there are certain features of cranial sexual dimorphism (we suggest principally a size difference and any shared allometric effects) common to all groups.

The results presented here strongly suggest that there is a degree of variation between the studied hominoid groups (even within a single species, *Homo*) in their patterns of cranial sexual dimorphism (contra 2). Whilst a male – female size difference is a common finding (indeed, it may account for the degree of interchangeability of variables), with males being larger than females, there are differences in the shape transformations between the sexes. We conclude that these differences in cranial sexual dimorphisms must be attributable to differences between the groups in their mechanisms (e.g. allometry) of development of sexual dimorphism, to similar mechanisms acting upon different ontogenies or to a combination of these. The present study casts no light upon the mechanism of development of the hominoid sexual dimorphisms but it does imply that great caution should be taken in using the sexual dimorphisms of living hominoids as models for the 'expected' dimorphisms of fossils.

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Muscle lipofuscin content and satellite cell volume is increased after high altitude exposure in humans

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Summary. Muscle ultrastructural changes during a typical expedition to the Himalayas were analyzed by taking muscle biopsies from seven climbers before and after their sojourn at high altitude (over 5000 m for 8 weeks). M. vastus lateralis samples were analyzed morphometrically from electron micrographs. A quantitative evaluation was made of lipofuscin, satellite cells and myonuclei. Significant increases of the volume densities of lipofuscin (+ 235%) and satellite cells (+ 215%) were observed.
Key words. Muscle; morphometry; human; hypoxia; lipofuscin; satellite cell; high altitude.

Recent studies on humans exposed to high altitude during traditional long-lasting expeditions to Mt. Everest and Lhotse have shown signs of muscle deterioration[4, 13]. The main observation made in these studies was that there was a significant reduction of muscle cross-sectional area, mainly due to a decrease in muscle fiber size. A loss of muscle oxidative capacity was also evident, as indicated by a decrease in the volume of muscle mitochondria. The capillary network was mostly unchanged, so that oxygen transport to the remaining muscle mitochondria appeared to be improved after prolonged high altitude exposure (fig. 1). These studies further showed qualitative evidence of lipofuscin accumulations, which were considered to be possible signs of degeneration of mitochondria and other membrane-rich cell organelles. However, these findings were not quantitated.

The first goal of the present study was to measure the previously suggested increase of lipofuscin granules with high altitude exposure[13], using muscle biopsy specimens of the same individuals described in the previous reports[9, 13]. In a survey of the literature we found quantitative measurements of lipofuscin in central nervous tissue, kidney and myocardial cells, but not in skeletal muscle tissue[2, 12, 23, 25, 30]. This study is thus, to the best of our

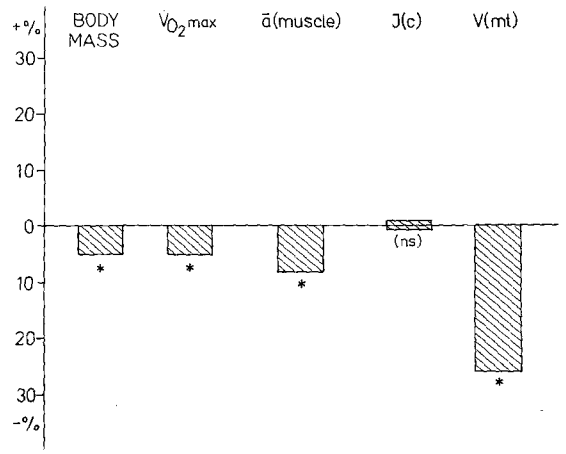

Figure 1. Relative changes of body mass, maximal oxygen uptake capacity ($\dot{V}_{O_2}$ max); thigh muscle cross-sectional area, a (muscle); capillary length, J(c) and mitochondrial volume, V(mt) with high altitude exposure in humans (*p = 0.05; original data reported in Ferretti[9] and Hoppeler[13]).

knowledge, the first morphometric report on changes of lipofuscin content in skeletal muscle fibers. As an additional aim we set out to quantitate possible structural markers of tissue regeneration such as satellite cells and myoblasts as it is well documented that the regenerative capacity of skeletal muscle is based on the proliferation of satellite cells, which are considered to be 'dormant' stem cells of mature muscle fibers[15,16].

