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Muscle plasticity of Inuit sled dogs in Greenland

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SUMMARY

This study examined flexible adjustments of skeletal muscle size, fiber structure, and capillarization in Inuit sled dogs responding to seasonal changes in temperature, exercise and food supply. Inuit dogs pull sleds in winter and are fed regularly throughout this working season. In summer, they remain chained to rocks without exercise, receiving food intermittently and often fasting for several days. We studied two dog teams in Northern Greenland (Qaanaaq) where dogs are still draught animals vital to Inuit hunters, and one dog team in Western Greenland (Qeqertarsuaq) where this traditional role has been lost. Northern Greenland dogs receive more and higher quality food than those in Western Greenland. We used ultrasonography for repeated muscle size measurements on the same individuals, and transmission electron microscopy on micro-biopsies for summer–winter comparisons of muscle histology, also within individuals. At both study sites, dogs' muscles were significantly thinner in summer than in winter – atrophy attributable to reduced fiber diameter. Sarcomeres from West Greenland dogs showed serious myofilament depletion and expansion of the sarcoplasmatic space between myofibrils during summer. At both study sites, summer samples showed fewer interfibrillar and subsarcolemmal mitochondria, and fewer lipid droplets between myofibrils, than did winter samples. In summer, capillary density was higher and inter-capillary distance smaller than in winter, but the capillary-to-fiber-ratio and number of capillaries associated with single myofibers were constant. Increased capillary density was probably a by-product of differential tissue responses to condition changes rather than a functional adaptation, because thinning of muscle fibers in summer was not accompanied by reduction in the capillary network. Thus, skeletal muscle of Inuit dogs responds flexibly to changes in functional demands. This flexibility is based on differential changes in functional components: mitochondrial numbers, lipid droplet size, and the number of contractile filaments all increase with increasing workload and food supply while the capillary network remains unchanged.

Key words: exercise, muscle ultrastructure, nutrition.

INTRODUCTION

Life in the Arctic is governed by strong seasonal fluctuations of environmental parameters, most noticeably temperature, light regime, and food and water availability. Animals living under such conditions must adapt or escape when environmental conditions turn harsh. Hibernation and migration are avoidance strategies, but those that stay need to adjust to cycling changes in their environment. Piersma and Drent (Piersma and Drent, 2003) have coined the term "life-cycle staging" for flexible responses of animals in seasonally fluctuating environments.

Most studies that have analyzed seasonal changes of activity and food supply in arctic and boreal mammals describe an abundance of food during summer resulting in seasonal obesity, and famine during winter (Lohuis et al., 2007; Mustonen et al., 2004; Nieminen et al., 2004). Inuit sled dogs (*Canis lupus familiaris* L.) differ from this model. During summer when sledding is impossible, they live chained to rocks with an intermittent food supply, and so cannot build up fat deposits for the winter season. During winter they receive more food, but this is also the period of maximal work load and low-temperature challenge. Therefore, studies on wild animals living in the same climate zone cannot be extrapolated to Inuit sled dogs. However, sled dogs may serve as an interesting model to tease apart the seasonal effects of activity, food supply and temperature on physiology and internal organ structure. Furthermore, Inuit sled dogs are easily accessible for repeated measurements of the same individuals in summer and winter. All individuals in a team live and work under the same conditions, ensuring uniformity within the experimental group.

Inuit sled dogs are the only domestic animal of traditional Inuit and were essential to the survival of these people for more than 1000 years. The working relationship between dogs and the Inuit is currently losing its importance and, in many places, people no longer hunt using dog sleds. We worked with dogs that are still used by active hunters in the northernmost settlement of Greenland, and compare these dogs with those kept for recreational activities in Western Greenland.

While being used as draft animals during winter and spring, sled dogs are fed regularly and more frequently. During summer and fall, the dogs are permanently chained to rocks and fed only one to three times per week. Because in North Greenland the dogs receive high energy food throughout the year, they remain in a balanced energy budget. In West Greenland the dogs receive low energy food during summer, accumulating an energy deficit. During winter, higher energy food permits balancing of their energy budget.

