Increased capillarity in leg muscle of finches living at altitude

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Increased capillarity in leg muscle of finches living at altitude. J. Appl. Physiol. 85(5): 1871–1876, 1998.—An increased ratio of muscle capillary to fiber number (capillary/fiber number) at altitude has been found in only a few investigations. The highly aerobic pectoralis muscle of finches living at 4,000-m altitude (Leucosticte arctoa; A) was recently shown to have a larger capillary/fiber number and greater contribution of tortuosity and branching to total capillary length than sea-level finches (Carpodacus mexicanus; SL) of the same subfamily (O. Mathieu-Costello, P. J. Agey, L. Wu, J. M. Szwczak, and R. E. MacMillen. Respir. Physiol. 111: 189–199, 1998). To evaluate the role of muscle aerobic capacity on this trait, we examined the less-aerobic leg muscle (deep portion of anterior thigh) in the same birds. We found that, similar to pectoralis, the leg muscle in A finches had a greater capillary/fiber number (1.42 ± 0.06) than that in SL finches (0.77 ± 0.05; P < 0.01), but capillary tortuosity and branching were not different. Also found in pectoralis, the resulting larger capillary/fiber surface in A finches was proportional to a greater mitochondrial volume per micrometer of fiber length compared with that in SL finches. These observations, in conjunction with a trend to a greater (rather than smaller) fiber cross-sectional area in A than in SL finches (A: 484 ± 42; SL: 390 ± 26 µm²; both values at 2.5-μm sarcomere length; P = 0.093), support the notion that chronic hypoxia is also a condition in which capillary-to-fiber structure is organized to match the size of the muscle capillary-to-fiber interface to fiber mitochondrial volume rather than to minimize intercapillary O₂ diffusion distances.

capillary anisotropy; mitochondria; hypoxia; capillarization; bird

AN INCREASE IN THE SIZE of the muscle capillary-to-fiber interface is thought to increase the O₂ conductance into muscle fibers (10), an adaptation that would be favorable for O₂ transport in hypoxia. Previous work has shown that chronic hypoxic exposure alone does not result in a change in either capillary-per-fiber number (28) or capillary geometry (15, 26) in limb muscles of mammals. However, there have been so far three reports of increased muscle capillary-per-fiber number in birds at altitude. Léon-Velarde et al. (12) found a greater capillary-per-fiber number in pectoralis and some limb muscles of Andean coots living at 4,200 m than in birds of the same species nesting at sea level. A greater capillary-per-number and altered geometry have recently been found in the flight muscle of both lowland pigeons kept at 3,800 m for 5 mo (18) and finches living and flying at 4,000-m altitude (Leucosticte arctoa; A) than in sea-level finches (Carpodacus mexicanus; SL) (20). One possible explanation for the changes in both capillary number and geometry in bird flight muscle was its high aerobic capacity, suggesting that capillary neogenesis at altitude may require a high O₂ demand. The primary objective of this investigation was to determine whether less aerobic leg muscle in the A finches demonstrated similar adaptations in fiber capillarization as the highly aerobic flight muscle (20), compared with the same leg muscles in SL finches. Specifically, we examined whether the size of the capillary-to-fiber interface was increased in leg muscle of A finches and, if so, what was the role of changes in capillary-per-fiber number, capillary geometry, and fiber size in this response. Furthermore, we wanted to determine whether the relationship between capillary-to-fiber surface and fiber mitochondrial volume in leg muscle would be altered at altitude.

METHODS

We have recently published data on the capillary-to-fiber geometry of the pectoralis muscle in SL and A finches (20). In this paper, we present data for capillary-to-fiber geometry and fiber ultrastructure of the leg muscle (deep portion of anterior thigh) of the same animals. The materials and methods are the same as those described in the previous paper, except for the muscle sampling of the leg, and are briefly described below.

Five house finches, C. mexicanus (2 adult females, body mass 18.2 and 21.8 g; and 3 subadults, body mass 18.3–22 g), and five gray-crowned rosy finches, L. arctoa (4 subadults, body mass 21.6–24.0 g; and 1 adult female, body mass 24.5 g), were used, with adult and subadult birds identified as described elsewhere (20). Both species belong to the same subfamily (Passeriformes: Fringillidae: Carduelinae) and demonstrate similar size and morphology as well as similar respiratory and metabolic responses to altitude or low temperature at rest, despite the large difference in altitude of their habitats (4).

