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Three-dimensional analysis of the arrangement and length distribution of fascicles in the triceps muscle of *Galea musteloides* (Rodentia, Cavimorpha)

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Abstract Non-uniformity of fascicle parameters (fascicle lengths and orientation) within one skeletal muscle is well known. These parameters have an effect on the physiological cross-sectional area and lengthening rate of the skeletal muscle. Using a binocular microscope with a table driver (q - and p -axes) and vertical drive (v -axis) as a tool for reconstruction of the spatial orientation of single muscle fascicles, we developed an approach for three-dimensional analysis of the arrangement and length distribution in the skeletal muscle of small mammals. Two subunits of the triceps brachii muscle of the *Galea musteloides* forelimb, triceps longum and triceps laterale, were quantified and compared. Our data show that in the triceps laterale the fascicles are significantly longer (10.23 mm, SD=1.19, $n=41$) than those in the triceps longum (6.58 mm, SD=2.88, $n=39$). In the triceps laterale, the fascicle orientation is more or less uniform, whereas, in the triceps longum, there are two areas with different orientation of fascicles: anterior and posterior ones. Different inner architecture of the subunits can be interpreted as an adaptation to the main locomotory function of the triceps muscle, namely production of propulsive force during limb transfer phase and keeping dynamic stability during stance phase. Comparison of our data on the fascicle length and geometry with our previous histochemical results on *G. musteloides*, shows that the anterior region of the triceps longum, which differs in the fascicle orientation, also contains a significantly larger percent of slow muscle fibres. It is hypothesised here that this small region is involved in keeping posture.

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A. Introduction

Intramuscular mechanics is complex and characterised by a variety of parameters, such as muscle mass, fascicle density, pennation angle, fascicle length, connective tissue properties and assumed activation levels (Spoor et al. 1989). It is generally accepted that architectural features differ across the muscles of the same functional group (Legreneur et al. 1997). Most features may have intramuscular differences, so-called inhomogeneities (van Eijden et al. 1997). Inhomogeneities in the fascicle length may effect work, force and lengthening rates (Jacobson et al. 1992; van Eijden et al. 1997). Local differences in the pennation angle influence lengthening rate (Garrett et al. 1988), operative joint angle range, force-length relationship (Savelberg and Schamhardt 1995), prevent compression of nerves and vessels, increase force exertion by packing in a larger number of fascicles (Alexander and McNeill 1992) and, together with tendon elasticity, reduce fascicle length variation (Legreneur et al. 1997).

Most previous studies on muscle architecture have been based on two-dimensional measurements. Further works dealing with modelling of physiological parameters of muscle also used previous two-dimensional measurements (see, for example, Gans and Bock 1965). However, if one has a good architectural model of a skeletal muscle at hand, it is somewhat easier to calculate its functional properties (for example, cross-sectional area) from its architecture rather than directly measuring forces.

In mammals, the triceps brachii muscle is mainly responsible for the dynamic joint stabilisation, which is a key factor in therian locomotion (Fischer 1999). As the main propulsion of the forelimb is caused by scapular movements, the main task of the elbow and shoulder joints is fine tuning the height of the scapular fulcrum and herewith the body's centre of gravity. Fine adjustments are achieved by biarticular muscles known as extensors but used mainly to counteract gravity (cf. Jacobs et al. 1993; van Ingen Schenau et al. 1995). The prevail-

ing task of a bi- and monoarticular extensor such as the triceps brachii muscle is to prevent flexion, for example, in the elbow joint during stance (Goslow et al. 1981; Fischer 1994, 1998); then the elbow joint can act as damping element in a multiarticular chain (Pandy et al. 1988).