We used the above differences in husbandry conditions as an experimental framework within which to explore the effects of exercise level, food supply and temperature on dog locomotor muscles. Activity (exercise) and nutrition are the strongest known determinants of skeletal muscle shape and size, which change fast and reversibly (Boonyarom and Inui, 2006; Hoppeler and Flück,

2002; Pette, 2001). To explore the separate effects of these two determinants on muscle morphology, we compared a suite of variables between seasons (summer and winter) and food supply, represented by two different locations (the West with low food supply *versus* the North with adequate food supply). These variables were muscle fiber diameter, capillary network and supply area, and myofibril ultrastructure (sarcomere shape, myofilament alignment and the sarcoplasmatic compartment), measured on biopsy samples using light and transmission electron microscopy.

First, to explore the combined effects of exercise and temperature, we compared muscle samples from summer with muscle samples from winter within each location. We hypothesized that while dogs were resting in summer, their muscle fibers would be atrophied relative to the winter condition. And in contrast to other arctic mammals, which downregulate muscle size in winter as a result of starvation (Josefsen et al., 2007), we expected Inuit dogs to upregulate their skeletal muscle size in response to increased work load, sufficient food supply and cold acclimatization. Such upregulation of muscle size is presumably based on changes in fiber size and architecture.

Second, The capillary network is one of the determinants of peripheral gas and substrate exchange, dictating the metabolic capacity of muscle tissue. The network is thus optimized to supply muscle metabolic demand (Baba et al., 1995; Hoppeler and Kayar, 1988), and adjusts to chronic electrical stimulation (Reichmann et al., 1985) or exercise. This being so, we expected that increased exercise would result in increased capillarization and a reduced capillary supply area in dogs in winter.

Third, we assessed the effects of food supply on exercised and resting muscle by comparing muscle samples between winter and summer, within each location (North and West Greenland) separately. In anorectic humans, loss of muscle bulk and muscle fiber atrophy are the most prominent effects of self-induced prolonged fasting (Lindboe et al., 1982). McLoughlin et al. (McLoughlin et al., 2000) also reported changes in blood plasma parameters in anorectic patients, including elevated levels of aspartate aminotransferase (AST), an enzyme associated with the transfer of nitrogen-containing groups between amino acids. Changes in the blood chemistry of sled dogs competing in a long distance race include significant increases of AST and creatine kinase (CK), indicating severe muscle breakdown (Burr et al., 1997). Here, we tried to clarify whether the seasonal changes in living conditions of the sled dogs result in similar changes of blood plasma parameters.

Although sled dogs offer an apparently unique and repeatable opportunity to study flexible responses of individual Arctic mammals to fluctuating conditions, our ‘experimental’ framework of seasonal and locational comparisons has certain constraints. A full cross-over design is not possible because of local traditions and physiological realities: ‘experimental’ groups cannot be reversed as dogs can neither work in summer nor fast in winter while working. It is beyond their physiological scope to work during summer or fast in winter while working. Moreover, biopsy sampling must be limited to a degree that dogs are not impaired and life of the hunters is not at risk.

MATERIALS AND METHODS

Research sites

Research was conducted at the Arctic Station of the University of Copenhagen in Qeqertarsuaq, Disko Island (69°15′N, 53°32′W) off the west coast of Greenland in summer 2005 and winter 2006. Temperatures at Disko Island are milder than in North Greenland, because of the relative warmth of Atlantic waters moving northwards with the West Greenland current. In summer 2007 and winter 2008, fieldwork was conducted in Qaanaaq, North Greenland (77°27′N 69°15′W).

Temperature recording

Environmental (air) temperatures close to the living area of the dog teams were recorded every 10 min throughout the fieldwork period using i-Button data loggers with an on-chip direct-to-digital temperature converter with 11-Bit (0.0625°C) resolution (DS2422 temperature/data logger, Maxim Integrated Products, USA). Mean daily temperatures were pooled for each season and location. Statistical analyses were done using SigmaStat 3.5 (Systat Software GmbH, Germany). Differences between seasons and between locations were assessed using Kruskal–Wallis one-way ANOVA on ranks, followed by pairwise multiple comparisons using Dunn’s method.

