Relationship between changes in haemoglobin mass and maximal oxygen uptake after hypoxic exposure

Philo U Saunders,1,2 Laura A Garvican-Lewis,1,2 Walter F Schmidt,3 Christopher J Gore1,2,4

ABSTRACT

Background Endurance athletes have been using altitude training for decades to improve near sea-level performance. The predominant mechanism is thought to be accelerated erythropoiesis increasing haemoglobin mass (Hbmass) resulting in a greater maximal oxygen uptake (VO2max). Not all studies have shown a proportionate increase in VO2max as a result of increased Hbmass. The aim of this study was to determine the relationship between the two parameters in a large group of endurance athletes after altitude training.

Methods 145 elite endurance athletes (94 male and 51 female) who participated in various altitude studies as altitude or control participants were used for the analysis. Participants performed Hbmass and VO2max testing before and after intervention.

Results For the pooled data, the correlation between per cent change in Hbmass and per cent change in VO2max was significant (p<0.0001, r² = 0.15), with a slope (95% CI) of 0.48 (0.30 to 0.67) intercept free to vary and 0.62 (0.46 to 0.77) when constrained through the origin. When separated, the correlations were significant for the altitude and control groups, with the correlation being stronger for the altitude group (slope of 0.57 to 0.72).

Conclusions With high statistical power, we conclude that altitude training of endurance athletes will result in an increase in VO2max of more than half the magnitude of the increase in Hbmass which supports the use of altitude training by athletes. But race performance is not perfectly related to relative VO2max, and other non-haematological factors altered from altitude training, such as running economy and lactate threshold, may also be beneficial to performance.

INTRODUCTION

The effects of training at moderate altitude on subsequent performance at altitude and near sea-level became important at the 1968 Mexico City Olympic Games (2300 m), and has subsequently been researched extensively. It is now common practice for elite endurance athletes across a range of sports to use altitude training to improve performance near sea-level. The prevailing paradigm of adaptation to hypoxia is that the lower partial pressure of oxygen associated with moderate altitude induces erythropoietin (EPO) production in the kidneys, which in turn stimulates the production of red blood cells in the bone marrow, facilitating an increase in maximal oxygen uptake (VO2max) and potentially improving endurance performance.1-3 Since VO2max depends on cardiac output and the arteriovenous O₂ difference, all factors that influence these physiological capacities may exert limiting effects on endurance capacity.4 The most important factor related to blood supply is the total blood volume, which may limit venous return and thus the stroke volume, as well as haemoglobin mass (Hbmass), which along with the capacity of muscles to extract and use O₂ determines the O₂-transport capacity and therefore the arteriovenous O₂ difference.5

Although some authors have explicitly related the change in near sea-level performance after an altitude training camp (hypoxic intervention) to the change in serum EPO1-2 at altitude, the correlation for the change in VO2max versus the change in red blood cell volume yielded an r²=0.14.5 Therefore, 86% of the variance in VO2max is attributable to factors other than the change in Hbmass. Further, it is important to be aware that VO2max is not the sole determinant of performance.5 Among elite athletes, other factors such as exercise economy and the fractional utilisation of VO2max are also important determinants of endurance performance.6 In addition to the increase in Hbmass and the subsequent increase in near sea-level VO2max, altitude training can enhance muscle efficiency, probably at a mitochondrial level, and change the muscle proteins involved in acid-base control; it can also increase the capacity for flux of lactate, bicarbonate and hydrogen ions from muscle to blood, all of which can be associated with better performance in endurance athletes and team sport athletes.7-9 A detailed review of non-haematological adaptations to hypoxia that can improve near sea-level endurance performance has been published recently.9 Given an adequate hypoxic dose (high enough, long enough and for enough hours/day),9 there will be an increase in Hbmass VO2max and possibly performance.8 However, there is evidence that although altitude training increases Hbmass, there may also be a parallel reduction in cardiac output, vascular regulation or some other mechanism that can limit the increase in VO2max.10 11 When evaluating the increases in Hbmass and VO2max resulting from altitude training, factors such as age, sex, training status and type of sport all must be considered. Many research studies have also used small sample sizes and a major strength of the current study is pooling data across many research studies to increase the sample size and to encompass a range of measures across athletes varying in the aforementioned characteristics. Recently, there has also been a controversy about whether altitude training, particular live high–train low (LHTL), is effective in improving Hbmass VO2max and endurance performance.12 Therefore, the aim of the current study was to investigate the
effectiveness of altitude (both live high–train high (LHTH) and LHTL) in increasing Hbmass and VO2max and the strength of the relationship between changes in Hbmass and VO2max in a large number of elite endurance athletes from different sports after different forms of altitude training. The substantial number of participants pooled for analysis offers more statistical power than individual smaller studies with modest sample sizes.