This study was undertaken to clarify whether these anti-gravity muscles (or anti-flexors) bear particular structural properties. Previous histochemical data on the muscle triceps brachii of *Galea musteloides* has revealed a distinct oxidative region (slow twitch type I fibres) within a matrix of glycolytic fibres (type IIa and IIb; von Mering and Fischer 1999). It is well known that the fibre composition of these anti-gravity muscles (for example, *M. supraspinatus*, *M. triceps brachii*, *M. triceps surae*) is different from other limb muscles [for example opossum *Didelphis virginiana* Kerr, 1792: see Hansen et al. 1987, mouse *Mus musculus* Linnaeus, 1758: see Brasseur et al. 1987, rat *Rattus norvegicus* (Berkenhout, 1769): see Armstrong and Phelbs 1984, rabbit *Oryctolagus cuniculus* (Linnaeus, 1758): see Lexell et al. 1994, cat *Felis catus* Linnaeus, 1758: see Collatos et al. 1977, cercopithecoid monkeys: see Jouffroy and Médina 1996]. The oxidative region can become segregated as a muscular entity described as a distinct head (*M. triceps brachii caput mediale*) or named muscle (for example *M. soleus*).

The concentration of oxidative fibres in distinct regions has been interpreted as morphological adaptation to improve muscle efficiency and to affect the direction of the muscle force (von Mering and Fischer 1999). But, whether different regions of this muscle have differences in fascicle orientation and fascicle length remained unknown.

The main goal of this study is to quantify inhomogeneities in the fascicle length and fascicle spatial orientation, which are important parameters for understanding mechanics of the skeletal muscle. For this purpose, a three-dimensional approach was developed to evaluate fascicle orientation and length distribution. Two muscles of the forelimb (triceps longum and triceps laterale) of the cui *Galea musteloides* Meyen, 1833 (Rodentia, Caviidae) were quantified and compared.

B. Materials and methods

I. Animals

Adult individuals of *G. musteloides* were obtained from our own laboratory. Immediately after killing, the skin and superficial fat were removed. Forelimbs were dissected from the trunk together with the clavicle by cutting the muscular connections. Forelimbs were fixed in alcoholic Bouin's solution for 48 h and washed in 70° ethanol. This fixative causes very few shrinking artefacts of muscle tissues, if compared to other fixatives. The limbs were firmly connected to the glass slides using thin copper wire.

II. Measurements

The idea to use a stereomicroscope with a table driver (q - and p -axes) and vertical drive (v -axis) originates from works on insect

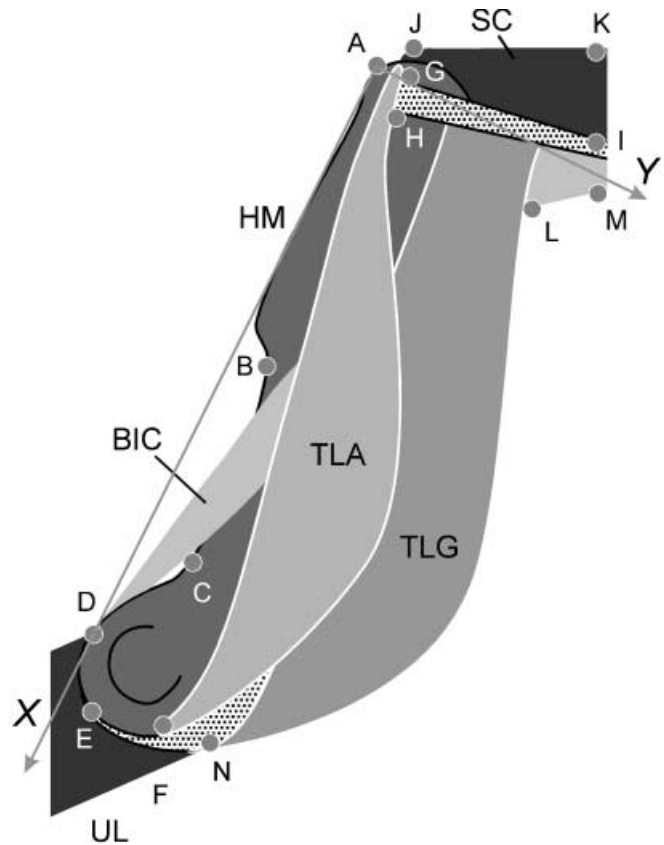


Fig. 1 Scheme of the left forelimb of *Galea musteloides*, lateral aspect, arrangement of points digitised. Some muscles are removed. *BIC* Biceps, *HM* humerus, *SC* scapula, *TIS* tendon of the muscle infraspinatus, *TLA* triceps brachii caput laterale, *TLG* triceps brachii caput longum, *X, Y, Z* spatial axes of the forelimb-fixed co-ordinate system, *A–N* reference points