Materials and methods

From seven male subjects participating in the Swiss Mt. Everest expedition of 1986 (for details of expedition characteristics, acclimatization profiles, timing of measurements etc.[4]) computed tomographs and muscle biopsies were obtained before departure and 10–15 days after the return from the expedition. The time spent at or above 5000 m was 8–10 weeks. The base camp was at 5350 m and all participants were exposed on several occasions to altitudes of 8000 m, with and without O_2 supplementation. Computed tomographs were only obtained from 6 subjects. The cross-sectional area of the left thigh muscle was determined by computed tomography (SOMATOM SF, Siemens, Erlangen, Germany). Scans were taken on approximately 8-mm-thick slices at two-thirds of the distance between the upper border of the patella and the greater trochanter, where the circumference of the thigh is nearly maximal.

Muscle biopsies from the vastus lateralis were taken at mid-thigh level using the technique of Bergström[3]. The muscle samples were processed for electron microscopy by fixation in a 6.25% solution of glutaraldehyde as previously described[14]. Morphometry of these samples was carried out on cross-sections from four randomly chosen tissue blocks from each biopsy. A magnification of × 30,900 was used to estimate the fractional volume of lipofuscin, myonuclei and satellite cells in myofibers. Systematic sampling was used for all stereologic procedures. Some 500 visual fields were analyzed in each biopsy using a 9-point test system lying in the picture plane of the electron microscope, in order to determine the reference volume (muscle fiber) directly on the microscope. If any of the structures of interest (lipofuscin, satellite cells, myoblasts, myonuclei; fig. 2) appeared within the defined measuring area, a micrograph was taken for later detailed analysis. Out of 100 visual fields that were analyzed, some 12 micrographs were taken. The stereological evaluation of these micrographs was made with a grid B100f, (400 test points, as indicated in detail in Weibel[29]). This procedure enabled us to obtain reliable estimates of the very low volume densities of fiber nuclei, lipofuscin inclusions and satellite cells. Myoblasts were still too rare to be quantitated. All stereological variables were obtained according to standard procedures[29].

We made no attempt to estimate the numerical density of satellite cells. This was because a model-based counting approach requires the dimensions of the structures to be counted to be known, and not to be changed by experimental procedures[29]. This requirement is certainly not fulfilled for satellite cells. We found rather diverse statements about satellite cell length; it is reported to range from 10 to 100 μm[18,28] with maximal values up to 600 μm in some species[10]. As it would be important to know whether muscle regeneration is achieved through satellite cell growth and/or mitosis[1], a project is currently being undertaken to adapt new stereological procedures, which allow for an unbiased estimation of particle size and number[11], to the problem of counting the relatively rare skeletal muscle satellite cells.

For the assessment of absolute values of morphometric data the assumption was made that all muscles of the thigh are equally affected by the stress of high altitude exposure and hence, that the structural changes observed in the vastus lateralis are representative for all thigh muscles. Absolute values of lipofuscin and satellite cell volumes were obtained by multiplying the volume density estimates of these variables by the volume of the entire thigh musculature calculated from the computed tomography scans (values given are calculated for a slice of muscle of 1-cm thickness[13]).

For statistical comparisons of group means (before and after the expedition) Student's t-test for paired samples was used; the level of statistical significance was set at 5%.

Results

The data on relative changes of body mass, maximal oxygen uptake capacity, thigh muscle cross-sectional area, capillary length, and mitochondrial volume have already been reported elsewhere[9,13], and are summarized in figure 1.

