

Economy of locomotion in high-altitude Tibetan migrants exposed to normoxia

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High-altitude Tibetans undergo a pattern of adaptations to chronic hypoxia characterized, among others, by a more efficient aerobic performance compared with acclimatized lowlanders. To test whether such changes may persist upon descent to moderate altitude, oxygen uptake of 17 male Tibetan natives lifelong residents at 3500–4500 m was assessed within 1 month upon migration to 1300 m. Exercise protocols were: 5 min treadmill walking at 6 km h⁻¹ on increasing inclines from +5 to +15% and 5 min running at 10 km h⁻¹ on a +5% grade. The data (mean ± S.E.M.) were compared with those obtained on Nepali lowlanders. When walking on +10, +12.5 and +15% inclines, net \dot{V}_{O_2} of Tibetans was 25.2 ± 0.7, 29.1 ± 1.1 and 31.3 ± 0.9 ml kg⁻¹ min⁻¹, respectively, i.e. 8, 10 and 13% less ($P < 0.05$) than that of Nepali. At the end of the heaviest load, blood lactate concentration was lower in Tibetans than in Nepali (6.0 ± 0.9 versus 8.9 ± 0.6 mM; $P < 0.05$). During running, \dot{V}_{O_2} of Tibetans was 35.1 ± 0.8 versus 39.3 ± 0.7 ml kg⁻¹ min⁻¹ (i.e. 11% less; $P < 0.01$). In conclusion, during submaximal walking and running at 1300 m, Tibetans are still characterized by lower aerobic energy expenditure than control subjects that is not accounted for by differences in mechanical power output and/or compensated for by anaerobic glycolysis. These findings indicate that chronic hypoxia induces metabolic adaptations whose underlying mechanisms still need to be elucidated, that persist for at least 1 month upon descent to moderate altitude.

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It has been long recognized that, with increasing altitude, acclimatized Caucasian lowlanders lose progressively greater fractions of their sea level maximal aerobic power ($\dot{V}_{O_{2peak}}$). By contrast, Tibetans, whose ancestors might have been living and thriving at altitudes between 3000 and 4500 m for more than 25 000 years (Dennell *et al.* 1988), are characterized by a peculiar pattern of adaptations to chronic hypoxia. In fact, compared with acclimatized lowlanders, Tibetan highlanders feature at peak exercise higher arterial O₂ saturation (S_{aO_2}), higher aerobic power ($\dot{V}_{O_{2peak}}$) and heart rate (HR_{peak}), the values of the two latter variables being at altitude in the range of those found at sea level in age-, gender- and fitness-matched individuals (Sun *et al.* 1990; Niu *et al.* 1995; Zhuang *et al.* 1996; Chen *et al.* 1997). In addition, compared with acclimatized Han Chinese long-time residents of 4700 m, Tibetans performing an incremental cycle ergometer exercise are characterized by lower O₂ consumption for given submaximal exercise loads, i.e. a better economy of locomotion, and can develop greater peaks of mechanical power output despite lower $\dot{V}_{O_{2peak}}$ levels (Ge *et al.* 1994). Interestingly, the same features were found at 3658 m

in lifelong Tibetan residents of 4400 m compared with 3658 m (Curran *et al.* 1998). On the other hand, second generation Tibetan lowland natives born at 1300 m, never before exposed to high altitude, acclimatize to 5050 m more quickly than Caucasians and within 1 month recover up to 92% (to be compared with 65% of Caucasians) of their initial $\dot{V}_{O_{2peak}}$ (Marconi *et al.* 2004).

So far, the mechanisms underlying a more efficient muscle oxidative metabolism in Tibetans have not been identified. Our working hypothesis is that the better economy of locomotion found in native Tibetan highlanders, independent of its origin, may be still operational at least in part also when these subjects transfer rapidly to low altitude. The present study was therefore aimed at comparing energy expenditure during submaximal constant-load exercises of a large group of Tibetan highlanders *lifelong* exposed to *severe* chronic hypoxia (3500–4500 m) in the *early* phase of adaptation to 1300 m (Kathmandu, Nepal) with that of Nepali lowlanders. The results confirm the validity of our hypothesis and raise questions concerning the underlying mechanisms.

Table 1. Age, height, body mass, body mass index (BMI), body surface area (BSA), body fat, blood haemoglobin concentration ([Hb]), and days at low altitude of Tibetan migrants and Nepali

| | Tibetan migrants | Nepali |
|----------------------------|------------------|-------------|
| <i>n</i> | 17 | 10 |
| Age (year) | 20.9 ± 0.9 | 20.0 ± 0.5 |
| Height (cm) | 167 ± 1 | 165 ± 1 |
| Body mass (kg) | 59.0 ± 0.9* | 54.6 ± 1.0 |
| BMI (kg m ⁻²) | 21.1 ± 0.4* | 20.0 ± 0.3 |
| BSA (m ²) | 1.66 ± 0.01* | 1.60 ± 0.02 |
| Body fat (%) | 10.7 ± 0.4* | 12.4 ± 0.7 |
| [Hb] (g dl ⁻¹) | 15.8 ± 0.3 | 15.1 ± 0.3 |
| Days at low alt. | 17 ± 11 | Life |

Values are means ± s.e.m. *Significantly different from Nepali.