METHODS
Subjects
The current study used elite endurance athletes from 10 separate studies spanning a 6-year period across four different sports (cycling, running, triathlon and race walking). The study comprised a total of 145 participants (94 males and 51 females), VO2max 67.7±7.2 (71.9±4.6 male and 61.4±5.9 female) mL/min/kg (mean±SD). The participants were at a minimum ‘nationally ranked’ athletes, but most had represented Australia in international competition. All participants were part of various altitude studies that were approved by the Australian Institute of Sport Ethics Committee. A summary of each of the 10 studies is listed in table 1.

Experimental overview
The current study investigated the relationship between changes in Hbmass and VO2max after various forms of altitude training, with the minimum requirement that both Hbmass and VO2max were measured pre-altitude and post-altitude. The altitude exposures included (1) classical LHTH at various locations (1350–2700 m), (2) simulated LHTL (3000 m, 14 h/day) in the altitude house at the Australian Institute of Sport (AIS, Canberra, Australian Capital Territory, Australia) or using altitude tents, (3) intermittent hypoxic training (IHT) in the altitude house at the AIS and (4) intermittent hypoxic exposure (IHE) using hypoxic breathing devices. A total of n=83 athletes completed altitude training interventions (1 and 2) and were treated as the Altitude group. The data from control participants and from altitude protocols that would not normally be expected to induce an increase in Hbmass (IHE and IHT alone) were (n=62) all treated as control data. Details of participants, type of altitude, dose of altitude, exposure duration and average VO2max are presented in table 1. The studies included were a combination of seven published13-19 and three previously unpublished works.

Maximal oxygen uptake
An incremental protocol to volitional exhaustion was used to determine VO2max. Protocols were specific to each sport and have been described in full previously.13-19 Expired ventilation samples were collected using a custom-built open-circuit indirect calorimetry system with associated in-house software for determination of oxygen uptake.20 The same open-circuit indirect calorimetry system was used in all studies. Additionally, the typical error (TE, SD of the difference scores divided by √(2)) for VO2max established in our laboratory for this system was 2.1–2.4%,18,21 which includes the combination of biological and analytical error.

Haemoglobin mass
Total Hbmass was measured with the optimised 2 min carbon monoxide (CO) rebreathing test adapted from Schmidt and Prommer22 for all the studies included in the analysis. Briefly, a CO dose of ~1.2 mL/kg body weight was administered and rebreathed for 2 min. Capillary fingertip blood samples were taken before the start of the test and at ~7 min post administration of the CO dose. Blood samples were measured a minimum of five times for determination of %HbCO using an OSM three hemoximeter (Radiometer, Copenhagen). Hbmass was calculated from the mean change in %HbCO before and after rebreathing CO. This parameter was measured prior to the intervention period and within 1 week after the completion of the intervention period. The TE of Hbmass was 1.8%,18 1.9%,13 2.0%,14,15,17 2.2%19 and 2.4%.16

Statistical analysis
A two-tailed Pearson correlation was used to compare the correlation between percentage changes in Hbmass and VO2max with statistical significance set at p<0.05. Linear regression analyses were also performed on the percentage changes in Hbmass and VO2max, and provided a regression equation, goodness of fit (r²) and significance of the slope (p value). Analyses were conducted both with the intercept allowed to vary freely and forced through the origin. Linear regression was also conducted for a cross-sectional comparison on the cumulative raw data (pre and post) of Hbmass (g/kg) and VO2max (mL/min/kg). All analyses were performed using Prism software (2007) V5.01 (GraphPad Software Inc., San Diego, California, USA).