Tissue preparation. All birds were collected in September, under scientific collector's permits issued by the US Forest Service, the US Fish and Wildlife Service, and the California Department of Fish and Game. House finches were caught by bait trap in Orange County, CA. Rosy finches were caught via mist nets in permanent snow fields at an elevation of 4,000 m in the White Mountains of Eastern California (Inyo County). All birds were anesthetized via intravenous injection of pentobarbital sodium (1–4 mg/100 g) within 24 h of capture, and the muscles were perfusion fixed in situ at a nonpulsatile pressure of 150–170 mmHg at the University of...
California, San Diego (house finches) and the Barcroft Laboratory of the University of California White Mountain Research Station (rosy finches), as detailed elsewhere (20). As in other studies of birds (18), this perfusion pressure is higher than that used in mammals (80–100 mmHg; Ref. 14) to match the higher blood pressure found in birds (30). Muscle samples (~7 × 4 × 1 mm) were obtained from the midbelly of the deep portion of the anterior thigh (femorotibialis medius muscle, which is of mixed fiber-type composition in other birds (31)]. All muscle samples were cut into thin longitudinal strips, stored in glutaraldehyde fixative (total osmolality: 1,100 mosM; pH 7.4), and processed for electron microscopy, as described previously (14).

Eight blocks from each muscle were cut into four transverse and four longitudinal 1-µm-thick sections with an LKB Ultratome III (14). All sections were stained with uranyl acetate and bismuth subnitrate. Electron micrographs were taken for morphometry on 70-mm films with a Zeiss 10 electron microscope.

Morphometry of capillaries in each fiber sectional area (i.e., capillary densities) were obtained by morphometry on transverse [Q(a)(0)] and longitudinal [Q(a)(π/2)] sections by using a 100-point eyepiece square-grid test at a magnification of ×400 on a light microscope, with muscle fibers as the reference space to avoid errors introduced by unreliable preservation of the intercellular spaces (14). An average of 13 ± 2 (SE) fields/sample in transverse sections and 38 ± 7 fields/sample in longitudinal sections were measured.

The capillary anisotropy coefficient [c(K,0)] was estimated as described previously (13), and capillary length per fiber volume [l(c,f)] was calculated as the product of Q(a)(0) and c(K,0). Capillary diameter [d(c), number of capillaries around a fiber (N_CAF), fiber cross-sectional area [a(f)], and fiber cross-sectional perimeter [b(f)] were measured by using an image analyzer (Videometric 150, American Innovision) on the same transverse sections used to estimate Q(a)(0). An average of 171 ± 23 fibers, randomly selected by systematic sampling, were measured per sample. The c(c) was determined as the shorter axis of capillary sections that were close to circular (~20% difference between shorter and longer diameters), which assumes capillary cross-sectional circularity, as suggested previously in bird (16) and rat muscles (14). An average of 165 ± 11 capillary profiles/sample were measured.

Capillary density and b(f) were normalized to a(i), 2.5 µm, because this value fell within the range found in this investigation (2.31–2.61 µm). Capillary-per-fiber number ratio [N_CAF(c,f)] was calculated as the product of Q(a)(0) and c(f). Capillary-to-fiber perimeter ratio [b(f)(0)] was estimated in transverse sections at a magnification of ×1,000 by using a 100-point square-grid test eyewitness system. On average, 24 ± 2 fields/sample were examined. Because c(K,0) was ~1.53 for all muscles, b(f)(0) was a direct estimate of capillary surface per fiber surface [S_v(c,f)] (21). Capillary surface per fiber volume [S_v(c,f)] was estimated by using an O (6 × 6) cycloid eyepiece test system (Cruz-Orive, Univ. of Bern, Switzerland) on vertical (i.e., longitudinal) sections, with the use of the same longitudinal sections used to estimate Q(a)(π/2). An average of 76 ± 1 fields/sample was measured at ×1,000 magnification. To test for internal consistency of the measurements, S_v(c,f) was also calculated from b(f)(0), i.e., S_v(c,f) = b(f)(0)·b(f)(a(f)) and c(f)·c(f). Mean capillary perimeter in muscle transverse sections [b(c)] was estimated by two independent methods: one making no assumptions about capillary shape, i.e., b(c) = b(f)(0)·b(f)(a(f)) and c(f)·c(f), and one assuming circular capillary cross sections, i.e., b(c) = π·c(K,0)·c(K,0)·d(c)·d(c). A significant difference between b(c) and c(f) would indicate noncircularity of the capillary cross sections (20).