Methods

Subjects

The study was conducted on a total of 27 male subjects with different characteristics, as follows.

Seventeen Tibetan highlander migrants (age 20.9 ± 0.9 years, mean value ± s.e.m.) born and living between 3500 and 4500 m until 2–32 days prior to the measurements were assessed. With the aid of an interpreter and the use of a map, the subjects traced the journey from their native villages to Kathmandu (Nepal, 1300 m), enabling us to evaluate the time elapsed since 3000 m altitude was left (day 0). We also assessed 10 Nepali (20.0 ± 0.5 years) born and living in the Kathmandu valley.

Age, anthropometrical characteristics and blood haemoglobin concentration of the subjects are shown in Table 1. Most of them were students. They were free of diseases or ailments as assessed by a preliminary clinical screening, which included history taking, physical examination, assessment of blood pressure, resting ECG and percentage of body fat from skinfold thickness. All subjects were non-smokers and their nutritional status, exercise habits and fitness levels were comparable. In particular, they used to walking on average about 2 h a day, but none of them was involved in specific training programs.

The subjects were acquainted with the aim of the study, instructed about the experimental procedure, and asked to sign an informed consent prior to participating in the study, which was carried out in accordance with the principles outlined in the Declaration of Helsinki (2000) of the World Medical Association. The ethical committees and research review boards of the National Research Council in Milan and of the Royal Nepal Academy of Science and Technology (RONAST) in Kathmandu approved the study.

Experimental procedure

The measurements were carried out at the Medical Clinic of the Tibetan Refugee Reception Center in Kathmandu, under the patronage of the Representative of the Dalai Lama in Nepal. Before performing the test, enough time was allowed for each subject to become familiar with the equipment, testing procedures, and, particularly, with treadmill walking and running. On the day of testing, all subjects were able to walk and run freely on the treadmill without holding the safety bar, and could maintain a constant stride. The subjects wore identical clothes and gym shoes suitable for performing the tests comfortably.

Three protocols were adopted.

Walking. On four different days, all subjects, whether Tibetan migrant or Nepali, after 5 min standing, performed a 5 min walk at 6 km h⁻¹ on an electrically driven treadmill (Lifestride 9100, LifeFitness, IL, USA) without holding the safety bar. The speed was frequently checked while subjects were walking. The slope was set randomly at a +5, +10, +12.5 or +15% gradient.

Five minute submaximal running. For 17 Tibetan migrants and 7 Nepali the speed of the belt was kept constant at 10 km h⁻¹ and the incline was set at +5%. After 5 min standing, the subjects jumped onto the treadmill and started running without holding the safety bar until the end of the test when steady-state for \dot{V}_{O_2} was attained. For three Nepali volunteers the initial speed and incline were adjusted differently and therefore these subjects were excluded from data analysis.

Incremental running. At the end of each running test, the incline of the treadmill was increased by +2.5% every minute until all subjects (17 Tibetan and 10 Nepali) attained voluntary exhaustion.

Gas exchange, heart rate, haemoglobin and blood lactate concentration

A computerized O₂–CO₂ analyser–flowmeter combination (Vmax 229, SensorMedics, Yorba Linda, CA, USA) was used for breath-by-breath assessment of tidal volume (V_T), pulmonary ventilation (\dot{V}_E), and gas exchange (\dot{V}_{O_2} and \dot{V}_{CO_2}). V_T and \dot{V}_E were calculated by integration of the flow tracings recorded at the mouth of the subject by means of a pair of heated stainless steel wires (Mass Flow Sensor). Volume and gas analyser calibrations were performed prior to each measurement using a 3 l syringe (Hewlett Packard 14278B) at three different flow rates, and by means of gas mixtures of known composition, respectively. Heart rate (HR) was monitored throughout the tests from ECG.

Early in the morning, 2 ml of blood were withdrawn from an antecubital vein of the subject sitting at rest for a duplicate assessment of blood haemoglobin concentration ([Hb]) by a photometric method (Pentra 80, ABX, UK). No vein occlusion was applied during sampling.

Before and at various intervals (1, 3, 5 and 7 min) throughout recovery after some of the tests, 20 μ l of capillary arterialized blood were taken from a pre-heated earlobe for lactate determinations ($[La_b]$), which were carried out by an electroenzymatic method (Biosen 5030, EKF, Germany).

Mechanical and metabolic power output

The mechanical external power output of Tibetan migrants and Nepali lowlanders was calculated from the data of Minetti *et al.* (1993, 1994) collected on Caucasians during walking and running on a treadmill at the same speeds and inclines adopted for the present study. In addition, metabolic power output of migrants and Nepali was calculated from aerobic energy sources, assuming the extreme caloric equivalents for $\dot{V}O_2$ of 20.9 kJ l⁻¹ (respiratory gas exchange ratio (R) = 1) and 19.7 kJ l⁻¹ (R = 0.7), respectively.

