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Acclimatization to 4100 m does not change capillary density or mRNA expression of potential angiogenesis regulatory factors in human skeletal muscle

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Summary

Increased skeletal muscle capillary density would be a logical adaptive mechanism to chronic hypoxic exposure. However, animal studies have yielded conflicting results, and human studies are sparse. Neoformation of capillaries is dependent on endothelial growth factors such as vascular endothelial growth factor (VEGF), a known target gene for hypoxia inducible factor 1 (HIF-1). We hypothesised that prolonged exposure to high altitude increases muscle capillary density and that this can be explained by an enhanced HIF-1α expression inducing an increase in VEGF expression. We measured mRNA levels and capillary density in muscle biopsies from vastus lateralis obtained in sea level residents (SLR; *N*=8) before and after 2 and 8 weeks of exposure to 4100 m altitude and in Bolivian Aymara high-altitude natives exposed to approximately 4100 m altitude (HAN; *N*=7). The expression of HIF-1α or VEGF mRNA was not changed with prolonged hypoxic exposure in SLR, and both genes were similarly expressed in SLR and HAN. In SLR, whole body mass, mean muscle fibre area and capillary to muscle fibre ratio remained unchanged during acclimatization. The capillary to fibre ratio was lower in HAN than in SLR (2.4±0.1 *vs* 3.6±0.2; *P*<0.05). In conclusion, human muscle VEGF mRNA expression and capillary density are not significantly increased by 8 weeks of exposure to high altitude and are not increased in Aymara high-altitude natives compared with sea level residents.

Key words: transcription, skeletal muscle, capillary density, hypoxia, HIF-1, VEGF.

Introduction

Ever since Krogh's findings in 1919 (Krogh, 1919), an increased capillary density has been considered advantageous for gas exchange at the tissue level due to a reduction in mean O_2 diffusion distance. Thus, an increased capillary density would be a logical adaptive mechanism to chronic hypoxic exposure. Indeed, several animal studies have reported significant increases in capillary density, while others found no change after chronic hypoxic exposure. An increased capillary to muscle fibre ratio has been found in birds living permanently at high altitude (Hepple et al., 1998; Leon-Velarde et al., 1993; Mathieu-Costello and Agey, 1997), and increased vascularization has been reported in rat skeletal muscle (Cassin et al., 1971; Smith and Marshall, 1999), rat lung (Howell et al., 2003) and rat brain (Boero et al., 1999; Harik et al., 1995; Kuo et al., 1999; LaManna et al., 1992) after 7–28 days of hypoxic exposure in low oxygen/hypobaric chambers. On the other hand, some animal studies have reported no effect of hypoxic exposure on capillarization in rat skeletal muscle (Abdelmalki et al., 1996; Olfert et al., 2001b; Sillau and Banchero, 1977, 1979; Sillau et al., 1980; Snyder et al., 1985). Two previous human studies in this field have examined muscle biopsies from high-altitude climbers in the Himalayas (Hoppeler et al., 1990) or subjects exposed to hypobaric hypoxia in pressure chambers (MacDougall et al., 1991). Both studies have described an increased capillarization. However, the increased capillary density was caused by atrophy of the individual muscle fibres (Hoppeler et al., 1990; MacDougall et al., 1991) and not by an increased number of capillaries. While muscle atrophy could be a consequence of hypoxic exposure *per se*, high-altitude expeditions are also frequented by gastroenteritis, malnutrition and low temperatures (Kayser, 1994), and in hypobaric chambers the limited space available may be associated with detraining atrophy. Thus, it is unclear whether or not the muscle atrophy and increased capillary density are caused by hypoxic signalling.