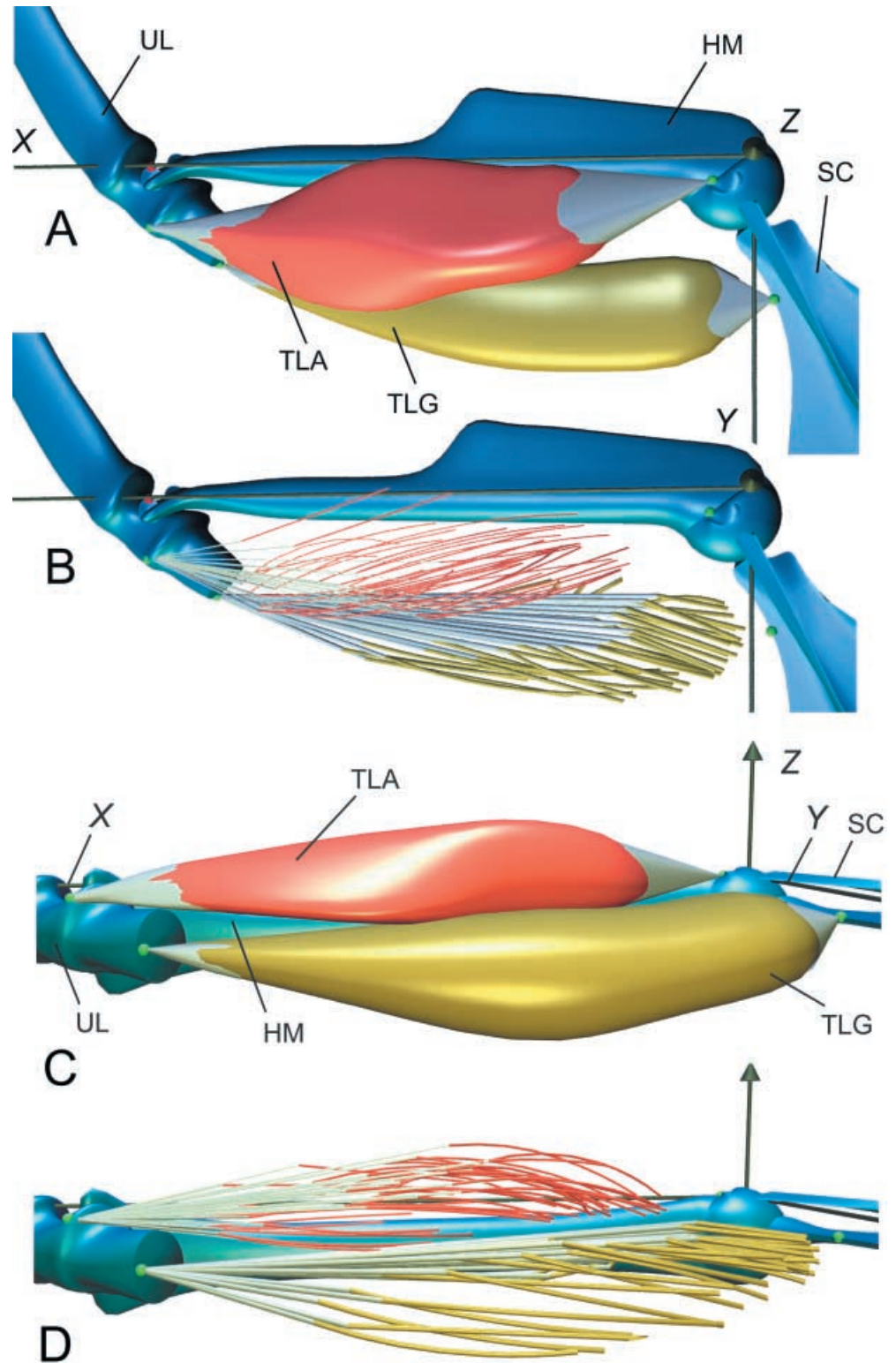
leg design (Gorb 1995; Frantsevich et al. 1996) and on fish myomeres (van Leeuwen 1999). Selected points on the bones and muscle fascicle points were observed under a binocular microscope with crossed hairs in one ocular; the positions of these points were read from the two scales of the table driver and the third scale on the vertical drive with an accuracy of 0.1 mm.