Data analysis and statistics

Both before and during exercise the breath-by-breath or the beat-by-beat measurements were averaged every 20 s. A prerequisite for steady state was that calculated values during the last 40–60 s of submaximal exercise were constant. At voluntary exhaustion after incremental running the highest measured value was considered 'peak' and not 'maximum' despite most criteria ($HR > 85\%$ of the maximum expected value, $R > 1.1$, blood lactate concentration > 8 mM) for 'maximal' exercise being fulfilled. The highest slope attained at exhaustion and maintained for at least 30 s was considered 'peak'. In order to calculate 'net' aerobic energy expenditure, standing $\dot{V}O_2$ values were subtracted from the submaximal steady-state values, according to standardized procedures (Minetti *et al.* 1993; Schepens *et al.* 2001).

Data are expressed as means \pm s.e.m. To determine the statistical significance of the differences between two means, unpaired Student's *t* test was performed. The level of significance was set at $P < 0.05$. Statistical analyses were performed by means of a commercially available software package (InStat, Graph Pad Software, San Diego, CA, USA).

Results

Walking test

Pulmonary oxygen uptake ($\dot{V}O_2$), heart rate (HR) and respiratory gas exchange ratio (R) during walking at

6 km h⁻¹ on increasing inclines are shown in Fig. 1. At each incline, $\dot{V}O_2$ of Tibetan migrants was significantly lower than that of Nepali (Fig. 1, upper panel). Also the slopes of the linear fittings of the experimental data ($y = 1.26x + 17.41$ versus $y = 1.67x + 16.43$, respectively) were significantly different ($P < 0.05$). HR (Fig. 1, middle panel) was significantly lower in migrants than in Nepali independent of the incline of the treadmill. HR of migrants ranged from 127 ± 4 to 167 ± 5 beats min⁻¹ on a +5 and +15% incline, respectively, whereas HR of Nepali ranged from 148 ± 4 to 192 ± 4 beats min⁻¹. At each metabolic level, the R value of both groups was close to or above 1 (Fig. 1, lower panel), with the values of migrants being significantly higher than those of Nepali, irrespective of the workload. Absolute and specific (expressed per unit of body surface area (BSA)) pulmonary ventilation

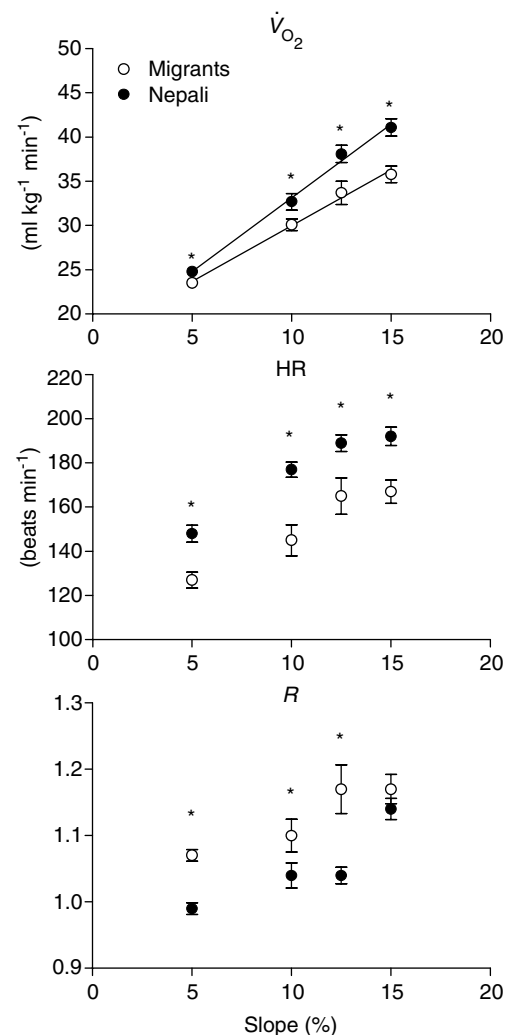


Figure 1 Pulmonary oxygen uptake ($\dot{V}O_2$), heart rate (HR) and respiratory gas exchange ratio (R) of Tibetan migrants and Nepali walking at 6 km h⁻¹ on increasing slopes.

values of Tibetan migrants and Nepali are shown in Fig. 2 (upper and middle panel, respectively), along with those of breathing rate (lower panel). It is noteworthy that at any given workload, pulmonary ventilation of migrants and Nepali were similar, irrespective of their somewhat different body size.