RESULTS
Changes in Hbmass and VO2max
The correlation between percentage changes in Hbmass and VO2max was significant for the altitude and control groups combined, with a slope (95% CI) of 0.48 (0.30 to 0.67) when the intercept was free to vary, and a slope of 0.62 (0.46 to 0.77) when the intercept was constrained through the origin (figure 1). The correlation was also significant for the altitude and control groups, separately (figure 2). For the altitude group, the slope was 0.57 (0.27 to 0.87) when free to vary and when constrained through the origin, the slope was 0.72 (0.51 to 0.92). The corresponding values for the Control group were 0.32 (0.06 to 0.58) and 0.38 (0.12 to 0.64). The Altitude group increased Hbmass by 3.3±3.0% (mean±SD) and VO2max by 2.7±4.4%; the corresponding changes in the Control group were 0.8±3.0% and 1.1±3.4%.

Cross-sectional relationship between Hbmass and VO2max
When the intercept was able to vary, the linear regression of cumulative raw data (control and altitude, pre and post) of Hbmass and VO2max was as follows

\[
VO2max (mL/min/kg) = 3.32 \times \%Hbmass (g/kg) + 22
\]

The slope was significant (p<0.0001, r=0.75) with 95% CI of 2.98 to 3.66. When constrained through the origin, the corresponding slope was 4.94 (4.89 to 4.99; figure 3).

DISCUSSION
The main findings of the current study were (1) a significant, very large cross-sectional relationship between Hbmass and VO2max and (2) a significant, moderate correlation between changes in Hbmass and VO2max in a large group of elite endurance athletes undertaking altitude training. Athletes who were part of LHTL and LHTH altitude interventions increased Hbmass and VO2max by ~3% such that each 1% change in Hbmass will result in a 0.6–0.7% change in VO2max. Even though significant, the correlation was weak and explained only 15% of the variation, indicating that other factors are still important in increasing VO2max apart from an increased Hbmass.
Cross-sectional relationship between Hbmass and VO2max

The current study yielded a strong relationship between Hbmass and VO2max (r=0.75), with a slope of ∼4.9 when constrained through the origin; this implies that every additional gram of Hb will increase VO2max by ∼4.9 mL/min. Other cross-sectional studies indicate a similarly strong relationship between Hbmass and VO2max, which is independent of sex or age.23 A close association between absolute Hb mass and VO2max (r=0.72) was reported in 131 males of varying training status.24 Likewise, strong correlations between Hb mass and VO2max have been reported across a range of sports and degrees of training status, with the slope of the regression line ∼4 in each instance, although in the present study the slope is closer to 5 when the regression is forced through the origin. In practical terms, this relationship translates to a change in VO2max of ∼4–5 mL/min for every 1 g change in Hb mass.

There were a total of 83 altitude athletes and 62 control athletes. VO2max values means±SD. CON, control; F, female; IHE, intermittent hypoxic exposure; IHT, intermittent hypoxic training; LHTL, live high–train low; LHTH, live high–train high; M, male.