Dog husbandry

The sex ratio in Inuit sled dog teams is artificially skewed towards one or two bitches per 10 male dogs. Females can be gravid or have puppies throughout the year. Therefore, and to avoid inflation of variances as a result of female reproductive status, we investigated only male dogs. A team of 12 male dogs (age between 2 and 4 years), was studied in July/August 2005 and in February/March 2006 in Qeqertarsuaq. In July/August 2007 and in February/March 2008 a total of 10 male dogs (age between 2 and 10 years) belonging to two different dog teams of active Inuit hunters were studied in Qaanaaq. In winter, the dogs pulled sleds once or twice per week in Qeqertarsuaq, and three to four times per week in Qaanaaq, but remained chained to their places for the remaining time. The feeding regime followed local practice. During winter, the dogs in Qeqertarsuaq received a daily meal (approximately 150–700 g per dog) of dried fish or frozen seal meat. In Qaanaaq, the dogs were fed every other day (meal size: about 2 kg per dog). The food consisted of thawed and heated walrus and seal meat. During hunting trips, the dogs were fed daily with commercially available food for sled dogs (Nukik Polar, A/S Arovit Petfood, Esbjerg, Denmark). Throughout the summer, the dogs were constantly chained to rocks. In Qeqertarsuaq, they were each fed 2.5–3.6 kg of fresh fish in a single meal every fourth day. In Qaanaaq, each received 1–2 kg of walrus and seal meat from the previous hunting period every second to third day. Although the total amount of food received per 4-day period did not differ much between winter and summer, the quality and energy content of the food did differ. In Qeqertarsuaq, the average daily energy intake in winter was 4134±934 kJ per dog, but only 3603±388 kJ during summer. In Qaanaaq, the quality of the dog food did not differ between seasons. Daily energy intake was about 5900 kJ per dog in summer and 11800 kJ per dog in winter (J.M.S., N.G. and S.J., unpublished data).

The general health of all dogs was assessed by repeated physical exams performed following standard procedures by a veterinarian. All dogs assessed in Qeqertarsuaq were underweight in summer and winter. They were heavily infested by intestinal parasites during the summer months and suffered from periodic diarrhea. (J.M.S., N.G. and S.J., unpublished data). The dogs studied in Qaanaaq were in good condition in summer and in winter.

Size measurements

Dog body masses were measured using a hanging scale (Kern CH 50K100, Kern and Sohn GmbH, Balingen, Germany; precision: 0.1 kg) mounted on a carrying rod. Dogs were placed in a sling that supported the chest and belly while two people lifted the sling. Head

length from the tip of the nose to the caudal tip of the crista sagittalis was used as an estimate of body size that was independent of body mass. The measuring tape was placed directly onto the dogs head and followed the outline of the head. Height was measured at the withers using a measuring rod.

Ultrasonography

For non-invasive measurements of muscle thickness, we used a portable ultrasonography machine equipped with a broadband 7.5–10 MHz linear scanner head (Titan, SonoSite, Bothell, WA, USA) (Starck et al., 2001). A 0.5% aqueous solution of a polyacrylic acid (sodium polymer PNC 430, Spinnrad, Norderstedt, Germany) was applied as acoustic coupling gel. The thickness of the shoulder muscle, m. supraspinatus, was measured precisely halfway along the spina scapulae on the dogs' left side while standing (Fig. 1). The measuring track (Fig. 2A) was arranged parallel to the spina scapulae so that the ultrasonograph image covered the widest dimension of the m. supraspinatus. Muscle thickness of portions of m. triceps brachii and m. brachialis lateral to the humerus of standing dogs was measured halfway along the humerus (Fig. 2B). The thickness of the hind leg muscles was measured from the lateral side halfway along the femur while dogs were standing (Fig. 2C). These measurements included parts of the m. biceps femoris as well as parts of the m. vastus lateralis of the m. quadriceps femoris. The axis of the ultrasound probe was positioned perpendicular to the spina scapulae at the shoulder and perpendicular to humerus and femur. At each location we took multiple images per session, and each dog was scanned for at least four repeated sessions. The daily averages of multiple measurements were pooled to obtain averages of all values for each dog, and compared within each location between seasons using one way ANOVA with season as fixed factor. Statistical analyses were performed using SPSS version 12.0.1. (SPSS, Chicago, IL, USA).