Net (steady-state – standing) \dot{V}_{O_2} values of Tibetan migrants and Nepali during a 6 km h^{-1} walk on +5, +10, +12.5 and +15% grades are shown in Table 2. Between +5 and +15%, net \dot{V}_{O_2} of migrants increased from 18.7 ± 0.4 to $31.3 \pm 0.9 \text{ ml kg}^{-1} \text{ min}^{-1}$, respectively. At the lowest workload, net \dot{V}_{O_2} of migrants and Nepali were not significantly different. By contrast, when raising the slope of the treadmill the net \dot{V}_{O_2} differences between migrants and Nepali progressively increased. On a +15%

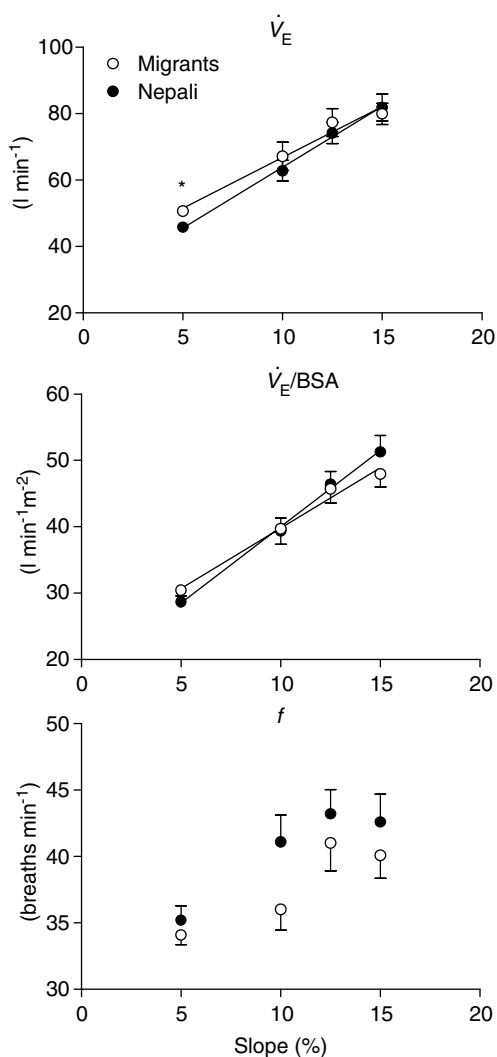


Figure 2

Absolute (upper panel) and specific (expressed per unit of BSA, middle panel) pulmonary ventilation and breathing rate (lower panel) of migrants and Nepali as a function of the slope of the treadmill, during walking at 6 km h^{-1} .

Table 2. Net (steady-state – standing value) pulmonary O_2 uptake ($\text{ml kg}^{-1} \text{ min}^{-1}$) of Tibetan migrants and Nepali during walking at a constant speed (6 km h^{-1}) on a +5, +10, +12.5 and +15% grade, and during running at a constant speed of 10 km h^{-1} on a +5% incline

| | Tibetan migrants | Nepali | Significance |
|-------------|---------------------|---------------------|--------------|
| Walking on: | | | |
| 5% | 18.7 ± 0.3 (31) | 19.6 ± 0.4 (20) | N.S. |
| 10% | 25.2 ± 0.7 (8) | 27.4 ± 0.8 (10) | $P < 0.05$ |
| 12.5% | 29.1 ± 1.1 (6) | 32.3 ± 0.8 (10) | $P < 0.05$ |
| 15% | 31.3 ± 0.9 (13) | 36.0 ± 1.3 (10) | $P < 0.01$ |
| Running on: | | | |
| 5% | 35.1 ± 0.8 (17) | 39.3 ± 0.7 (7) | $P < 0.01$ |

Values are means \pm S.E.M. The number of measurements for any given condition is shown in parentheses.

incline, average net \dot{V}_{O_2} difference between the two groups was $4.6 \text{ ml kg}^{-1} \text{ min}^{-1}$ ($P = 0.002$), i.e. -13% of the value found in Nepali. In the above condition, blood lactate concentration was lower in Tibetan migrants than in Nepali (6.0 ± 0.9 versus $8.9 \pm 0.6 \text{ mm}$, respectively; $P < 0.05$).

Five minute submaximal running

Mean net (steady-state – standing values) pulmonary O_2 uptake of all migrants and of seven Nepali during running at a constant speed of 10 km h^{-1} on a +5% slope is also shown in Table 2. Net \dot{V}_{O_2} was significantly lower in Tibetan migrants than in Nepali (-11% ; $P < 0.01$). In the above conditions, \dot{V}_E values of migrants and Nepali were similar (88.1 ± 3.2 versus $88.1 \pm 2.6 \text{ l min}^{-1}$, respectively), whereas HR was lower in migrants than in Nepali (172 ± 3 versus $194 \pm 3 \text{ beats min}^{-1}$, respectively).

Incremental running

Tibetan migrants ($n = 17$) attained exhaustion when running at 10.0 km h^{-1} on an average incline of $+11.7 \pm 0.6\%$. By contrast, Nepali lowlanders ($n = 10$) attained exhaustion at a lower, albeit not significantly different, average speed ($9.6 \pm 0.4 \text{ km h}^{-1}$) and incline ($+10.5 \pm 1.0\%$). Mean values of \dot{V}_{O_2} , R , \dot{V}_E , V_T , respiratory frequency (f), and HR at exhaustion are shown in Table 3. No significant differences were found between Tibetan migrants and Nepali as far as peak values of absolute and specific \dot{V}_{O_2} , R and the ventilatory pattern (\dot{V}_E , V_T , and f) are concerned. Average R values ranged from 1.14 to 1.21. By contrast, HR_{peak} of Tibetan migrants was significantly ($P < 0.05$) lower than that of Nepali lowlanders (189 ± 2 versus $202 \pm 3 \text{ beats min}^{-1}$, respectively).