**Table 1 Individual study summary**

<table>
<thead>
<tr>
<th>Year</th>
<th>Sport</th>
<th>Study N (M/F)</th>
<th>Altitude type (N)</th>
<th>Level (m)</th>
<th>Duration (d)</th>
<th>Time at altitude</th>
<th>VO2max (mL/min/kg)</th>
<th>Test mode (Ergometer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006 (13)</td>
<td>Cycling</td>
<td>11 (11/0)</td>
<td>LHTL (11)</td>
<td>3000</td>
<td>21</td>
<td>14 h/day</td>
<td>65.32±5.29</td>
<td>Cycle (tode bike)</td>
</tr>
<tr>
<td>2007 (14)</td>
<td>Cycling</td>
<td>9 (9/0)</td>
<td>LHTH (5) CON (4)</td>
<td>2700</td>
<td>21</td>
<td>24 h/day</td>
<td>72.16±4.66</td>
<td>Cycle (tode bike)</td>
</tr>
<tr>
<td>2007 (17)</td>
<td>Running</td>
<td>32 (21/11)</td>
<td>LHTL (18) CON (14)</td>
<td>3000</td>
<td>21</td>
<td>14 h/day</td>
<td>71.60±6.05</td>
<td>Run (treadmill)</td>
</tr>
<tr>
<td>2008 (18)</td>
<td>Running</td>
<td>17 (13/4)</td>
<td>LHTL+TH (8) IHT (9)</td>
<td>3000+2200 2200</td>
<td>21+9 9</td>
<td>14 h/day+60 min 60 min</td>
<td>67.91±7.47</td>
<td>Run (treadmill)</td>
</tr>
<tr>
<td>2008 (19)</td>
<td>Walking</td>
<td>16 (8/8)</td>
<td>LHTL (6) CON (10)</td>
<td>3000</td>
<td>21</td>
<td>14 h/d</td>
<td>62.17±7.44</td>
<td>Race walk (treadmill)</td>
</tr>
<tr>
<td>2009 (16)</td>
<td>Triathlon</td>
<td>18 (14/4)</td>
<td>LHTL (5) IHE (8) CON (5)</td>
<td>3000 3500–6000</td>
<td>17 17</td>
<td>14 h/day 60 min</td>
<td>72.49±4.74</td>
<td>Run (treadmill)</td>
</tr>
<tr>
<td>2010 (15)</td>
<td>Cycling</td>
<td>9 (0/9)</td>
<td>LHTL (5) CON (4)</td>
<td>3000</td>
<td>26</td>
<td>14 h/day</td>
<td>62.20±6.68</td>
<td>Cycle (tode bike)</td>
</tr>
<tr>
<td>2011</td>
<td>Walking</td>
<td>10 (4/6)</td>
<td>LHTL (5) CON (5)</td>
<td>1850</td>
<td>28</td>
<td>14 h/day</td>
<td>64.96±6.59</td>
<td>Race walk (treadmill)</td>
</tr>
<tr>
<td>2011</td>
<td>Walking</td>
<td>6 (2/4)</td>
<td>LHTL (3) CON (3)</td>
<td>3000</td>
<td>28</td>
<td>14 h/day</td>
<td>60.73±6.60</td>
<td>Race walk (treadmill)</td>
</tr>
<tr>
<td>2012</td>
<td>Walking</td>
<td>17 (12/5)</td>
<td>LHTH (7) LHT (10)</td>
<td>3000/1350 1350</td>
<td>21 21</td>
<td>9 h/day/15 h/day 24 h/day</td>
<td>66.50±6.17</td>
<td>Race walk (treadmill)</td>
</tr>
</tbody>
</table>

**Figure 1** Linear regression (and 95% CI) for changes between preintervention and postintervention for Hbmass and VO2max, n=145. Regression slope (solid line) and 95% CIs (dashed line) are shown. Top panel is for intercept free to vary, and bottom panel intercept constrained through the origin. Open circles are control participants and filled circles are altitude participants.

Cross-sectional relationship between Hbmass and VO2max

The current study yielded a strong relationship between Hbmass and VO2max (r=0.75), with a slope of ∼4.9 when constrained through the origin; this implies that every additional gram of Hb will increase VO2max by ∼4.9 mL/min. Other cross-sectional studies indicate a similarly strong relationship between Hbmass and VO2max, which is independent of sex or age.23 A close association between absolute Hbmass and VO2max (r=0.72) was reported in 131 males of varying training status.24 Likewise, strong correlations between Hbmass and VO2max have been reported across a range of sports and degrees of training status, with the slope of the regression line ∼4 in each instance, although in the present study the slope is closer to 5 when the regression is forced through the origin. In practical terms, this relationship translates to a change in VO2max of ∼4–5 mL/min for every 1 g change in Hb mass.25 26 Furthermore, the relationship is consistent with the theoretical calculation of O2 transport during maximal aerobic exercise. The strong relationship between Hbmass and VO2max has two important implications; first, that a high Hbmass is an important prerequisite for a high VO2max, and second, that alterations to Hbmass have the potential to alter VO2max and possibly performance.