The volume density of lipofuscin (volume of lipofuscin material per volume of muscle fiber) was increased by over 200% ($p \leq 0.05$; table). The calculated absolute volume of lipofuscin was thus significantly increased by

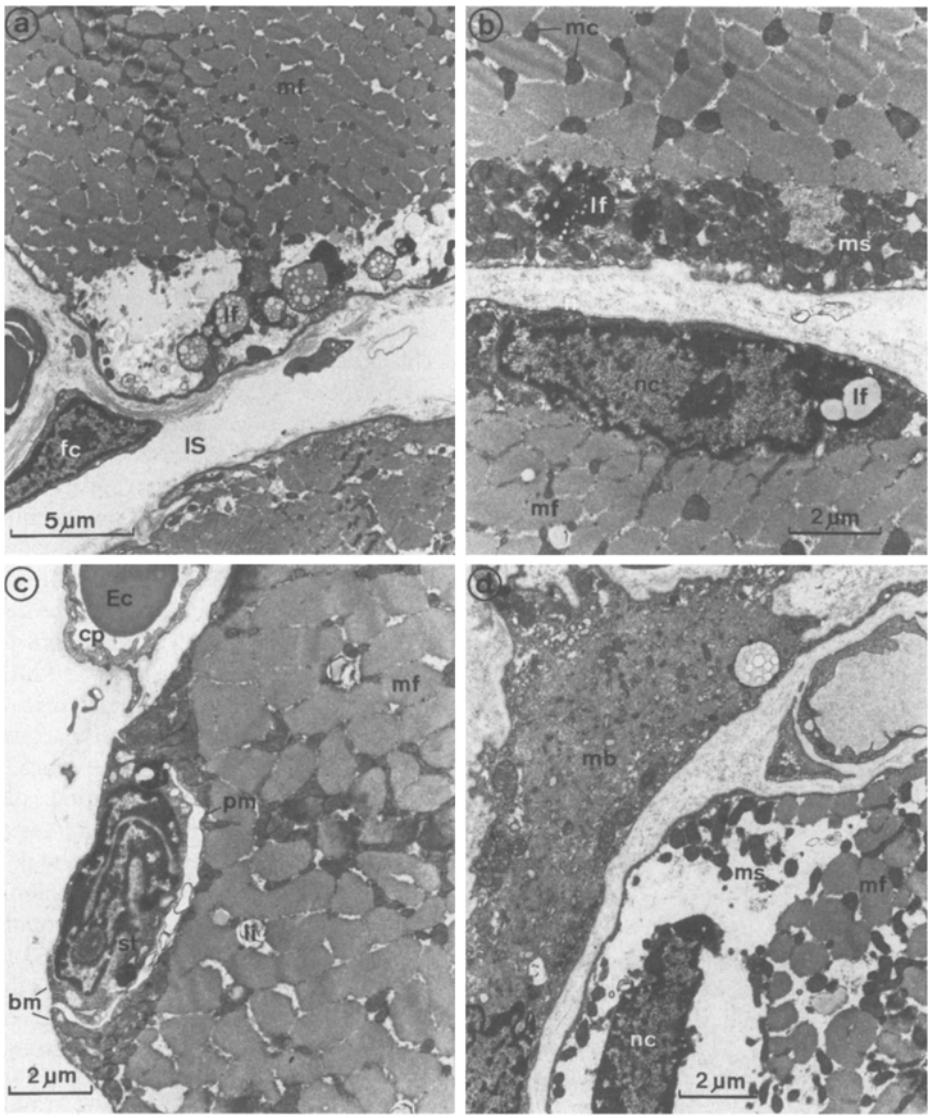

Figure 2. *a* Lipofuscin accumulations (lf) in subsarcolemmal location of a muscle fiber in a biopsy obtained after return from the Everest expedition. *b* Lipofuscin granules were mostly found in the subsarcolemmal region near the poles of myonuclei (nc). *c* Satellite cell (st) representing stem cells of muscle fibers, wedged between the basement membrane (bm) and the plasma membrane (pm) of the skeletal muscle fibers. *d* Myoblast (mb) in interstitial space (cp = capillary; Ec = erythrocyt; fc = fibrocyt; IS = interstitial space; li = lipid; mc = central mitochondria; mf = myofibril; ms = subsarcolemmal mitochondria).