Duration of the exposure to normoxia

The relationships between the average net \dot{V}_{O_2} ($\text{ml kg}^{-1} \text{ min}^{-1}$) values of Tibetan migrants walking at

Table 3. Oxygen consumption (\dot{V}_{O_2}), respiratory gas exchange ratio (R), pulmonary ventilation (\dot{V}_E), tidal volume (V_T), respiratory frequency (f), and heart rate (HR) of Tibetan migrants ($n = 17$) and Nepali ($n = 10$) in standing conditions and at exhaustion

| | Tibetan migrants | | Nepali | |
|--|------------------|-------------|-------------|-------------|
| | Standing | Exhaustion | Standing | Exhaustion |
| \dot{V}_{O_2} (l min ⁻¹) | 0.28 ± 0.01 | 2.73 ± 0.08 | 0.26 ± 0.01 | 2.70 ± 0.09 |
| \dot{V}_{O_2} (ml kg ⁻¹ min ⁻¹) | 4.7 ± 0.1 | 45.9 ± 1.2 | 4.8 ± 0.2 | 49.3 ± 1.0 |
| R | 1.13 ± 0.02* | 1.21 ± 0.02 | 1.03 ± 0.03 | 1.17 ± 0.03 |
| \dot{V}_E (l min ⁻¹) | 14.8 ± 0.4* | 111.0 ± 4.3 | 13.0 ± 0.6 | 104.8 ± 4.8 |
| \dot{V}_E (l min ⁻¹ m ⁻²) | 8.89 ± 0.30 | 66.6 ± 2.4 | 8.11 ± 0.29 | 65.6 ± 2.6 |
| V_T (l) | 0.67 ± 0.02 | 2.22 ± 0.06 | 0.64 ± 0.04 | 2.12 ± 0.07 |
| f (breaths min ⁻¹) | 22.3 ± 0.8 | 50.3 ± 1.6 | 21.4 ± 1.9 | 50.1 ± 2.7 |
| HR (beats min ⁻¹) | 86 ± 4* | 189 ± 2* | 99 ± 3 | 202 ± 3 |

Values are means ± s.e.m. *Significantly different from Nepali.

constant speed on different slopes and the number of days spent at altitudes below 3000 m are shown in Fig. 3. No significant differences could be found in aerobic energy expenditure of Tibetan migrants examined earlier after descent to 1300 m compared with those tested later.

Mechanical and metabolic power output

As shown in Fig. 4, during walking on the treadmill, the metabolic power output of both groups of subjects increased linearly as a function of the mechanical external power output. At any given walking condition and during running on a +5% grade, the metabolic power output of the Tibetan migrants was always lower than that of Nepali control subjects, independent of the type of oxidized substrates.

Discussion

The present investigation is part of a wider project, which also included muscle proteome mapping, aimed at assessing possible determinants of muscle energetics in high-altitude Tibetans (Gelfi *et al.* 2004). Two major novel

findings originated from this study. First, the absolute and specific (with the exception of walking on a 5% incline) \dot{V}_{O_2} of Tibetan migrants during steady-state submaximal walking at 6 km h⁻¹ on the tested inclines (+ 5, +10, +12.5 and +15%) and running (10 km h⁻¹ on a +5% incline) was significantly lower than that of Nepali lowlanders (shown in Fig. 1 and Table 2). This finding indicates that the mechanism(s) underlying the better economy of locomotion reported by Ge *et al.* (1994) in high-altitude native Tibetans compared with acclimatized lowlanders is (are) still operational when the former moved to moderate altitude (1300 m), corroborating our working hypothesis. Second, the reduction of \dot{V}_{O_2} found in Tibetan migrants during submaximal walking appears to be independent of the duration of exposure to normoxia, at least within the first month (shown in Fig. 3).

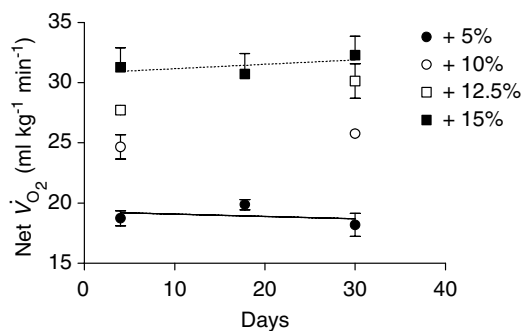


Figure 3 Net (steady state – standing value) \dot{V}_{O_2} of Tibetan migrants during walking at a constant speed (6 km h⁻¹) on a +5, +10, +12.5 and +15% grade as a function of the days at 1300 m.