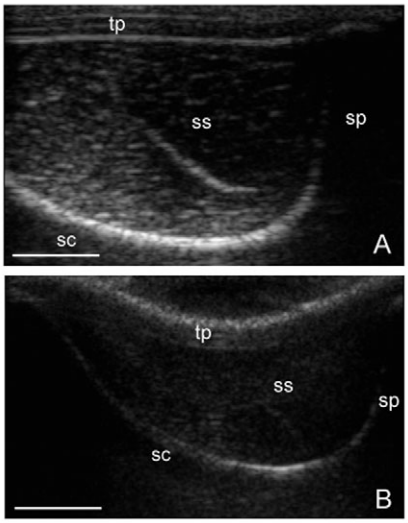

Fig. 1 Ultrasonographs of the supraspinatus muscle of Inuit dogs from Qeqertarsuaq in (A) winter and (B) in summer condition. The ultrasound probe was placed lateral and perpendicular to the scapula (sc), so that the spina scapulae (sp) is seen on the right side of the image. Top of the image shows layers of the skin, superficial fascia and trapezius muscle (tp). The supraspinatus muscle (ss) is characterized by a fascia which can be recognized on all ultrasonographs. Note the convex shape of the m. supraspinatus above the scapula (sc) in winter compared with the concave shape in summer. These are representative sonographs of the range from the different dogs. Scale bars, 1 cm.

Biopsy sampling

A 14 gauge spring-loaded side-cutting needle with a sampling notch of 1.5 cm (Temno; Allegiance Healthcare, McGaw Park, IL, USA) was used for needle biopsy sampling of the m. adductor magnus. Biopsies were taken from standing dogs in the field under local Lidocaine anesthesia (1–1.5 ml per dog Xylocain 2% local infiltration; AstraZeneca, Wedel, Germany), except in winter 2006, when they were obtained from dogs in lateral recumbency while under full anesthesia for other procedures. We took focused biopsy samples from the caudal mid-belly region of the m. adductor magnus. We took three biopsies from one incision site. This procedure minimized a possible negative effect of biopsy sampling on dog performance. An extensive random sampling as described by Mayhew (Mayhew, 2008) was neither possible nor intended. Care was taken to obtain cross-sectional samples at a 90 deg. angle to the muscle fibers. After biopsy sampling all dogs received subcutaneous Carprofen injections for analgesia (4 mg kg^{-1} Rimadyl, Pfizer GmbH, Karlsruhe, Germany). Bacterial inflammation was suppressed by a single subcutaneous dose of amoxicillin/clavulanic acid (10 mg kg^{-1}; Synulox RTU; Pfizer GmbH, Karlsruhe, Germany).

Histology

Muscle biopsies were preserved in 2.5% glutaraldehyde in 0.1 mol l^{-1} phosphate buffer at pH 7.4 and stored at 4°C until processing for histology. Embedding followed standard protocols for transmission electron microscopy. Biopsy samples were carefully oriented for later longitudinal and cross-sections. First, samples were washed in phosphate buffer, then postfixed in 1% osmium tetroxide in 0.1 mol l^{-1} phosphate buffer (pH 7.4) for 2 h, and dehydrated in a graded series of ethanol and pure acetone. Following dehydration, samples were embedded in epoxy resin (Epon, Carl Roth GmbH, Karlsruhe, Germany). Semithin sections of 500 nm thickness were stained with Rüdeberg solution (Methylene Blue–Thionin). Ultrathin sections of 60 nm thickness were counterstained with uranyl acetate and lead citrate. We used a Zeiss EM 10 transmission electron microscope to examine the sections.

Histological morphometry

When sectioning, careful attention was paid to obtain cross-sections and longitudinal sections of the samples. For cross-sectional morphometry we measured only fibers that showed no indication of oblique sectioning, i.e. elongated fiber diameter with an orientation in one direction. For longitudinal morphometry, we used only fibers that were running across the entire length of the section without major change in shape. To obtain quantitative measures of muscle architecture, we (1) measured the smallest diameter of all myofibers per section (100–150 myofibers per dog), (2) counted the number of fibers per area (myofiber density), and (3) measured the extracellular distance between myofibers. As a measure of capillarization, we (1) counted the number of capillaries surrounding each myofiber, and (2) counted the number of capillaries per unit area (capillary density). Because capillaries supply more than one fiber, we (3) calculated a capillary-to-fiber ratio by dividing the number of capillaries per mm^2 by the number of fibers per mm^2. (4) The capillary supply area was determined as a circle with the radius being half the mean intercapillary distance around the capillary. To obtain a random selection of measuring points for the distance between myofibers (μm), a grid of five lines was laid over longitudinal sections. Measuring points were defined where grid lines crossed intercellular space. We used SigmaScanPro (version 5, Jandel Scientific, SPSS Inc., Chicago, USA) for morphometry