The signal transduction pathways underlying capillarization are undoubtedly complex. The transcription factor hypoxia-inducible factor-1α (HIF-1α) is increased with hypoxic exposure in many tissues, including hypoxic rat muscle (Stroka et al., 2001). HIF-1α regulates many of the genes that are

augmented in hypoxia, and it is thought that the overall purpose of these genes and their products is to increase O_2 delivery to the cells. HIF-1α has been recognized to cause an increased expression of at least one important angiogenic factor, vascular endothelial growth factor (VEGF; Jin et al., 2000). VEGF mRNA levels have been reported to increase with hypoxia in mouse brain (Chávez et al., 2000; Kuo et al., 1999) but also to decrease in rat muscle (Olfert et al., 2001b). In human muscle, VEGF is increased with acute exercise (Gustafsson et al., 2002; Richardson et al., 1999). However, it has not previously been studied whether VEGF expression is increased in human muscle in response to hypoxia, and simultaneous determination of these signalling factors and morphological characterization of capillary density in human skeletal muscle has not previously been performed in lowlanders acclimatizing to altitude or in high-altitude natives.

The purpose of the present study was twofold. First, to test whether skeletal muscle HIF-1α and VEGF mRNA content and morphologically determined capillarization increase in lowlanders during the course of acclimatization to high altitude compared with sea level. Second, to test whether expression of HIF-1α and VEGF mRNA and capillarization are higher in high-altitude natives compared with the levels observed in lowlanders at both sea level and after prolonged acclimatization. Human skeletal muscle is an interesting model because (1) it may be studied repeatedly in the same subject by intravital biopsies, (2) skeletal muscle microvasculature is characterized by homogenous structure, which facilitates evaluation of changes, and (3) oxygen extraction from capillary blood is higher in exercising skeletal muscle than in any other organ. To address specifically whether any changes in capillarization were caused by hypoxia *per se*, care was taken to avoid cold exposure, malnutrition, gastroenteritis and detraining.

Materials and methods

Subjects

Eight sea level residents (SLR; six male and two female) [$\dot{V}_{O_2max}$=4.2±0.8 l min^{-1} (at sea level), height=186±6 cm, mass=78.4±8 kg, age=25±8 years] and seven male Aymara natives [HAN; $\dot{V}_{O_2max}$=2.6±0.8 l min^{-1} (at 4100 m), height=164±13 cm, mass=63±16 kg, age=30±11 years] were included in the study.

After being given both written and oral information on the experimental protocol and procedures, the subjects gave their informed, written consent to participate. The study conformed with guidelines laid down in the Declaration of Helsinki and was approved by the Copenhagen and Frederiksberg Ethics Committees (KF11-050/01) and by El Tribunal de Honor del Colegio Médico Departamental de La Paz and the Ministerio de Previsión Social y Salud Pública, La Paz, Bolivia.

Acclimatization and conditions in high altitude

The subjects were flown from Copenhagen (sea level) to La Paz in Bolivia. Here, acclimatization started with two nights in a hotel at 3800 m, from where the subjects moved to a well-equipped heated apartment in El Alto at 4100 m, where they resided for the next 8 weeks. Subjects had ample access to food and followed a varied diet rather similar to their usual diet before the expedition. Subjects remained active throughout the stay with activities such as cycling, soccer, basketball, hiking and rock climbing, to match their reported activity level at sea level. The Bolivian Aymara high-altitude natives were all born and raised around El Alto and La Paz and had lived their whole lives at altitudes between 3700 and 4100 m. They were all employed or active students and were physically active. The oxygen uptake per body mass at high altitude was similar in the lowlanders and high-altitude natives.

Experimental procedure

All sampling was performed in hospital settings, either at sea level at the Copenhagen Muscle Research Centre or at high altitude in a clinic at 4100 m situated a few kilometres from the apartment. After local anaesthesia (lidocaine), a resting muscle biopsy was obtained from the middle portion of the vastus lateralis muscle. The muscle specimen was immediately frozen in liquid nitrogen, transported to Copenhagen on dry ice and stored at –80°C for later analysis. The lowlanders had biopsies taken at sea level (SLR) and after 2- (CH2) and 8-weeks (CH8) exposure to 4100 m of altitude. The Aymara natives had one resting biopsy taken at high altitude. The subjects were not physically active 24 h prior to biopsies.

Arterial blood sampling

Blood samples were obtained at rest from the femoral artery at sea level and after breathing 12.6% O_2 balanced in N_2 for 15 min at sea level (acute hypoxia) and after 2 and 8 weeks of acclimatization to 4100 m. Blood sampling from the high-altitude natives was done at 4100 m. For further details see Lundby et al. (2004).