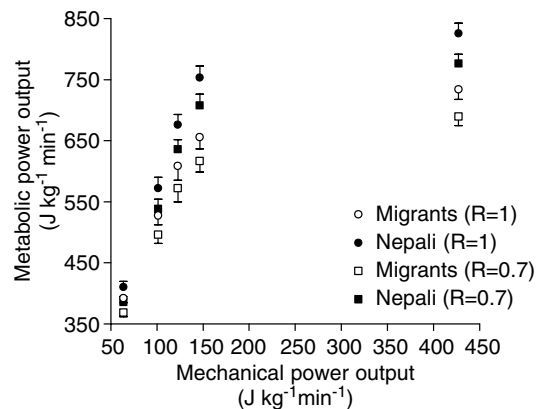


Figure 4. Metabolic power output as a function of the corresponding mechanical external power output in Tibetan migrants and Nepali lowlanders during walking at a constant speed (6 km h⁻¹) on a +5, +10, +12.5 and +15% grade and during running (10 km h⁻¹ on a +5% grade)

Metabolic power output was calculated from aerobic energy sources, assuming the extreme caloric equivalents for \dot{V}_{O_2} of 20.9 kJ l⁻¹ ($R = 1$) and 19.7 kJ l⁻¹ ($R = 0.7$). Mechanical external power output during walking and running was calculated from data by Minetti *et al.* (1993, 1994, respectively).

Choice and physiological characteristics of the subjects

Tibetan migrants were recruited in the Tibetan Refugee Reception Center at Kathmandu. Most of them were students at religious schools in Tibet. Nepali were college students enrolled on the basis of age, gender and level of spontaneous physical activity comparable to those of Tibetans. Unfortunately, Nepali did not match properly Tibetans since they were lighter and with a lower lean body mass. Nevertheless, group matching was considered acceptable, considering the difficulty of the selection and that the mean absolute and specific $\dot{V}_{O_{2peak}}$ values of Tibetan migrants were close or only slightly lower than those of Nepali control subjects (shown in Table 3).

To our knowledge, this is the first assessment of maximal aerobic power on a large number of high-altitude Tibetan natives exposed acutely to 1300 m. The latter condition, from the standpoint of $\dot{V}_{O_{2peak}}$ is almost comparable, for non-athletic subjects, to normobaric normoxia (Terrados *et al.* 1985). Their mean $\dot{V}_{O_{2peak}}$, $45.9 \pm 1.2 \text{ ml kg}^{-1} \text{ min}^{-1}$, was similar to the value ($48.9 \pm 2.6 \text{ ml kg}^{-1} \text{ min}^{-1}$) recently found at 1300 m on seven Sherpas born and living until a few days prior to the measurements at altitudes of 2800–3200 m (Marconi *et al.* 2004). Based on the personal history of the subjects, many features indicate that the investigated group of migrants is representative of an average age-matched, physically active, native Tibetan population. In fact, their physical characteristics (age, height and body mass) were similar to those of a group of Tibetans living at 3680 m investigated by Niu *et al.* (1995) and of other groups living permanently in the Tibetan Plateau, whose $\dot{V}_{O_{2peak}}$ was in the range from ~ 41 to $\sim 52 \text{ ml kg}^{-1} \text{ min}^{-1}$ (Sun *et al.* 1990; Huang *et al.* 1992; Niu *et al.* 1995). Moreover, their mean Hb concentration (15.8 g dl^{-1}) was close to that of Tibetan natives resident at 3800 and 4065 m (Beall *et al.* 1998). It seems therefore reasonable to infer that the $\dot{V}_{O_{2peak}}$ gain associated with the rapid altitude drop of the present group of Tibetan migrants was negligible, perhaps less than 10%, i.e. of the same order of magnitude as that found in seven altitude Sherpas moving from 5050 to 1300 m (15%, Marconi *et al.* 2004), in 50 altitude natives of La Paz (3600 m) undergoing acute hypobaric normoxia (8%, Favier *et al.* 1995), and in seven Andean 3700 m natives after 3 weeks exposure to sea level (10%, Hochachka *et al.* 1991). Thus, the gain of $\dot{V}_{O_{2peak}}$ undergone by Tibetan migrants descending to moderate altitude is by far smaller than the corresponding loss of peak aerobic power Caucasians are subjected to, after 4 weeks exposure to 3600–4800 m (–35 to –40%; Cerretelli & Hoppeler, 1996). This allows us to conclude that, compared with Caucasians, the maximal aerobic power of native Tibetans within the altitude range of their habitual settings is barely impaired by low barometric pressure. This may be the result, on the one hand, of an

optimization of oxygen convective and diffusive transport and of gas exchange (Marconi *et al.* 2004) and, on the other, of metabolic adaptations occurring at the muscle level.