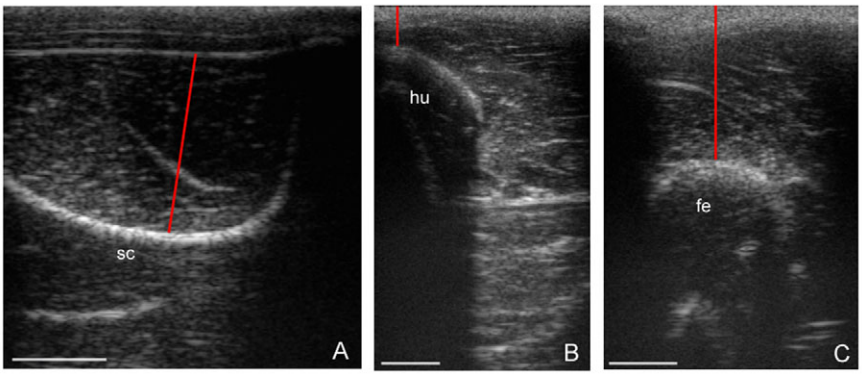

Fig. 2. Ultrasonographs of measurement sites and muscle thickness of (A) m. supraspinatus above the scapula (sc), (B) muscles (m. triceps brachii, m. brachialis) lateral of the humerus (hu), and (C) muscles (m. biceps femoris, m. vastus lateralis) lateral of the femur (fe); the measured distance from the bone (A: scapula; B: humerus; C: femur) is indicated by a red line. Layers of skin can be seen at the top of the images. Scale bars, 1 cm.

of semithin sections in longitudinal and transversal alignment to the fibers. Two-way ANOVAs with season and location of the dogs as fixed factors were performed and pairwise comparisons (Holm–Sidak method, overall significance level=0.05) applied to detect differences between groups.

Myofiber ultrastructure

The histological examination of ultrastructure was based on TEM images of longitudinal sections through myofibers. Of course, morphometry of myofiber ultrastructure is affected by a cascade of factors ranging from biopsy sampling under non-standard conditions in the field (e.g. extremely low temperatures during winter) and lack of precise stereological information of biopsy sampling position (no ultrasound control possible), to dehydration and embedding artifacts during standard TEM-histology (see Zumstein et al., 1983). Thus, morphometric measurements on myofiber ultrastructure are semi-quantitative and need to be interpreted with caution. We applied three different qualitative and quantitative measures to analyze the ultrastructure of muscle fibers.

(1) Sarcomere shape. We assigned the outer line of the sarcomeres to two different categories; either parallel/convex or concave. All sarcomeres, that were fully visible in the TEM images, were categorized and the percentage of sarcomeres in each category was calculated. Furthermore, we measured the width of the sarcomeres at the M-line and at the Z-line and calculated the M/Z ratio of individual sarcomeres. The M/Z ratio is a simple estimate of sarcomere shape: a ratio <1 indicates that the sarcomere has a concave shape, which indicates a depletion of myofilaments in the sarcomere, while a ratio ≥1 is typical for straight or convex sarcomere, indicating a normal structure of the myofilaments. We measured the M/Z ratio of all fully visible sarcomeres on each TEM-image and then calculated the percentage of the sarcomere that were either normal or concave. Because sample size differs between years and locations it is always given with the results. We ran a two-way ANOVA to test for effects of season and location as fixed factors on the measure of sarcomere shape. When the model was significant, pairwise multiple comparisons (Holm–Sidak method) were made to recognize significant differences between groups.

(2) The alignment of myofilaments within each sarcomere is another character that can be used to qualitatively describe possible effects of workload and/or nutrition on sarcomere structure. We defined two categories. The first category was characterized by all myofilaments arranged in a parallel fashion, which is usually associated with a normal (full) sarcomere. The second category was characterized by myofilaments in a diffuse arrangement, usually because of depleted numbers of myofilaments. Transmission electron micrographs were screened for the arrangement of myofilaments in each sarcomere. Sarcomeres were assigned to each category and counted to calculate the percentage of each category in samples of dogs from summer and winter.