RNA isolation, reverse transcription and PCR

To determine the mRNA content of HIF-1α and VEGF, total RNA isolation, reverse transcription (RT) and PCR were carried out as follows. Total RNA was isolated from ~25 mg of muscle tissue by a modified guanidinium thiocyanate–phenol–chloroform extraction method adapted from Chomczynski and Sacchi (1987), as described previously (Pilegaard et al., 2000). RNA was resuspended overnight (4°C) in 2 μl mg^{-1} original tissue mass in diethyl pyrocarbonate (DEPC)-treated H_2O containing 0.1 mmol l^{-1} EDTA. RT of 11 μl of total RNA sample was performed using the Superscript II RNase H system (Invitrogen, Carlsbad, CA, USA) as previously described (Pilegaard et al., 2000). RT products were diluted in nuclease-free H_2O to a total volume of 150 μl.

The mRNA content of the selected genes was determined by fluorescence-based real-time PCR (ABI PRISM 7700 Sequence Detection System; Applied Biosystems, Foster City, CA, USA). Forward (FP) and reverse (RP) primers and TaqMan probes were designed from human specific sequence

data (Entrez-NIH and Ensembl, Sanger Institute) using computer software (Primer Express; Applied Biosystems). The following sequences were used to amplify a fragment of HIF-1α FP: 5′-GCCCCAGATTCAGGATCAGA-3′; RP: 5′-TGGGACTATTAGGCTCAGGTGAAC-3′; probe: 5′-ACCTAGTCCTTCCGATGGAAGCACTAGACAA-3′. The following sequences were used to amplify a fragment of VEGF FP: 5′-CTTGCTGCTCTACCTCCACCAT-3′; RP: 5′-AGGAACAGATAAAAGAGAAAAGGCATT-3′; probe: 5′-CCAAGGTGTGCGACTGCTGCGAC-3′. The probes were 5′ 6-carboxyfluorescein (FAM) and 3′ 6-carboxy-*N*,*N*,*N*′,*N*′-tetramethylrhodamine (TAMRA) labelled. Prior optimization was conducted for each set of self-designed oligos determining optimal primer concentrations, probe concentration and verifying the efficiency of the amplification. For each of the target genes, the expected size of the PCR product was confirmed on a DNA 2.5% agarose gel. β-actin was also amplified for use as an endogenous control using a pre-developed assay reaction (Applied Biosystems). PCR amplification was performed (in triplicate) in a total reaction volume of 25 μl. The reaction mixture consisted of 2.5 μl diluted template, forward and reverse primers and probe as determined from the prior optimization, 2× TaqMan Universal MasterMix optimized for TaqMan reactions (Applied Biosystems; containing AmpliTaq Gold DNA polymerase, AmpErase uracil *N*-glycosylase, dNTPs with dUTP, ROX as passive reference and buffer components) and nuclease-free water. The following cycle profile was used for all genes: 50°C for 2 min + 95°C for 10 min + 40 cycles of 95°C for 15 s + 60°C for 1 min. Serial dilutions were made from a representative sample, and these samples were amplified together with the unknown samples and used to construct a standard curve. The mRNA contents were normalized to β-actin mRNA levels, and samples expressed relative to the sea level samples, which were set to 1. β-actin mRNA has previously been shown not to change in bronchial epithelium and leukocytes in healthy subjects exposed to hypoxia (Mairbaurl et al., 2003).