III. Data processing

A computer program [Studio 8.0 for Silicon-Graphics Workstations (Alias-Wavefront)] transformed digitised points from the microscope-fixed system to the limb-fixed one. Three-dimensional orientation of muscle fascicles is described in the forelimb-fixed co-ordinate system. Axes of this system were defined by three reference points: A, D and G (Fig. 1). The origin of the co-ordinate system was the point A. The longitudinal axis *X* runs through A and D, positive to the distal direction. The transverse axis *Y* runs through A parallel to the line, which is running through G perpendicular to the *X* axis, positive to the caudal direction. The vertical axis *Z* is perpendicular to both axes *X* and *Y*, positive to the lateral direction.

The basic assumption is that each fascicle of the muscle begins on one tendon and runs to other tendon. Spatial orientations of fascicles were expressed as the triplet of directing cosines (or angles) of the line connecting initial and end points. The directing cosine is a cosine of an angle between the line, that connects origin and insertion points of each fascicle, and each axis of the limb-fixed co-ordinate system. Using data on the three points for each fasci-

Fig. 2A–D Three-dimensional reconstruction of the fascicles arrangement in triceps brachii caput laterale (*TLA*) and in triceps brachii caput longum (*TLG*). **A, B** Lateral aspect. **C, D** Posterior aspect. *HM* Humerus, *SC* scapula, *UL* ulna; *X, Y, Z* spatial axes of the fore-limb-fixed co-ordinate system. **A, C** Topography of the whole muscles. **B, D** Arrangement of selected fascicles



cle (the beginning, end and approximate middle points), the lengths of fascicle arcs were calculated.

IV. Three-dimensional visualisation

Data obtained from measurements were visualised using computer program Studio 8.0 for Silicon-Graphics Workstations (Alias-

Wavefront; for details see Haas and Fischer 1997). Three-dimensional models were compared with those obtained from previous histochemical studies on the same muscles in *G. musteloides* (von Mering and Fischer 1999).