(3) The packing of myofibrils within the myofiber was assessed by defining two groups (‘large’ and ‘narrow’, according to the size of the sarcoplasmatic compartments between the myofibrils) that categorized the distance between the myofibrils. Sarcoplasmatic compartments were labeled ‘large’ when they were wider than one third of the width of adjacent myofibrils, and ‘narrow’ when thinner than one third of the width of myofibrils. Again, we counted distance categories on each image and calculated percentages for samples of dogs from summer and winter.

Blood sampling

Blood was obtained from the cephalic vein of the left front leg using a 21-gauge needle, and spun down in heparinized 2 ml tubes at 3000 ***g*** for 10 min to gather plasma within 1 h after collection. Care was taken to prevent the blood samples from freezing. The supernatant was frozen immediately and stored at –20°C. Blood was sampled from dogs in postabsorptive condition, in summer, this was at the end of a 4-day fasting interval in Qeqertarsuaq, and in winter and both seasons in Qaanaaq, this was 24 h after feeding. Measured parameters were liver function and integrity (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), urea, triglycerides, glucose, cholesterol and bilirubin); kidney function [sodium (Na^+), potassium (K^+), creatinine, urea, phosphate (PO_4^{3-})]; muscles and bones [creatine kinase (CK), lactate dehydrogenase (LDH), calcium (Ca^{2+}), magnesium (Mg^{2+}), triglyceride]; and digestion (glucose, fructosamine and cholesterol). Additionally, we measured chloride (Cl^-), total protein, albumin and fatty acids (Qeqertarsuaq: N=12 in summer, N=11 in winter; Qaanaaq: N=10 in summer and winter).

RESULTS

Temperature

Winter temperatures at Qeqertarsuaq in West Greenland (median –8.5°C, 25% percentile –12.1°C, 75% percentile –2.1°C) were significantly higher than winter temperatures at Qaanaaq in North Greenland (median –21.4°C, 25% percentile –24.4°C, 75% percentile –18.4°C; difference of ranks: 32.4, q: 3.3, P<0.05), but significantly below the summer values at both study sites (difference of ranks: $64.6_{Qeqertarsuaq}$, $42.6_{Qaanaaq}$; q: $6.5_{Qeqertarsuaq}$, $4.5_{Qaanaaq}$; P<0.05). During summer, the temperatures at Qeqertarsuaq (median 12.1°C) did not differ statistically from temperatures measured at Qaanaaq (9.4°C).

Size measurements

The body mass of dogs from Qeqertarsuaq averaged 27.3±2.7 kg during winter, which is about 30% above the mean summer mass (19.1±1.6 kg). The body mass of dogs from Qaanaaq did not differ between the seasons (33.2±3.0 kg in winter, 33.7±2.7 kg in summer). A two-way ANOVA with season and location as factors revealed significant differences for both factors (season: d.f.=1, F=25.3, P<0.001; location: d.f.=1, F=176.9, P<0.001). Interactions between the factors were significant (d.f.=1, F=31.4, P<0.001), i.e. location as a factor determined whether dogs differed in body mass between seasons or not. Body mass differed between seasons in Qeqertarsuaq (difference of means=8.2, t=7.8, P<0.001), but not in Qaanaaq. Dogs from Qaanaaq (61.9±3.2 cm) were significantly taller than dogs from Qeqertarsuaq (56.6±1.4 cm; d.f.=1, F=9.6, P=0.01). However, the head length of the dogs did not differ between the study sites (Table 1).

Ultrasonography

Ultrasonography of dogs from Qeqertarsuaq showed that shoulder muscles were on average 19% thicker during winter than during summer (d.f.=1, F=50.4, P<0.0001) (Fig. 1), foreleg muscles were 44% thicker during winter than during summer (d.f.=1, F=18.1, P=0.0002), and hindleg muscles were 39% thicker during winter than during summer (d.f.=1, F=175.5, P<0.0001). By contrast, the thickness of shoulder and foreleg muscles of dogs from Qaanaaq did not differ between seasons, but, their hindleg muscles were significantly (10%) thicker during winter than during summer (d.f.=1, F=13.1, P=0.004).