Capillarization and fibre distribution

Serial sections (10 μm) of the muscle biopsy samples were cut in a cryostat (Zeiss, HM 560) at –20°C, and routine ATPase histochemistry analysis was performed after pre-incubation at pH 4.37, 4.60 and 10.30 (Brooke and Kaiser, 1970). Five different fibre types were defined: types 1, 1/2a, 2a, 2ax and 2x (Andersen and Aagaard, 2000). Capillary density was determined using the double staining method combining *Ulex europaeus* agglutinin I lectin (UEA-I) and a collagen IV antibody as previously described (Qu et al., 1997). The serial sections of the various ATPase and capillary stainings were visualized and analysed for fibre type percentage, fibre size and capillary density, expressed as capillaries fibre^{-1} and capillaries mm^{-2}, using a TEMA image analyzing system (TEMA, Hadsund, Denmark) as earlier described in detail (Andersen and Aagaard, 2000; Qu et al., 1997). An average of 67±7 fibres was included in the analysis of fibre type and size. An average of 197±9 capillaries around 59±3 fibres was included in the separate analysis of capillary density.

Statistics

Values reported are mean ± S.D. One-way analysis of variance (ANOVA) for repeated measurements was used to evaluate the effect of duration at altitude. Student's *t*-test was used for comparisons between the lowlanders and the Aymara high-altitude natives. To conform to normal distribution criteria, mRNA ratios were log transformed before statistical analysis. The significance level was $P<0.05$, using the Bonferroni correction where relevant.

Results

Haematocrit, haemoglobin and arterial oxygen content

In lowlanders, haematocrit (Htc), haemoglobin (Hb) and arterial oxygen content (Ca_{O_2}) increased markedly during the first 2 weeks of acclimatization and did not increase further over the subsequent 6 weeks at altitude (Table 1). The Htc, Hb and Ca_{O_2} in the high-altitude natives were not significantly different from those observed in the lowlanders after 2 and 8 weeks of acclimatization.

Body mass, fibre size and fibre type

Body mass was 78.4±9.1 kg at sea level and 74.6±9.6 and 77.4±10.2 kg after 2 and 8 weeks acclimatization, respectively. At sea level, the mean fibre area was 6492±1448 μm^2 in the lowlanders and was not significantly changed after 8 weeks of acclimatization to 4100 m (6060±1765 μm^2) (Table 2). The mean fibre area for the high-altitude natives was 4474±497 μm^2 ($P<0.05$ *vs* lowlanders), a 31% lower mean fibre area compared with lowlanders before altitude exposure. The muscle fibre type distribution was not affected by altitude exposure in the lowlanders; while high-altitude natives tended to have a decreased fraction of type 1 fibres, this did not reach statistical significance (Table 3).

Capillarization

At sea level, lowlanders had 4.0±0.6 capillaries fibre^{-1}, and this ratio was not significantly changed with acclimatization

Table 1. *Haematocrit (Htc), hemoglobin (Hb) and arterial oxygen content (Ca_{O_2}) in lowlanders studied at sea level (SLR), in acute hypoxia (AH) and after 2 (CH2) and 8 (CH8) of acclimatization to 4100 m and high-altitude natives (HAN) living at 4100 m*

	Hct (%)	Hb (g l^{-1})	Ca_{O_2} (ml l^{-1})
SLR	42.4±2.0	139±6	191±8
AH	41.4±2.5	131±11	160±17*
CH2	49.2±2.8*	163±8*	204±17
CH8	47.6±2.8*	159±8*	201±11
HAN	50.2±3.4*	167±11*	203±17

Values are means ± S.D. *$P<0.05$ compared with SLR.

Table 2. *Muscle morphology in sea level residents at sea level (SLR) and after 8 weeks exposure to 4100 m altitude (CH8) and in high-altitude Aymara natives (HAN)*

	Capillaries fibre^{-1}	Capillaries mm^{-2}	Mean area (μm^2)
SLR	4.0±0.6	556±158	6492±1448
CH8	3.6±0.6	579±79	6060±1765
HAN	2.4±0.3*,†	491±53†	4474±497*

Values are means ± S.D. *P<0.05 compared with SLR; †P<0.05 compared with CH8.

(Table 2). By comparison, the high-altitude natives had 2.4±0.3 capillaries fibre^{-1} (P<0.05 compared with lowlanders). Despite a smaller mean fibre area in the high-altitude natives, the capillary density tended to be smaller in high-altitude natives than in lowlanders, and the difference reached significance when comparing with the 8-week value for the lowlanders (P<0.05).