The volume density of mitochondria, myofibrils, and lipid droplets per volume of muscle fiber was estimated by point-counting on 40 fields/sample (20 fields on each of two blocks/sample) by using electron micrographs of ultrathin transverse sections projected on a 144-point square-grid test of a microfilm reader (Documate DL 2, Jenoptik, Jena, Germany). Mitochondrial volume per micrometer of fiber length [V_Mt(f,t)] was calculated as the product of mitochondrial volume density [V_Mt(f,t)] and b(f).

Statistics. Data are means ± SE. Group means were compared by unpaired Student’s t-test. Repeated-measures ANOVA and paired Student’s t-test were used to compare estimates of S_v(c,f) and c(f), respectively, obtained in the same samples via different methods. Body mass was compared between groups by using a two-way ANOVA to account for the effects of maturational level (i.e., subadult or adult) on this variable. Linear-regression analysis was used to interpolate [c(f)] at a similar [V_Mt(f,t)] (i.e., 8%) in each group. A two-way ANOVA was subsequently used to compare [V_Mt(f,t)] between A and SL finches as a function of sampling site (i.e., leg muscle (this study)) and data in pectoralis muscle of the same birds (20).

RESULTS

Body mass and fiber size. As previously reported, body mass was significantly greater in A (23.1 ± 0.5 g) than in SL birds (20.0 ± 0.8 g; P < 0.05), with no systematic difference between subadult and adult in either group (P = 0.50). Whereas b(f) in transverse sections normalized to 2.5-µm a(i) was significantly larger in A (97 ± 4 µm) than in SL birds (85 ± 3 µm; P < 0.05), muscle a(f) at 2.5-µm a(i) only demonstrated a trend to being larger in A finches (P = 0.09). Fiber shape factor, i.e., shape factor = 4·π·a(f)(b(f))(2), was not different between A (0.64 ± 0.02) and SL birds (0.68 ± 0.01; P = 0.14).

Capillary number, surface, and geometry. Figure 1 shows light micrographs of transverse and longitudinal sections obtained from the leg in A and SL finches. Q(a)(0) normalized to 2.5-µm a(i) (Table 1) was significantly greater in A (2,990 ± 208 capillaries/mm²) than in SL finches (1,989 ± 121 capillaries/mm²; P < 0.01). This was because of the greater number of capillaries per fiber, i.e., 56% greater N_CAF in A (4.2 ± 0.1) than in SL finches (2.7 ± 0.1; P < 0.01), and 84% greater N_CAF in A (1.42 ± 0.06) than in SL birds (0.77 ± 0.05; P < 0.01). The greater increase in N_CAF in A finches resulted in a 15% reduction of the capillary sharing factor, i.e., number of fibers sharing a capillary = N_CAF/N_CAF(c,f) (24). This corresponds to a greater proportion of capillaries being shared by three rather than four fibers. There was no difference in c(f) between the muscles examined, nor was there a difference in c(K,0) between A and SL birds (Fig. 2). For comparison, the relationships between c(K,0) and a(i) in leg of mammal (rat soleus) and pectoralis of bird (pigeon) are also shown in Fig. 2. The 55% greater J v(c,f) in A finches
was due entirely to the greater QA(0), with no difference in the contribution of capillary tortuosity and branching to capillary length. SV(c,f) measured with a cycloid grid was 32% larger in A than in SL finches, but the difference did not reach significance (P = 0.073). SS(c,f) was 41% greater in A than in SL finches (P = 0.01). The d(c) was not different between groups (P = 0.27). As found in other birds (18, 22), d(c) was smaller (range: 2.9–4.3 µm) than in mammals [e.g., rat, range: 4.3–6.1 µm (14)], consistent with the smaller short axis of red blood cells (RBC) in small birds (2, 8). Although RBC dimensions were not measured in this study, the RBC long axis averaged 11.5 ± 0.2 µm and the short axis 6.1 ± 0.2 µm in blood smears from house finches (4 females: 2 adults and 2 subadults) found in the same