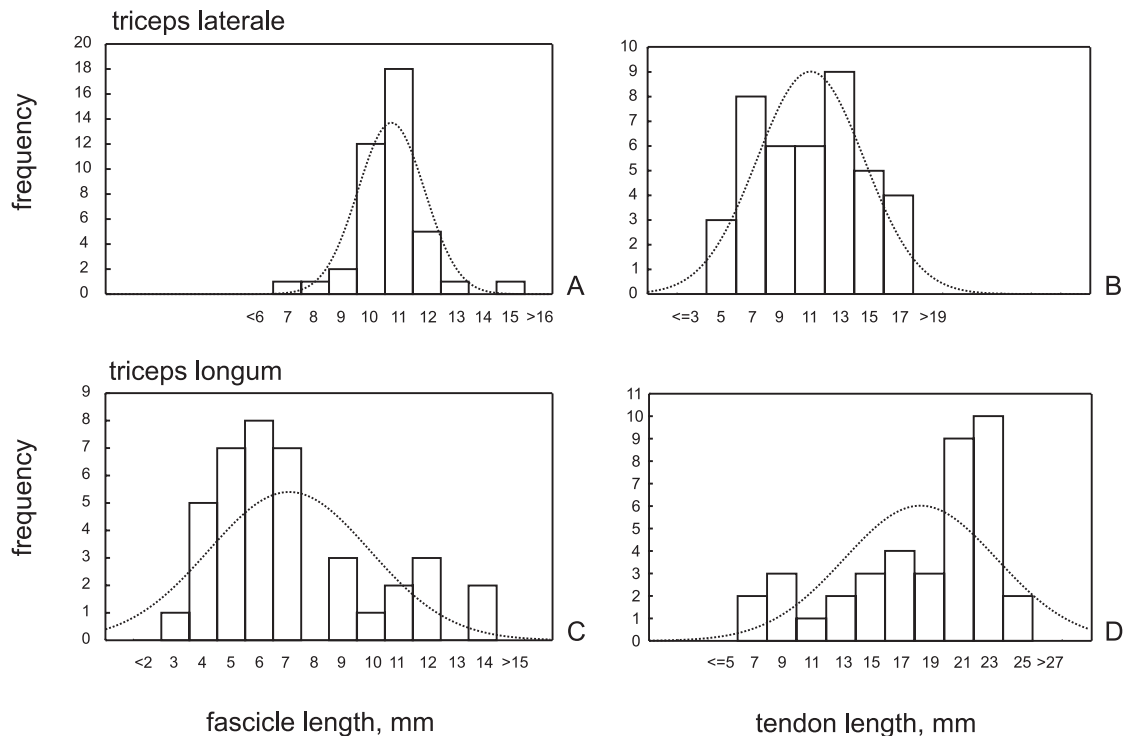
C. Results

The *M. triceps brachii* in *G. musteloides* consists of the massive caput longum (TLG), a smaller caput laterale (TLA) and the short caput mediale (TMD). The triceps tendon arises as an internal tendinous sheet at the latero-humeral side of the caput longum. The long head of the *m. triceps brachii* arises from the margo caudalis of the scapula, then runs along the humerus down to the elbow joint, where it attaches, together with the capita laterale et mediale, to the olecranon of the ulna. Three-dimensional models of the forelimb together with the fascicle orientation are given in Fig. 2 at different aspects.

I. Fascicle and tendon lengths

In the triceps laterale, the fascicles are significantly longer (10.23 mm, $SD=1.19$, $n=41$) than those in the triceps longum (6.58 mm, $SD=2.88$, $n=39$) (Figs. 3, 4). Lengths of TLA fascicles are also not as widely distributed as those of the TLG. Since the overall lengths of both muscles are comparable, one can expect a longer tendinous part in the TLG fascicles. Indeed the tendinous parts of the TLA fascicles are significantly shorter (11.05 mm, $SD=3.63$, $n=41$) than those in the TLG (18.4 mm, $SD=5.17$, $n=39$) (Fig. 4). Also the length variability in the TLG tendons was somewhat higher. In the TLG close

Fig. 3 Frequency histograms of the lengths of fascicles (A, C) and tendons (B, D) in triceps brachii caput laterale (A, B) and in triceps brachii caput longum (C, D). In the triceps longum, fascicles of the anterior area have been not taken into account



to the humerus, there is an area containing very short fascicles. These fascicles also have different orientations from other fascicles (see below).

However, the fascicles with different lengths are not randomly distributed over the muscle. Fascicles and tendon lengths depend on their location within the muscle. In the triceps laterale, the somewhat shorter fascicles with shorter tendons are located more distally (higher X). In the triceps longum, the fascicles are somewhat longer and tendons shorter along this axis. In the triceps laterale, the shorter fascicles and shorter tendons are located more posteriorly (higher Y). In the triceps longum, the fascicles are somewhat longer and tendons shorter along this axis. The lengths also differed in fascicles located at different depths in the muscle (Z -dependence). In triceps laterale, deeper-located fascicles and their tendons were somewhat shorter. In triceps longum, deeper-located fascicles are longer and their tendons are shorter (Fig. 5). As one may expect, the fascicle length is a well-correlated parameter with length of the tendinous part of the fascicle.