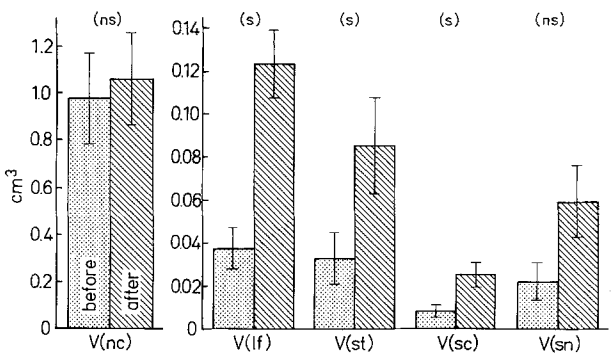

Figure 3. Absolute volumes (V) of myonuclei (nc), lipofuscin (lf) and satellite cells (st) in a slice of M. vastus lateralis of 1-cm thickness before and after prolonged high-altitude exposure (sc = satellite cell cytoplasm, sn = satellite cell nuclei; means ± 1 SE; s = significant, $p \leq 0.05$, paired t-test, n = 6).

almost three-fold (fig. 3). The volume density of satellite cells showed a significant increase, close to three-fold (table). This increase was due to a similar proportional increase in the volume of the cytoplasm of the satellite cells (significant) and the nuclei of the satellite cells (not significant). The absolute volumes of satellite cells and their component spaces are shown in figure 3. Both the volume density and the absolute volume of myonuclei were not significantly changed with high altitude exposure (table, fig. 3).

Discussion

The focus of the present analysis was the quantitative evaluation of lipofuscin content and satellite cell volume with high altitude exposure in humans. Because of the

Volume densities of lipofuscin, myonuclei and satellite cells (units: percent of muscle fiber volume; means ± SE; * $p \leq 0.05$, two-sided paired t-test).

		Volume density of lipofuscin	Volume density of myonuclei	
Before	(n = 7)	$2.14 \cdot 10^{-4} \pm 4.0 \cdot 10^{-5}$	$6.05 \cdot 10^{-3} \pm 4.6 \cdot 10^{-5}$	
After	(n = 7)	$7.17 \cdot 10^{-4} \pm 7.9 \cdot 10^{-5}$ *	$6.49 \cdot 10^{-3} \pm 9.8 \cdot 10^{-5}$	
		Volume density of satellite cells	Volume density of satellite cytoplasm	Volume density of satellite nuclei
Before	(n = 7)	$1.74 \cdot 10^{-4} \pm 6.3 \cdot 10^{-5}$	$4.80 \cdot 10^{-5} \pm 1.6 \cdot 10^{-5}$	$1.26 \cdot 10^{-4} \pm 4.8 \cdot 10^{-5}$
After	(n = 7)	$5.46 \cdot 10^{-4} \pm 1.2 \cdot 10^{-4}$ *	$1.64 \cdot 10^{-4} \pm 3.0 \cdot 10^{-5}$ *	$3.81 \cdot 10^{-4} \pm 9.2 \cdot 10^{-5}$

scarcity of these components reliable quantitation required special attention. Satellite cells have their long axis aligned with the major axis of the muscle fibers[10]; this makes sampling on cross-sections of muscle tissue more efficient, by a factor which is given by the ratio of the longitudinal divided by the cross-sectional diameter of these cells. The drawback of cross-sections is that they do not readily lend themselves to the qualitative description of pathological changes of muscle cells. This is because many of the pathological changes concerning the ultrastructure of muscle fibers are related to disorders of the sarcomeric organization of myofilaments, and can best be seen in longitudinal sections[27]. Pathological signs which can be identified in cross-sections, such as split fibers or central nuclei, were not more prominent in biopsies after high altitude exposure than before.

Lipofuscin accumulation. The accumulation of lipofuscin is one of the most widespread age-dependent cytological alterations in a variety of long-lived (post-mitotic) cells, such as neurons, liver cells, myocardial cells and striated muscle fibers[2,7,20,23,25,30]. Some of the investigations reported have shown that lipofuscin accumulation occurs in individuals with a chronic wasting disease, in functionally overstressed tissue (e.g. cardiac hypertrophy) and in organs with chronic toxic alterations (e.g. phenacetin abuse, malnutrition). However, lipofuscin accumulation concomitant with muscle fiber degeneration, as seen with chronic hypoxia exposure in humans, has so far not been documented.