| Table 1. Fiber size and capillarization in femorotibialis medius muscle of sea-level and altitude finches |
|-----------------------------------------------|----------------|----------------|----------------|
| Sarcomere length (l0), µm                     | SL             | A              | P Value       |
| Fiber Area [a(f)2.5]*, µm²                    | 2.46 ± 0.01    | 2.49 ± 0.06    | NS            |
| Perimeter [b(f)2.5]*, µm                      | 85 ± 3         | 97 ± 4         | <0.05         |
| Capillary Density [QA(0)2.5]*, capillaries/mm²| 1,989 ± 121    | 2,990 ± 208    | <0.01         |
| Anisotropy coefficient [c(K, 0)]              | 1.05 ± 0.01    | 1.08 ± 0.02    | NS            |
| Length/fiber volume [l√(c, f)], mm⁻²          | 2.063 ± 140    | 3,204 ± 206    | <0.01         |
| Diameter [d(c)], µm                           | 3.85 ± 0.19    | 3.52 ± 0.20    | NS            |
| Surface/fiber volume [Sv(c, f)], mm⁻¹         | 37.6 ± 2.2     | 49.7 ± 5.5     | NS (0.073)    |
| Number around a fiber [NCAF]                  | 2.7 ± 0.1      | 4.2 ± 0.1      | <0.01         |
| Per fiber ratio [NCAF], µm⁻¹                  | 0.77 ± 0.05    | 1.42 ± 0.06    | <0.01         |
| Sharing factor (SF)                           | 3.52 ± 0.12    | 2.98 ± 0.06    | <0.01         |
| Surface/fiber surface [SS(c, f)]              | 0.17 ± 0.01    | 0.24 ± 0.01    | <0.01         |

Values are means ± SE. SL, sea level; A, altitude. *Normalized to 2.5-µm sarcomere length.

(P < 0.01) was due entirely to the greater QA(0), with no difference in the contribution of capillary tortuosity and branching to capillary length. SV(c,f) measured with a cycloid grid was 32% larger in A than in SL finches, but the difference did not reach significance (P = 0.073). SS(c,f) was 41% greater in A than in SL finches (P < 0.01). The d(c) was not different between groups (P = 0.27). As found in other birds (18, 22), d(c) was smaller (range: 2.9–4.3 µm) than in mammals [e.g., rat, range: 4.3–6.1 µm (14)], consistent with the smaller short axis of red blood cells (RBC) in small birds (2, 8). Although RBC dimensions were not measured in this study, the RBC long axis averaged 11.5 ± 0.2 µm and the short axis 6.1 ± 0.2 µm in blood smears from house finches (4 females: 2 adults and 2 subadults) found in the same

Fig. 1. Light micrographs of portions of muscle bundles in transverse (A and C) and longitudinal (B and D) sections of leg muscle (femorotibialis medius) of altitude (A) (A and B) and sea-level finches (SL) (C and D). Capillaries appear as empty spaces after vascular perfusion fixation. Note higher capillary density in A (A and B) than in SL finches (C and D) at similar sarcomere lengths (2.43 and 2.45 µm, respectively). Bar, 20 µm.

Fig. 2. Plot of capillary orientation coefficient [c(K, 0)] against muscle sarcomere length in leg muscle of A and SL finches. Included for comparison are relationships shown previously for sea-level rat leg (21) and pigeon pectoralis muscles (16).
Table 2. Fiber ultrastructure in the femorotibialis mediobasal muscle of sea-level and altitude finches