II. Fascicle orientation

Frequency distribution of directional cosines of digitised fascicles are given in Fig. 6. The mean vectors of fascicle direction in the forelimb-fixed co-ordinate system were calculated for the two muscles studied. For the TLA, they were $\cos(x)=0.965$, $SD=0.013$; $\cos(y)=0.227$, $SD=0.047$; $\cos(z)=0.105$, $SD=0.071$, $n=41$. For the TLG they were $\cos(x)=0.953$, $SD=0.029$; $\cos(y)=-0.150$, $SD=0.22$; $\cos(z)=0.070$, $SD=0.135$, $n=39$.

In the triceps laterale, the fascicle orientation is more or less uniform, especially along the X -axis (Fig. 6). In the

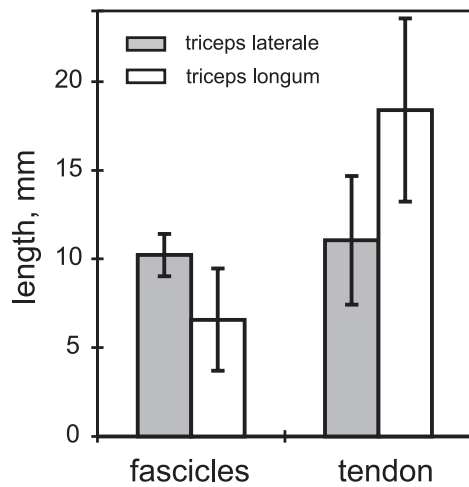


Fig. 4 Lengths of fascicles and their tendons in the two muscles studied. Means and standard deviation are provided

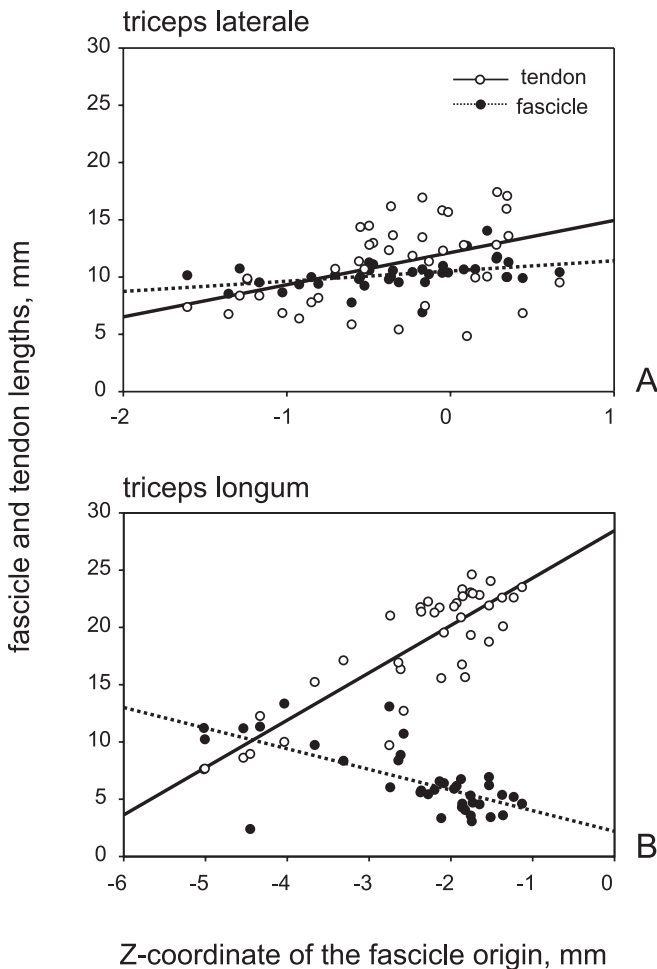


Fig. 5 Dependence of the fascicle and tendon lengths on the fascicle location in muscles triceps brachii caput laterale (A) and triceps brachii caput longum (B)

triceps longum, there are two areas with different orientation of fascicles (Figs. 2, 6): anterior area [$\cos(x)=0.914$, $SD=0.049$; $\cos(y)=0.316$, $SD=0.146$; $\cos(z)=-0.200$, $SD=0.103$] and posterior area [$\cos(x)=0.960$, $SD=0.016$; $\cos(y)=-0.240$, $SD=0.076$; $\cos(z)=-0.118$, $SD=0.065$]. The anterior area is composed of short fascicles terminating on the tendon (see above).