Successful endurance performance is highly correlated with an athlete’s VO2max.28–32 Other physiological and performance factors are important and include sustaining a high percentage of VO2max for the event duration (fractional utilisation or lactate threshold)33 34 and exercising with relatively low energy expenditure (good economy).30 33 35 These factors in isolation are not necessarily better predictors of performance than VO2max, although in elite athletes where an already high VO2max is present, these factors may become more important. A three-factor model (VO2max, running economy, lactate threshold) was reported to highly predict the within-subject changes in performance (measured by peak running speed) during a 17-week training period in well-trained distance runners.26 When holding each variable constant, to ascertain the effect of a predictor variable independently of all the other predictor factors, a regression analysis can be performed.
variables, \(\dot{V}O_2\text{max}\) was the best predictor of performance with a 1% improvement in \(\dot{V}O_2\text{max}\) resulting in a 0.5% improvement in peak running speed, but the running economy was also a moderate predictor of performance.36

Changes in Hbmass and \(\dot{V}O_2\text{max}\)

Interestingly, the Hb mass–\(\dot{V}O_2\text{max}\) relationship appears to uncouple somewhat following altitude training, with disproportionate changes being reported in Hbmass and \(\dot{V}O_2\text{max}\). A weak association (r=0.32) between changes in Hbmass and \(\dot{V}O_2\text{max}\) was reported following 3 weeks of LHTL simulated altitude training combined with hypoxic training.18 In fact, when the individual data are examined, some athletes displayed substantial increases in Hbmass (>5%) with no change in \(\dot{V}O_2\text{max}\), whereas others who experienced minor reductions in Hbmass increased \(\dot{V}O_2\text{max}\) by \(\sim 5\%\).18 Similarly, despite a \(\sim 4\%\) increase in Hbmass observed in elite runners following \(\sim 400\) h of simulated LHTL (\(\sim 2900\) m), only a trivial change in \(\dot{V}O_2\text{max}\) was observed and, not surprisingly, the relationship between changes in Hbmass and \(\dot{V}O_2\text{max}\) was also trivial (r=0.04).37 It has been reported that after 24 days of LHTL, the Hbmass increased by 5.3% (\(\sim 44\) g) in a group of orienteers, accompanied by a 4.1% increase in \(\dot{V}O_2\text{max}\) (\(\sim 145\) mL/min), which in terms of the mean data appears in line with the expected increase in \(\dot{V}O_2\text{max}\)/g of Hbmass.38 Indeed, the relationship between the change scores was \(\sim 0.7\) when the group was divided into men (r=0.75) and women (r=0.68); however, when the group data are combined, the relationship becomes much weaker (r=0.35, p=0.29). Clark et al report a trivial correlation between changes in Hbmass and \(\dot{V}O_2\text{max}\) in well-trained cyclists following 21 days of simulated LHTL (r=0.09, p=0.32); however, the slope of the regression line appears to indicate that a 1% increase in Hbmass is associated with a 0.8% increase in \(\dot{V}O_2\text{max}\). The only study to report a significant (albeit weak) correlation (r=0.4, p=0.02)
between changes in red cell volume (measured using Evan’s Blue) and VO2max was in a group of collegiate runners following 4 weeks of LHTL, where 5% and 9% increases in red cell volume and VO2max were reported.1

The current study suggests that the relationship between increases in Hbmass and VO2max is slightly stronger in athletes undertaking altitude training compared to control athletes, although the variance explained was ≈15%, which is similar to that reported previously (r^2=0.14) for a 9% increase in red cell volume associated with a 5% increase in VO2max. When participants (n=18) received recombinant EPO injections (50 IU/kg 3×/week) for a period of 25 d, they increased VO2max more proportionately with the increase in Hbmass (r^2=0.28) when compared with the current data set,39 adding support to this apparent uncoupling of the Hbmass–VO2max relationship with altitude training. On the other hand, when non-athletic, altitude adapted participants return to near-sea-level, they show a VO2max similar to lowlanders despite a 13% increase in Hbmass.40 The lack of adaptation in VO2max during training at hypoxia may be related to impairment in vascular regulation and reduced cardiac output after altitude training.10 11 Interestingly, 23 consecutive nights of LHTL simulated altitude exposure (3000 m) depressed VO2max by 7% with only a trivial increase in Hbmass.41 This, along with other non-haematological adaptations that occur with training at altitude, may explain why VO2max does not increase more proportionately with the increase in Hbmass. Finally, it should be considered that even with a small TE of measurement for VO2max and Hbmass, it is quite likely that in some of the studies with smaller samples, as well as in the current study, these errors contribute to an obfuscation of the relationship between the two. However, the results of the current study are quite likely to be more robust, given the relatively large sample size and hence greater statistical power, as well as using only one method for measuring Hbmass.