<table>
<thead>
<tr>
<th></th>
<th>SL</th>
<th>A</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>Total mitochondrial volume density [V_v(mt, f)], %</td>
<td>7.1 ± 0.6</td>
<td>9.4 ± 0.4</td>
<td>&lt; 0.05</td>
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<tr>
<td>Subsarcolemmal mitochondrial volume density [V_v(ms, f)], %</td>
<td>2.7 ± 0.3</td>
<td>3.9 ± 0.3</td>
<td>&lt; 0.05</td>
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<tr>
<td>Intermyofibrillar mitochondrial volume density [V_v(mi, f)], %</td>
<td>4.4 ± 0.4</td>
<td>5.5 ± 0.2</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Lipid volume density [V_v(li, f)], %</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Myofibrillar volume density [V_v(my, f)], %</td>
<td>84.4 ± 0.2</td>
<td>85.1 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Mitochondrial volume per µm fiber length, [V_v(mt, f)2.5],* µm³</td>
<td>28.1 ± 3.4</td>
<td>45.9 ± 5.8</td>
<td>&lt; 0.05</td>
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Values are means ± SE. * Normalized to 2.5-µm sarcomere length.

area as the birds used in this study (unpublished observations).

Fiber ultrastructure. Table 2 presents data for fiber ultrastructure in the leg of A and SL finches. The 32% greater total V_v(mt, f) in A finches (P < 0.05) was proportionally distributed between subsarcolemmal and intermyofibrillar populations of mitochondria. V_v(mt, f)2.5, calculated as the product of V_v(mt, f) and a(f) normalized to 2.5-µm l_f, was significantly greater in A (45.9 ± 5.8 µm³) than in SL birds (28.1 ± 3.4 µm³; P < 0.05). To determine the relationship between S_2(c,f) and mitochondrial volume, we examined the quotient of S_2(c,f) and V_v(mt, f). In this ratio, V_v(mt, f) was not normalized to l_f because both S_2(c,f) and V_v(mt, f) were measured at the same l_f in each sampling site and, therefore, variability due to l_f canceled out. The ratio S_2(c,f)/V_v(mt, f) was similar in A (0.0056 ± 0.0007) and SL finches (0.0064 ± 0.0010; P = 0.55), indicating that the slope of this relationship was maintained at altitude compared with sea level.

Figure 3 shows the plot of J_v(c,f) against V_v(mt, f) in A and SL finches. For comparison, group mean values in mammals (23) and pectoralis muscle of the same birds (20) are also shown. For a given V_v(mt, f), J_v(c,f) in SL finch leg was not different from that in rat soleus, bat leg, or bat pectoralis (1-way ANOVA, P = 0.68). Thus they were added to the original data of that regression, yielding the regression equation, J_v(c,f) = [246 × V_v(mt, f)] + 234 (r = 0.991; P < 0.01). The use of that regression and comparison with data in pectoralis of the same birds (20) revealed a greater J_v(c,f) in A finch leg (2-way ANOVA, P < 0.01), independent of the muscle sampling site (i.e., pectoralis or leg). It yielded values for J_v(c,f) of 2.861 ± 243 and 2.275 ± 166 mm²⁻² for the leg in A and SL finches, respectively, at 8% V_v(mt, f).

Capillary shape. Capillary perimeter estimated with no assumption of shape, B(c)_f, was significantly greater than B(c)_b, which assumed circular capillary cross sections, in both A (16.8 ± 1.0 vs. 11.9 ± 0.6 µm; P < 0.05) and SL finches (19.2 ± 1.4 vs. 12.8 ± 0.6 µm; P < 0.01), indicating that capillary cross section was not circular in either group. Taking the quotient of B(c)_f/(K_0·c(K_0,K'_0)) and π·d(c) revealed that capillary cross-sectional perimeter was 42 ± 12 and 50 ± 6% greater than for circular capillary profiles in A and SL birds, respectively. There was no difference in either estimate of capillary cross-sectional perimeter between groups, consistent with the d(c) measurements made in transverse sections. Similarly, S_v(c,f) and S_v(c,f) measured without assumption of capillary shape, were not significantly different, indicating internal consistency of the data. In contrast, S_v(c,f) (i.e., using d(c)), was significantly smaller than both S_v(c,f) and S_v(c,f), confirming the noncircularity of capillary cross sections in both groups.