If we pool all cosine (or angle) data together and compare them, it seems that fascicle orientation in both muscles is quite similar along both X- and Z-axes, but significantly differ along the Y-axis (Fig. 7). Since the Y-axis corresponds approximately to the morphological longitudinal body axis, the main difference in direction of produced force by both muscles would be also expected along this axis.

D. Discussion

The variability of the fascicle length and pennation angle within a muscle (Scott et al. 1993; Zuurbier and Huijig 1993) and between muscles (Lieber et al. 1990) have been shown already by other authors. This study is a part of the forelimb dynamics project integrating data from kinematics (Fischer 1994, 1998; Fischer and Lehmann 1998; Schilling and Fischer 1999; Schmidt and Fischer 2000), inverse dynamics (Biltzinger and Fischer 1998; Witte et al. submitted for publication), muscle histochemistry (von Mering and Fischer 1999), muscle activation pattern (Schumann et al. 1998; Fischer 1999) and muscle architecture (Gorb and Fischer 1999). Our results are the first three-dimensional quantitative data set obtained from the forelimb skeletal muscle in a small mammal.

Our data show that two heads of the muscle triceps brachii have different inner architecture, which can be interpreted as an adaptation to the main locomotory function of the triceps muscle, namely production of propulsive force during limb transfer phase and keeping dynamic stability during stance phase. Electrophysiological data show the highest activity of these muscles at touch down when non-anticipated irregularities on the ground may cause trouble (Fischer 1999). It has been previously shown that such locomotory-relevant muscles such as medial gastrocnemius of the hindlimb of the cat *F. catus* have compartments with two pennation patterns (Gareis et al. 1992). Our study also revealed the presence of two regions with different fascicle size and orientation in the TLG. Additionally, the TLG and TLA parts of the triceps have different fascicle arrangements along the longitudinal body axis. Presumably, this may effect on the force tuning during touch down.

Previous three-dimensional histochemical analysis (von Mering and Fischer 1999) has revealed very little regionalisation in the lateral head of the triceps muscle. The long head, however, shows clear regions with different fibre patterns. This head consists mainly of the fatigable type II fibres. A higher proportion of oxidative type I fibres has been revealed only in the very deep ar-

Fig. 6 Frequency histograms of directional cosines of fascicles in the two muscles studied