In ultrathin sections, lipofuscin granules appear as rounded or irregularly shaped bodies. These consist mainly of highly electron-dense material of a granular nature, and usually also contain one or more medium-density or lucent lipid droplets (fig. 2a and b)[26]. On rare occasions recognizable remnants of organelles and electron-dense membranous material may be discerned, which supports the view that lipofuscin granules belong to the category of lysosomal bodies[5,8,30]. This is also indicated by studies, demonstrating the presence of lysosomal enzymes[8,19]. The current view is therefore that lipofuscin represents the accumulation of indigestible material in lysosomes after autophagy. This ingested material may accumulate when the rate of autophagy exceeds the capacity for digestion or when the membranous material has been chemically altered, for example by lipid peroxidation[2,5], which could render it more difficult or even impossible to degrade. The lipofuscin granules include some 60% of protein as a matrix for the oxidized lipid polymers. They also contain copper and iron ions, as decomposition products of enzymes of oxidative metabolism. These substances remain undigested as pigmented granules in cells[21].

In the present study the absolute volume of lipofuscin inclusions in muscle cells increased significantly, by about three-fold. The material mainly appeared close to the sarcolemma near the poles of the myonuclei. Hoppeler et al.[13] found a marked decrease in mitochondrial volume after a prolonged sojourn at high altitude. Since it appears that lipofuscin may originate from cellular organelles such as mitochondria[5,8,30], a relationship between these findings could be postulated. An observation supporting this hypothesis is that the loss in mitochondrial volume is mainly due to the reduction of subsarcolemmal mitochondria which also occurs at the location where the lipofuscin is preferentially found[13].

The group of subjects participating in the expedition was heterogeneous, comprising experienced mountaineers with previous climbing experience in the Himalayas and less experienced mountaineers on their first expedition. We did notice that some of the muscle samples from the experienced climbers contained more lipofuscin before the expedition than did samples from inexperienced members upon return from the expedition. These findings are compatible with the idea that lipofuscin, once accumulated, remains in the muscle fibers. However, the number of subjects analyzed in these subgroups were too small and the experimental errors too large for these relationships to be statistically significant.

Satellite cells, myonuclei and myoblasts. The second aim of our study was to look for signs of muscle fiber regeneration. Satellite cells are known to be a population of dormant stem cells endowing muscle fibers with the potential for regeneration, which cannot be supplied by the post-mitotic myonuclei [6,16,17]. The fusiform satellite cells are wedged between the basement membrane and the plasma membrane of cross-striated skeletal muscle fibers [10,16]. It is believed that the nuclei of these cells account for 4–10% of the cell nuclei present in muscle fibers[18,22]. In order to safely distinguish them from 'ordinary' myonuclei it is necessary to use the resolution

afforded by the electron microscope. Muscle fiber hyperplasia and hypertrophy have been related to the activation of satellite cells[1]. Satellite cell activation has also been demonstrated in ischemia-induced damage in rat extensor digitorum longus muscle[15].

Satellite cells underwent a significant, close to three-fold increase in volume density with similar proportional increases in the volume of cytoplasm and nuclei (only the former increase reached the level of statistical significance). An unbiased estimate of the number of satellite cells could not be carried out, owing to technical problems discussed in the methods section. In order to understand better the role played by satellite cells in muscle regeneration, it would seem worthwhile to develop techniques which would enable one to obtain reliable and unbiased satellite cell counts.

The present analysis does not allow us to draw any conclusions as to the time-course of the regenerative events. It remains to be tested whether the satellite cell activation already occurred during the stay in the Himalayas or only after return of the subjects to near sea-level conditions. The volume density and the absolute volume of myonuclei remained unchanged after high altitude exposure. Myoblasts proved to be much too rare for quantitation; they were only occasionally seen either in pre- or in post-expedition biopsies (fig. 2d).

In conclusion, the morphometric analysis of human muscle biopsy samples before and after an expedition to Mt. Everest showed a striking three-fold increase in lipofuscin inclusions in muscle fibers, thought to be a consequence of degenerative events in the muscle. It is unclear whether muscle deterioration occurred because of malnutrition, or exercise under conditions of hypoxia, or as a result of reduced protein synthesis during the extreme environmental stress. Muscle regenerative events were indicated by an almost three-fold increase in satellite cell volume, while myonuclei and myoblasts remained unchanged with high altitude exposure.

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