**DISCUSSION**

We report here for the first time a greater N_v(c,f) and fiber mitochondrial volume in the leg muscle of A compared with SL finches of the same subfamily (Carduelinae). In contrast to pectoralis muscle in the same birds (20), the greater N_v(c,f) in A finches was not associated with a greater contribution of tortuosity and branching to total capillary length at the l_f examined (range: 2.31–2.61 µm). The greater N_v(c,f) resulted in a larger S_2(c,f) (41%) in leg muscle of the A finch. This was accompanied by 63% greater fiber V_v(mt, f), due to both a greater V_v(mt, f) (32%) and a trend to larger muscle a(f) (24%; P = 0.09) in the A finch. As a result, S_v(c,f)/fiber V_v(mt, f) did not differ between A and SL finches. These findings are consistent with those in pectoralis muscle in the same birds (20) and support the notion that capillary-to-fiber structure is organized to match the size of the capillary-to-fiber interface to fiber mitochondrial volume rather than to minimize intercapillary O₂ diffusion distances in chronic hypoxia. Furthermore, the much lower J_v(c,f) (range: 1.703–3.584 mm²⁻²) and V_v(mt, f) (5.3–11.1%) in the femorotibialis mediobasal muscle compared with that in the pectoralis muscle (range: 5.584–12.009 mm²⁻² and 15.3–31.6%, respectively (20)) suggests that high capillarization and O₂ demand are not the sole factors determining capillary neogenesis at altitude.
Capillarization and muscle O\textsubscript{2} demand. Studies of adaptation to chronic hypoxia alone in mammals, including humans, have found no increase in N\textsubscript{N}(c,f) (7, 15, 28). However, Léon-Velarde et al. (12) reported for the first time greater N\textsubscript{N}(c,f) in altitude birds (Andean coots) compared with that in the same species nesting at sea level. In that study, capillary geometry was not examined, and no difference was found in either glycolytic or oxidative enzyme activity between altitude and sea-level coots.

Recent investigations of flight muscle in birds showed increased N\textsubscript{N}(c,f) and altered capillary geometry both in sea-level pigeons kept at altitude (3,800 m, inspired PO\textsubscript{2} = 91 Torr) for 5 mo (18) and in small birds (finches) living at 4,000 m (20). The much higher capillary density in flight muscle of pigeon (~3,000–6,000 mm\textsuperscript{-2}) and finch (~5,000–11,000 mm\textsuperscript{-2}), compared with that in limb muscle of mammals [e.g., rodents: ~1,400–3,000 mm\textsuperscript{-2} (14, 15)] and coot [500–1,700 mm\textsuperscript{-2} (12)], suggested that a high O\textsubscript{2} demand may be required to induce changes in capillary geometry at altitude. The results of this investigation confirmed this hypothesis. Whereas the increased N\textsubscript{N}(c,f) in leg muscle of the A finch, as found earlier in some leg muscles of coot (12), showed that the level of capillarization and O\textsubscript{2} demand are not the sole determining factors for capillary neogenesis, there was no change in capillary geometry in the leg (this study), unlike previous studies of more aerobic flight muscle in pigeon (18) or the same finches used in this study (20).

Effect of activity on capillarization. Previous investigations have shown that exercise training in hypoxia increases muscle N\textsubscript{N}(c,f) and mitochondrial enzyme activities in humans (5, 6) and rats (3). The A finches used in this study were observed to be flying uphill into strong descending head winds to reach permanent snow fields located in cirque basins on White Mountain, thus imposing high O\textsubscript{2} demands on the flight muscle and likely accounting for the greater N\textsubscript{N}(c,f) and altered geometry compared with SL finches (20). Interestingly, the leg muscle of the A finch also shows greater N\textsubscript{N}(c,f) and fiber mitochondrial volume than SL finch leg muscle, but, in contrast to their own (A finch) flight muscle, no alteration of capillary geometry. The change in geometry in flight muscle, reflecting a greater contribution of venular capillary branches to total capillary length, was thought to be due to the lower PO\textsubscript{2} (and thus greater stimulus for adaptation) in venular capillaries (20). Although the rosy (A) finches forage, while hopping, on insects thawing from snow fields in this cold microenvironment, the combined increased aerobic demands of hopping locomotion and elevated thermogenesis apparently are less than those imposed by flight in this hypoxic environment. These observations further suggest the existence of a rather remarkable degree of vascular plasticity that fits a given muscle system in a single individual to the aerobic requirements imposed on that system by the physical conditions of its environment. They also suggest that it is not the absolute O\textsubscript{2} demand per se but rather the proportion of available muscle aerobic capacity which is utilized for activity at altitude that stimulates capillary proliferation.