HIF-1α and VEGF mRNA content

No significant changes were found in HIF-1α and VEGF mRNA content after 2 and 8 weeks hypoxic exposure in the lowlanders, and HIF-1α and VEGF mRNA levels were similar in the high-altitude natives (Fig. 1).

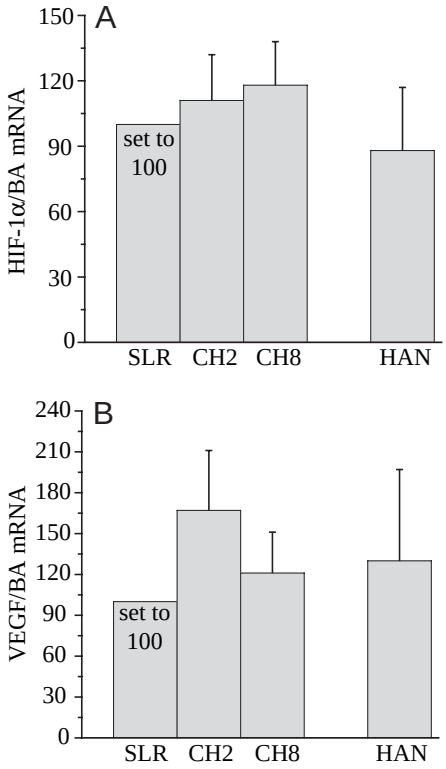

Fig. 1. (A) Hypoxia-inducible factor 1α (HIF-1α) and (B) vascular endothelial growth factor (VEGF) mRNA content in sea level residents (SLR) and after 2 (CH2) and 8 weeks (CH8) of hypoxic exposure and in high-altitude natives (HAN). BA, β-actin.

Discussion

The primary findings of the present study are: (1) in lowlanders, neither 2 nor 8 weeks of exposure to the hypoxia of high altitude (4100 m) caused any significant change in HIF-1α or VEGF mRNA; (2) in lowlanders, mean muscle fibre area and capillary density were not significantly changed by 8 weeks at high altitude and (3) mean fibre area was smaller in high-altitude natives than in lowlanders but, despite this, capillary density tended to be lower, not higher, in high-altitude natives compared with lowlanders.

Body mass and mean muscle fibre area

Previous high-altitude mountaineering expeditions have reported 5–10% reductions in body mass (Hoppeler et al., 1990), probably caused by reductions in food intake (Guilland and Klepping, 1985; Kayser, 1992) and gastroenteritis (Kayser, 1994). To prevent undernutrition-induced weight loss, Butterfield et al. (1992) imposed hypercaloric diets on subjects sojourning on the top of Pike's Peak for 3 weeks (4300 m). Despite the hypercaloric diet, these subjects lost a significant 3% of body mass during the 3 weeks, most likely an effect of inactivity at the spatially restricted high-altitude laboratory on Pike's Peak. Two previous human biopsy studies have reported a decrease of 20–26% in mean fibre area during an 8-week expedition to the Himalayas (Hoppeler et al., 1990) and in Operation Everest II (OEII), a chamber study lasting 7 weeks (MacDougall et al., 1991). It is likely that the catabolic state associated with gastroenteritis contributed to the muscle atrophy reported with Himalayan mountaineering, and the reported muscle fibre atrophy in OEII could have been at least partly caused by decreased physical activity while the subjects were confined to the hypobaric chamber. It has been hypothesized that hypoxia causes overall downregulation of protein synthesis in skeletal muscle with the purpose of decreasing fibre size to facilitate oxygen diffusion (Hochachka et al., 1996). However, decreased fibre size is not a universal finding. Five months at 3800 m caused no change in the cross-sectional area of pigeon muscle fibres compared with those at sea level (Mathieu-Costello et al., 1997), and rat skeletal muscle fibre cross-sectional area was preserved after 7–9 weeks of exposure to 12–13% O_2 (Abdelmalki et al., 1996; Olfert et al., 2001b). In humans, it was recently reported that a 21-day expedition to Mt Denali, USA (reaching the summit at 6194 m after 18 days climbing effort from 2160 m) did not

Table 3. *Muscle histochemistry in sea level residents before and after hypoxic exposure and in high-altitude natives*

	Type 1	Type 1/2a	Type 2a	Type 2ax	Type 2x
SLR	71.1±13.0	ND	26.4±13.0	2.2±4.0	0.4±1.1
CH8	70.3±10.5	ND	25.2±14.1	4.2±9.9	ND
HAN	57.1±11.9	ND	32.0±14.0	5.8±9.0	5.2±5.8

Values are means ± S.D. Abbreviations as in Table 2. ND, not detected.

induce any significant change in mean fibre area (Green et al., 2000).