The differences in the changes in the Hbmass–VO2max relationship between the altitude and control athletes (figure 2) are most likely a result of individual variation.17 However, using the component estimates of TE, the combined error of measurement for changes between successive measures of Hbmass and VO2max at the 95% level could be as large as ±8% (1.96√2×√(2^2+2.2^2)), which is a consequence of analytical and biological variation in both tests. Factors such as illness, training (or detraining), fatigue and iron stores may all potentially affect changes in Hbmass and VO2max.42 43 Interestingly, for the control group, there was a scatter of data (for changes in Hbmass and VO2max) about the origin in positive and negative directions suggestive of measurement error, whereas the Altitude group showed a preponderance of points in the positive/positive quadrant for both measures, inferring that altitude is an effective intervention to increase Hbmass and hence VO2max. In addition, the significant relationship for changes in Hbmass and VO2max found for the Control group most likely reflects not just measurement error but also real changes in these markers secondary to factors such as training/detraining.

When acutely exposed to hypoxia, it is not just VO2max that is affected. All the functional systems of the body are affected, including the central nervous system, respiratory system, cardiovascular system and muscles, a process that is mediated at the tissue level through rapid oxygen sensing.44 The transcription factor hypoxia-inducible factor-1 (HIF-1), present in every tissue of the body, is the global regulator of oxygen homeostasis and plays a critical role in the acute cardiovascular and respiratory responses to hypoxia.45 Improvement in many of these responses may be a factor in their variable response to increasing VO2max after exposure to altitude.

In summary, the current data indicate that increases in Hbmass were significantly, albeit weakly, correlated with increases in VO2max in elite endurance athletes after altitude training interventions with an approximate 1% increase in Hbmass resulting in a 0.6–0.7% increase in VO2max. However, since the relationship is not perfect, other factors must be considered, which affect changes in VO2max in addition to other responses from altitude that may impact on performance.

What are the new findings?

- This study confirms the strong cross-sectional relationship between haemoglobin mass (Hbmass) and maximal oxygen uptake (VO2max) in a sample of 145 elite athletes.
- Adequate moderate altitude exposure is effective for increasing Hbmass and VO2max in elite endurance athletes by ~3%. But the correlation between these changes is weak and explains less than one-sixth of the variation, indicating that other factors are still important in increasing VO2max after altitude training, apart from an increased Hbmass.

How might it impact on clinical practice in the near future?

- Altitude training can be undertaken as a helpful method to improve endurance performance in already highly trained athletes, but it should be incorporated as part of an annual plan.
- Race performance may improve post-altitude as a result of increased haemoglobin mass and maximal oxygen uptake, but other non-haematological factors are also quite likely important. Therefore, altitude training studies should not be limited to measuring these two variables alone.

Acknowledgements The authors would like to acknowledge Dr Sally Clark, Dr Clare Humberstone-Gough, Dr Eileen Robertson, Dr David Martin and Dr David Pyne for their contribution to various studies used for the current manuscript.

Contributors PUS was involved in the conception and design, acquisition of data, analysis, interpretation of data, drafting of the article and approved the final version. LAG-L was involved in the acquisition of data, analysis and interpretation of data, and critically revised the article and approved the final version. WFS was involved in the conception and design, analysis and interpretation of data, and critically revised the article and approved the final version.

Competing interests None.

Ethics approval Australian Institute of Sport Ethics Committee (for all 10 studies).

Provenance and peer review Not commissioned; externally peer reviewed.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 3.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/3.0/

REFERENCES

2 Levine BD, Stray-Gundersen J, Gore CJ, et al. Point: counterpoint: positive effects of intermittent hypoxia (live high:train low) on exercise are not mediated primarily


Relationship between changes in haemoglobin mass and maximal oxygen uptake after hypoxic exposure

Philo U Saunders, Laura A Garvican-Lewis, Walter F Schmidt and Christopher J Gore

doi: 10.1136/bjsports-2013-092841

Updated information and services can be found at:
http://bjsm.bmj.com/content/47/Suppl_1/i26

These include:

References
This article cites 45 articles, 12 of which you can access for free at:
http://bjsm.bmj.com/content/47/Suppl_1/i26#BIBL

Open Access
This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 3.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See:
http://creativecommons.org/licenses/by-nc/3.0/

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
Open access (266)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/