oxygen consumption (VO$_2$max), red cell volume (RCV), and performance by splitting subjects into responders and nonresponders based on change in time trial performance. Responders had bigger changes in VO$_2$max and RCV than nonresponders, but these differences could be due at least partly to the responders’ higher intensity of interval training or to fluctuations in RCV unrelated to altitude exposure. Levine and Stray-Gundersen also used a model to argue that the changes in RCV and hemoglobin concentration accounted accurately for the changes in VO$_2$max in the two groups. The accuracy is probably accidental, because this model does not accurately predict the changes in one or more of their altitude groups (3).
Levine and Stray-Gundersen provided two references (3, 5) for their claim that “economy never changed” in their research on “n > 100” runners. Reference 5 is an early review in which we could find no assertions or data for changes in economy, despite exhaustive searching of all the references (mostly conference abstracts) therein. Reference 3 is a study in which there were no statistically significant differences in change in economy between a control and two attitude groups, each of 13 runners. Confidence limits for the changes would presumably be consistent with real improvements of ~1-2% after altitude training. We can think of no other straightforward way to reconcile this study with the six studies we cited showing clear-cut improvements in economy.

We grant Levine and Stray-Gundersen some stimulation of erythropoiesis, especially at high altitude, but its contribution to performance enhancement is unclear. They claim that low doses of exogenous erythropoietin produce effects on RCV that are virtually identical to those of altitude training, whereas other researchers (1) have shown that high doses of erythropoietin are needed to produce the large changes in RCV that Levine and Stray-Gundersen report.

Finally, Levine and Stray-Gundersen’s claim that low iron stores prevent altitude-associated performance enhancement of \( V_O^{2\text{max}} \) and performance would be their best evidence, were true. A low iron store unquestionably prevents an increase in RCV, but astonishingly, neither reference cited to support their claim (2, 7) included data on \( V_O^{2\text{max}} \) or performance. Similarly, the reference for their claim that infection inhibits erythropoiesis and prevents an increase in \( V_O^{2\text{max}} \) or performance (8) actually only suggests the possibility, based on other studies, of erythropoiesis and only in tissue cultures and animals. Even their claim that “no other effects of altitude acclimatization... can be manipulated independently... to improve athletic performance” is wrong: some kinds of resistance training enhance endurance performance by increasing economy selectively (6).

In summary, the evidence presented by Levine and Stray-Gundersen is consistent with an unclear role for erythropoiesis, RCV, and \( V_O^{2\text{max}} \) in the effects of altitude training on endurance performance. The role of the only plausible alternative, exercise economy, is also unclear. Furthermore, a fall in fractional utilization could partially or completely offset the effects of increases in \( V_O^{2\text{max}} \) and economy. We therefore repeat our call for more research to clarify the relative contribution of these three aerobic determinants of endurance in altitude studies.

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POINT:COUNTERPOINT CALL FOR COMMENTS
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