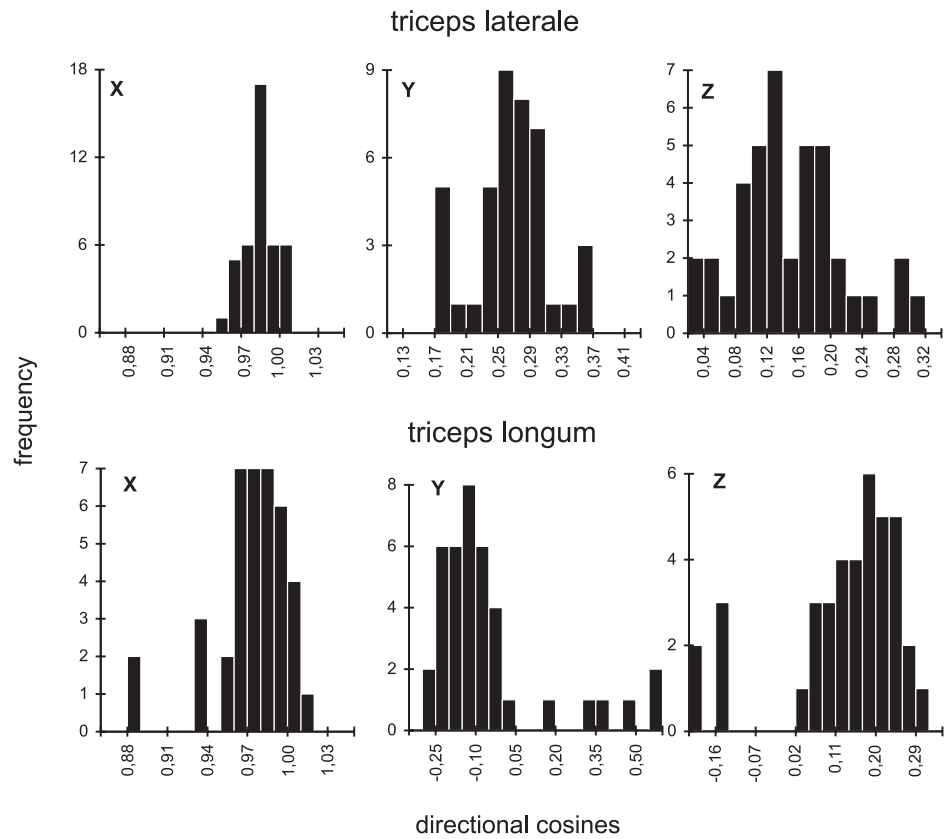
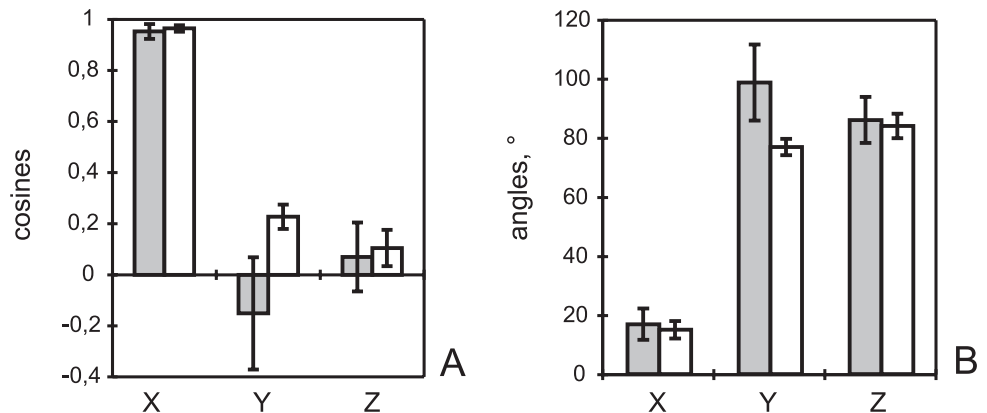


Fig. 7 Cosines (A) and angles (B) between fascicles and three axes of the forelimb-fixed coordinate system in the two muscles studied. Means and standard deviation are provided



eas of the muscle (von Mering and Fischer 1999). Close to the scapula and humerus, type I fibres aggregate into a tiny oxidative region and insert on the intramuscular tendinous sheet, which is distally transformed into the triceps tendon. Comparison of our data on the fascicle length and geometry with previous histochemical results on *G. musteloides* (see von Mering and Fischer 1999) shows that the anterior region of the TLG, which differs in the fascicle orientation and length, also contains a significantly larger percent of sustainable type I muscle fibres. Presumably, this small region is involved in keeping posture.

Knowing that stress limits for vertebrate muscles are about 30 (Alexander and McNeill 1992)–50 $N \cdot cm^{-2}$

(MacGill et al. 1988), our data may be later used for further calculations of the maximal force potentially produced by the whole muscle or by one particular muscle region. However, it is suggested here that use of the physiological cross-sectional area for calculation of force production is not exact, because it is impossible to obtain a cross-section of any fascicle of the muscle in one particular plane. Additionally, a physiological cross-sectional area does not take into account fascicle length, the essential parameter for the evaluation of force production.

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