Reduced submaximal oxygen consumption (enhanced economy of locomotion)

This is the first time that aerobic energy expenditure has been determined during steady-state submaximal constant-load walking and running in Tibetan highlanders recently migrated to 1300 m after an entire life spent at altitudes ranging from 3500 to 4500 m. During both types of locomotion Tibetan migrants were characterized by average \dot{V}_{O_2} levels lower than those of lowlanders. Interestingly, when walking at 6 km h^{-1} on +10 and +15% inclines the net O_2 consumption of the Nepali control subjects (27.4 ± 0.8 and $36.0 \pm 0.9 \text{ ml kg}^{-1} \text{ min}^{-1}$, respectively), was close to that found at sea level by Margaria (1938) in Caucasian lowlanders walking at the same speed and incline (27 and $36 \text{ ml kg}^{-1} \text{ min}^{-1}$, respectively). Tibetan migrants were also able to run at the same submaximal speed and incline as Nepali, despite a significantly lower \dot{V}_{O_2} value. In the above condition, i.e. running at 10 km h^{-1} on a +5% incline, the mean net O_2 consumption of Nepali was $39.3 \pm 0.7 \text{ ml O}_2 \text{ kg}^{-1} \text{ min}^{-1}$, i.e. the same found at sea level by Margaria *et al.* (1963) in Caucasians ($\sim 40 \text{ ml O}_2 \text{ kg}^{-1} \text{ min}^{-1}$), and 11% higher than that of Tibetan migrants (see Table 2). Thus also running proved to be more economical in Tibetan migrants than in Nepali lowlanders. Taken together, these results indicate that the mechanisms underlying the reduced energy expenditure of Tibetan migrants compared with Nepali lowlanders are independent of the type of locomotion. This conclusion is consistent also with the recent finding that the metabolic cost of carrying a 1 kg load over a distance of 1 m is lower in Sherpa porters than in acclimatized Caucasian mountaineers (Bastien *et al.* 2005).

Compared with acclimatized lowlanders, a more economical submaximal cycle ergometer performance was found in high-altitude Tibetans (Ge *et al.* 1994; Curran *et al.* 1998) and Andeans (Matheson *et al.* 1991). A slight improvement of the economy of sea-level cycling and/or running was reported also in Caucasians after a 3- to 4-week sojourn at high altitude (Grassi *et al.* 1996; Green *et al.* 2000b) or after prolonged intermittent exposure to simulated altitudes above 2000 m (Gore *et al.* 2001; Stray-Gundersen *et al.* 2001; Katayama *et al.* 2004; Saunders *et al.* 2004b). By contrast, no economy change was observed in elite climbers upon return from a Himalayan mountaineering expedition (Boutellier *et al.* 1984) or in elite distance runners after a 4-week sojourn at altitudes ranging from 1500 to 2000 m (Bailey *et al.* 1998). In addition, 4 months of intermittent normobaric hypoxia (5-min : 5-min 10–12% O_2 breathing-to-normoxia ratio

for 70 min, 5 times per week) failed to improve economy in highly trained distance runners (Julian *et al.* 2004).

Economy of walking and/or running, as is well known, is affected by a number of mechanical and metabolic variables (Williams & Cavanagh, 1987; Saunders *et al.* 2004a). In order to reduce confounding factors, care was taken to standardize type of footwear, duration of exercise practice (the same for all subjects), time of testing during the day, and quality of nutrients, all factors potentially affecting energy expenditure during locomotion on the treadmill. It is of note that upon their first visit to the laboratory, Tibetan migrants were poor runners compared with lowlanders, a handicap that was rapidly compensated for by specific training. In any case, a less efficient treadmill running would have increased and not reduced their energy expenditure compared with Nepali lowlanders.

Mechanics of locomotion

External and internal mechanical work was not assessed in the present study. This would have required having to take into account variables such as displacement of the body centre of mass (COM), of body segments relative to COM, co-excitation of antagonist muscles responsible for variable amounts of positive and negative work, elastic energy storage and release, tendon stiffness, etc., which were out of our reach. Thus, the rather simplistic but reasonable assumption was made that no measurable differences in mechanical power existed between Tibetan migrants and Nepali control subjects walking and running on the treadmill. This assumption is supported by the finding that: (i) no difference in the economy of walking and running (authors' unpublished observations) or cycling (Kayser *et al.* 1994) was found between second generation Tibetans born at 1300 m and Nepali lowlanders; (ii) in Caucasians, the intra- and interindividual variability of the external work of running at different speeds on a treadmill is very low (Avogadro *et al.* 2004); (iii) the external work of walking is not affected by conditions such as ageing (O. S. Mian, J. M. Thom, L. P. Ardigo, M. V. Narici & A. E. Minetti, personal communication), that are associated with considerable structural and functional changes of the locomotor apparatus. Therefore, the mechanical external power output of Tibetan migrants and Nepali lowlanders could be calculated on the basis of the above assumption from published data (Minetti *et al.* 1993, 1994). In addition, metabolic power output of migrants and Nepali was calculated from aerobic energy sources, assuming the extreme caloric equivalents for \dot{V}_{O_2} of 20.9 kJ l⁻¹ ($R = 1$) and 19.7 kJ l⁻¹ ($R = 0.7$), respectively. In the present conditions, in fact, the actual R values could not be used, since they were equal to or higher than 1, probably because of the increased amount of CO₂ deriving from bicarbonate buffering of H⁺. It is of note that R