Factors affecting capillary and mitochondrial adaptations at altitude. Within a muscle, N\textsubscript{N}(c,f) is closely related to fiber size (29). Similarly, an increased N\textsubscript{N}(c,f) accompanying the increased fiber size that occurs with maturational growth (1, 28) or functional overload (25) has been well documented in mammals. Whereas we found a 14% greater S(f) and a trend toward a greater a(f) (24%; P = 0.093) in leg muscle of the A finch, N\textsubscript{N}(c,f) was 84% greater in A finches, strongly suggesting that the higher N\textsubscript{N}(c,f) was not simply a function of greater fiber size. Rather, S\textsubscript{S}(c,f) and fiber mitochondrial volume were proportionally greater in A finch leg muscle, supporting the suggestion that, like exercise training (27) and electrical stimulation (19), chronic hypoxia may be another condition in which S\textsubscript{S}(c,f) is regulated as a unique function of fiber mitochondrial volume or muscle O\textsubscript{2} demand (20).

Interestingly, when J\textsubscript{V(c,f)} was examined in relation to V\textsubscript{V(mt,f)}, we found that the A finch demonstrated greater J\textsubscript{V(c,f)} in both pectoralis and leg muscles (Fig. 3). Whereas J\textsubscript{V(c,f)} and V\textsubscript{V(mt,f)} are known to be strongly related over a broad range of species and muscle aerobic capacity (11, 17), a greater J\textsubscript{V(c,f)} for a given V\textsubscript{V(mt,f)} has been observed following the adaptation to endurance training in rats (27). This suggests that the difference between the A and SL finch is an adaptive response consequent to the high metabolic stress associated with flying and feeding at altitude (see description of A finch behavior in Effect of activity on capillarization) and argues against this being an interspecies difference per se. It is likely the capacity to develop these differences in muscle structure fairly rapidly under the influence of elevational changes that is genetically fixed, as suggested from previous work in pigeons (18). As we have already mentioned, behavioral traits are also likely operating in the A finches, in their feeding largely while hopping on snow banks, with both hypoxia and cold operating to produce the observed differences in muscle structure.

Without corresponding respiratory data from both groups of finches, O\textsubscript{2} consumption rates during maximal activity are not known. However, if we assume that the absolute O\textsubscript{2} costs of flying and hopping are the same at altitude and sea level (or possibly higher for flight at altitude due to thinner air), it is likely that the relative metabolic stress placed on the muscle (e.g., as a proportion of maximal O\textsubscript{2} consumption) would be greater at altitude (before adaptation) because of the lower inspired PO\textsubscript{2}. It is known that exercise while the subject is breathing hypoxic gas mixtures leads to a greater perturbation of intracellular metabolism, as indicated by ADP/ATP and P\textsubscript{i}/phosphocreatine ratios, than does a similar absolute workload performed while the subject is breathing normoxic gas (9). Furthermore, Desplanches et al. (5) have shown that exercise training in hypoxia produces greater changes in muscle N\textsubscript{N}(c,f) and fiber mitochondrial volume than does the same training in normoxia. Thus a greater relative metabolic stress on the muscle at altitude, coupled with the
The leg muscle (femoralis medius) of A. finches demonstrated greater N(f)(c) and mitochondrial volume than that of SL finches of the same subfamily (Carduelinae). In contrast to flight muscle in the same birds (20), there was no difference in leg muscle capillary geometry between A and SL finches. In conjunction with a trend toward larger, rather than smaller, muscle a(f) in leg of A. finches, the unchanged quotient of S(a)(f) and V(a)(mt)(f) in leg supported findings in pectoralis muscle (20) and supported the notion that capillary-to-fiber structure is organized to match fiber mitochondrial volume rather than to minimize intercapillary O2 diffusion distance at altitude.

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