By design, our subjects remained physically active and controlled their own diet. In contrast to previous studies, this regimen was accompanied by a non-significant 1% reduction in whole body mass and a non-significant 4% reduction in mean muscle fibre area during 8 weeks of acclimatization to 4100 m. An unchanged fibre area was an important prerequisite for interpretation of the capillary density in human skeletal muscle before and after hypoxic exposure. If fibre size had been reduced by hypoxia *per se* or alternative mechanisms, this would improve oxygen diffusion and diminish any potential hypoxia-induced stimulation of growth factor expression and neoformation of capillaries.

Compared with the lowlanders, the mean fibre area in the high-altitude natives was significantly lower (by 30%). This is in agreement with previous published data from a similar experimental population (Desplanches et al., 1996). Whether this is related to high-altitude residence or perhaps an evolutionary adaptation remains unknown but, interestingly, similar small muscle fibre areas have been observed in high altitude Sherpas from the Himalayas (Kayser et al., 1991).

Fibre type distribution

Typically, untrained subjects have an approximately equal distribution of the major fibre types in the vastus lateralis muscle (Saltin and Gollnick, 1983). The Danish lowlanders studied in the present investigation were physical education college students active in a variety of sporting activities and this may, to some extent, explain the rather high distribution of type 1 fibres (Table 3). However, although physically active, their $\dot{V}_{O_2max}$ (approximately 54 ml min^{-1} kg^{-1}) indicates that they were not competing athletes. It has been speculated that it would be favourable to modify the fibre composition of the muscle to more oxidative fibres in hypoxic conditions. However, in agreement with previous studies (Green et al., 1989), we did not find any changes in muscle fibre type histochemistry with acclimatization in the lowlanders. The Aymara high-altitude natives tended to have a smaller fraction of type I fibres compared with the lowlanders, which may in part, but not entirely, correspond to their smaller mean fibre area.

HIF-1 and VEGF

The biological activity of HIF-1 is determined by the expression and activity of the HIF-1α subunit. Hypoxia has been demonstrated to increase HIF-1α mRNA expression (Wang et al., 1995; Wiener et al., 1996; Yu et al., 1998). Perhaps even more importantly, hypoxia disrupts the usual cytoplasmic HIF-1α ubiquination (inactivation), and instead HIF-1 is stabilized and translocates to the nucleus (Semenza, 2000). Analysis of rat brain exposed to hypoxia revealed an increase in HIF-1α protein after 6 h exposure that persisted for 14 days (Chávez et al., 2000). It has also been shown that the response of HIF-1α to hypoxia is very tissue specific and that brain and spleen are much more sensitive than liver and kidney (Stroka et al., 2001). Recently, human muscle HIF-1α mRNA was found to increase following intermittent exposure to normobaric hypoxia, equivalent to an altitude of 3850 m (Vogt et al., 2001). In the present investigation, we observed no increases in HIF-1α mRNA after either 2 or 8 weeks of hypoxic exposure. While most animal studies finding increased HIF-1α mRNA levels have been conducted under severe hypoxia, with $F_{I_{O_2}}$s (fractional O_2 concentrations in incurrent gas) of 0.00 or 0.01 (Wang et al., 1995; Yu et al., 1998), the human study by Vogt et al. (2001) was conducted with a somewhat milder hypoxic exposure than used in the present study. The most obvious differences between the present study and that of Vogt et al. were the intermittent nature of hypoxia used by Vogt and co-workers, and the fact that Vogt et al. combined the hypoxic exposure with exercise whereas our subjects abstained from exercise for 24 h prior to the biopsy procedure. Exercise in a hypoxic environment may cause severe arterial and tissue deoxygenation (Calbet et al., 2003), and this could explain the increases in HIF-1α mRNA found by Vogt et al. (2001). It would have been an advantage if we had been able to quantify the HIF-1α protein in our biopsies, but western blotting analysis of this particular protein proved difficult in pilot experiments and was most likely related to the limited amount of muscle tissue. The *VEGF* gene is well accepted to be a target gene for HIF-1α and the fact that VEGF mRNA did not change significantly is an important indirect indication that HIF-1α activity was not increased in skeletal muscle in our study.