values higher than expected were also found in Caucasians during constant-load exercises carried out after a 3-week expedition to 6194 m (Green *et al.* 2000b). The results of the above analysis are shown in Fig. 4. It appears that during walking on the treadmill, the metabolic power output of both groups increased linearly as a function of the mechanical external power output, being lower in Tibetan migrants than in Nepali, independent of the type of oxidized substrates. An estimate of the Tibetans' walking efficiency yields average values ranging from 0.163 to 0.223 and from 0.173 to 0.238 depending on the R value (1 or 0.7, respectively) and the incline (from 5 to 15%). For the same given conditions Nepali's walking efficiency was lower, ranging from 0.155 to 0.195 and from 0.165 to 0.207, respectively. In both groups of subjects the increase in efficiency as a function of the incline was probably the consequence of a progressive reduction of the fraction of negative mechanical external work (Margaria, 1938; Minetti *et al.* 1993). By contrast, the greater efficiency found in Tibetans compared with Nepali has no evident explanation and, particularly, cannot be accounted for by a greater compensatory reliance on anaerobic energy sources. In fact, blood lactate concentration was generally lower in Tibetan migrants than in Nepali (6.0 ± 0.9 versus 8.9 ± 0.6 mM, respectively, $P < 0.05$, after a 6 km h⁻¹ on a +15% incline walk). In addition, it appears to be highly unlikely that ventilatory and cardiac work for the same external mechanical power output by locomotor muscles be lower in migrants compared with Nepali. In fact, during submaximal walking or running, Tibetan migrants and Nepali had similar \dot{V}_E values, which, at the most, were ~80% of the peak values attained at exhaustion. By contrast, submaximal HR was always significantly lower in migrants than in Nepali (during submaximal running it was 172 ± 3 versus 194 ± 3 beats min⁻¹, respectively). Even so, assuming at peak exercise myocardial \dot{V}_{O_2} ($M\dot{V}_{O_2}$) of both investigated groups to be 0.0019 ml O₂ per 100 g of left ventricular mass and per beat (Sheffield, 1988), the difference in $M\dot{V}_{O_2}$ between Tibetan migrants and Nepali would be negligible for the overall energy balance.

How can Tibetan migrants perform more economically than lowlanders?

No ultrastructural characteristics of the muscle can explain the lower aerobic energy expenditure of Tibetans during submaximal exercise. In fact, as is well known, the widely investigated vastus lateralis muscle of Sherpas (Kayser *et al.* 1991), second generation Tibetans (Kayser *et al.* 1996) and Quechuas from the Andes (Rosser & Hochachka, 1993), is characterized, compared with Caucasians, by equal distribution of fibre types, normal capillary density and reduced muscle fibre cross-sectional area. The latter adaptation may enhance convective and diffusive O₂ flow to contracting fibres, but obviously does not influence

their metabolic efficiency. On the other hand, Tibetan highlanders, despite reduced mitochondrial volume density, display maximal O₂ consumption : mitochondrial volume ratio higher than acclimatized Caucasians (Kayser *et al.* 1991). These findings seem to indicate that an optimization of the metabolic machinery occurs in Tibetans and that the mitochondrial function and, particularly, the respiratory chain activity, are well preserved.

So far, however, the mechanisms underlying the above metabolic changes and the more efficient muscle oxidative metabolism in Tibetans have not been identified. A tighter coupling of ATP demand and supply was proposed by Hochachka *et al.* (1991) and by Matheson *et al.* (1991) to explain the better performance of Andean natives. On the other hand, a down-regulation of some active transport systems was found in Caucasians after 3 weeks at 6194 m (Green *et al.* 2000a,c). It is therefore likely that chronic exposure to hypoxia induces adaptations at the molecular level that may be more effective in lifelong exposed populations. For these reasons, a proteomic analysis was carried out on muscle samples taken from some subjects participating in the present study. The results showed a greater expression of various proteins (myoglobin, antioxidant compounds, etc.) in Tibetan migrants compared with lowlanders (Gelfi *et al.* 2004), which might be related to the improved function of the oxidative machinery (Antunes *et al.* 2004; Giuffrè *et al.* 2005).

In conclusion, it is likely that the better economy of cycling of high-altitude Tibetans compared with acclimatized lowlanders (Ge *et al.* 1994) or to Tibetans living at lower altitude (Curran *et al.* 1998), as well as the better economy of walking and running of the Tibetan migrants of the present study be related to some, so far unknown, hypoxia-induced metabolic adaptations. At low altitude, the improved efficiency of the oxidative metabolic machinery can be maintained for at least for 1 month. However, it is likely that it declines progressively. In fact, second generation Tibetans born at 1300 m are characterized by the same economy of walking and running (authors' personal observation) and cycling (Kayser *et al.* 1994) as Nepali lowlanders. A more efficient aerobic metabolism at altitude may explain the anecdotal great athletic ability of Incas and the exceptional working capacity of Andean natives and Sherpa porters.

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