VEGF mRNA levels have been found to increase in human muscle following an exercise bout (Gustafsson et al., 2002) and in humans exercising in hypoxia (Vogt et al., 2001); however, it was unknown whether hypoxia *per se* would lead to an increased VEGF mRNA content in resting human skeletal muscle. With prolonged hypoxic exposure, an increase in VEGF mRNA was found in rat brain after 6 h of 10% oxygen exposure (Chávez et al., 2000) and remained elevated for 14 days but had returned to normoxic levels after 21 days. In rat skeletal muscle, a surprising attenuation of resting VEGF mRNA was reported after 8-weeks exposure to 12% O_2 (Olfert et al., 2001a,b). In the present study, there was a trend towards an increase in VEGF mRNA levels after 2 weeks. However, this change did not reach statistical significance, and the VEGF mRNA levels at 8 weeks and in high-altitude natives were very similar to the levels obtained at sea level in the lowlanders. It is noteworthy that HIF-1α mRNA and VEGF mRNA were easily detectable in all biopsies.

Capillarization

Even after prolonged exposure to high altitude, we observed no indication of neoformation of capillaries in human skeletal muscle. Similar findings have been reported in animal skeletal muscle after hypoxic exposure (Abdelmalki et al., 1996; Olfert et al., 2001a,b; Sillau and Banchero, 1977, 1979; Sillau et al., 1980; Snyder et al., 1985). Two animal studies report increased muscle fibre capillarization after exposure to hypoxia (Cassin et al., 1971; Smith and Marshall, 1999). However, at least part

of the results can be attributed to body mass differences in the animals. In humans exposed to altitudes between 5000 and 8500 m for 3–8 weeks, no net capillary neoformation has been reported (Green et al., 2000; Hoppeler et al., 1990; MacDougall et al., 1991). It is unknown whether neoformation of capillaries in human skeletal muscle would occur during acclimatization of lowlanders beyond 8 weeks. Interestingly, an increased capillary to fibre ratio has been found in animals living permanently at high altitude (Hepple et al., 1998; Leon-Velarde et al., 1993; Mathieu-Costello and Agey, 1997; Sillau and Banchero, 1979). However, in the present study, the capillary to fibre ratio in the Aymara natives was lower than that found in the lowlanders, which is in agreement with previous reported data from a similar population (Desplanches et al., 1996). It could be speculated that if the natives had a similar mean muscle fibre area to the lowlanders, this would require an increased capillary number (in order to achieve a similar capillarization/mm^2). Maybe the capillarization per mm^2 of muscle tissue is of greater importance than capillarization/muscle fibre and could explain why the natives have a lower capillarization per muscle fibre, i.e. due to the smaller mean area, the tissue is sufficiently supplied as it is.

Conclusion

In summary, HIF-1α and VEGF mRNA levels in lowlanders are not increased after 2- and 8-weeks exposure to 4100 m, and similar levels are found in high-altitude natives living permanently at this altitude. Eight weeks of acclimatization did not induce any detectable angiogenesis in human skeletal muscle. Skeletal muscle fibre type distribution and mean muscle fibre area are not altered by altitude acclimatisation. Altogether, these findings indicate that, at 4100 m, the acclimatization-dependent increase in haemoglobin concentration is sufficient to maintain adequate levels of muscular oxygen supply and that angiogenesis is not necessary in order to preserve oxygen delivery to skeletal muscle.

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