Histological morphometry

For both locations in winter, muscle fibers were packed tightly in clusters surrounded by capillaries in a thin endomysium (Fig. 3C,D, Table 2). Light microscopy showed numerous mitochondria clustered along the margin of each fiber next to the capillaries. The intermyofibrillar space was partially filled with lipid droplets. Distribution of lipids differed between fiber types, which cannot be discriminated in standard light microscopy. Dogs from Qeqertarsuaq in summer had rounded muscle fibers loosely packed in an endomysium with more space between the fibers than in winter. The margin of each myofiber showed no concentration of mitochondria and the intermyofibrillar space contained only few lipid droplets (Fig. 3A). By contrast, the muscle fibers of dogs from Qaanaaq sampled in summer (Fig. 3B) did not differ much from winter muscle. Although light microscopy is semi-quantitative, the margin of the muscle fibers appeared to contain fewer mitochondria in summer than in winter, and the intermyofibrillar space fewer lipid droplets.

Morphometric measurement of muscle fiber diameter showed that this was always smaller in summer than in winter, and within each season, was smaller in Qeqertarsuaq than in Qaanaaq. A two-way ANOVA showed that the effects of season and location were highly significant but no interactions between factors were observed (season: d.f.=1, F=15.2, P<0.001; location: d.f.=1, F=12.6, P<0.001). At both locations, the myofiber density was significantly higher (d.f.=1, F=11.1, P=0.002) in summer than in winter. No differences were found for the location and no interaction was detected between season and location.

The distance between myofibers was largest in muscle samples from dogs from Qeqertarsuaq. When tested in a two-way ANOVA with season and location as the main effects, the model was highly significant (season: d.f.=1, F=12.7, P=0.001; location: d.f.=1, F=35.5, P<0.001). However, we detected a significant interaction between factors, thus an interpretation of the main factor would be difficult, i.e. the effect of season will depend on whether the dogs originate from Qeqertarsuaq (difference of means=2.8, t=5.0, P<0.001) or Qaanaaq (no significant difference).

The number of capillaries surrounding one fiber remained constant in summer and winter at both locations. Significantly more (d.f.=1, F=36.4, P<0.001) capillaries surrounded one fiber in Qaanaaq than in Qeqertarsuaq. The capillary density differed significantly between the seasons and between the locations (season: d.f.=1, F=9.5, P=0.004; location: d.f.=1, F=16.7, P<0.001), but no interaction was detected between the two factors. The capillary-to-fiber ratios calculated from the densities of capillaries and fibers were constant throughout the year, but significantly higher (d.f.=1, F=39.1, P<0.001) in Qaanaaq than in Qeqertarsuaq. No interactions between the two factors were detected.

Significant seasonal differences (d.f.=1, F=31.3, P<0.001) were found for the distance between neighboring capillaries, but no difference were observed for the factor 'location' and no interaction between the factors. The area supplied by each capillary consequently differed significantly between seasons (d.f.=1, F=30.5, P<0.001), but not between areas, and no interactions between the factors were observed.

Sarcomere ultrastructure

In winter, the sarcomeres were full with a straight or convex outline (Fig. 4C,D). The myofilaments were densely packed in straight and parallel arrangement to each other and to the long axis of the sarcomeres. Between the myofilaments numerous lipid droplets were stored close to the intermyofibrillar mitochondria. This ultrastructure of myofibrils holds for muscle samples from winter for both locations.

Table 1. Body mass, size measurements and muscle thickness of Inuit dogs in summer and winter conditions

	Qeqertarsuaq (N=12)		Qaanaaq (N=10)	
	Winter 2006	Summer 2005	Winter 2008	Summer 2007
Body mass (kg)	27.3±2.7	19.1± 1.6	33.24±3.03	33.69±2.71
Withers height (cm)	56.6±1.4		61.9±3.2	
Head length (cm)	25.7±1.2		25.8±1.1	
Thickness of shoulder muscle* (cm)	2.7±0.2	2.2±0.2	2.3±0.1	2.3±0.2
Thickness of muscles lateral of 50% of the length of the humerus (cm)†	0.9±0.2	0.5±0.2	1.0±0.2	0.9±0.1
Thickness of muscles lateral of 50% of the length of the femur (cm)‡	2.3±0.2	1.4±0.2	2.2±0.2	2.0±0.2

Values are means ± s.d.

*M. supraspinatus measured at half the length of the spina scapulae at the left side of a standing dog.

†Combination of m. triceps brachii and m. brachialis.

‡Combination of m.biceps femoris and m.vastus lateralis.

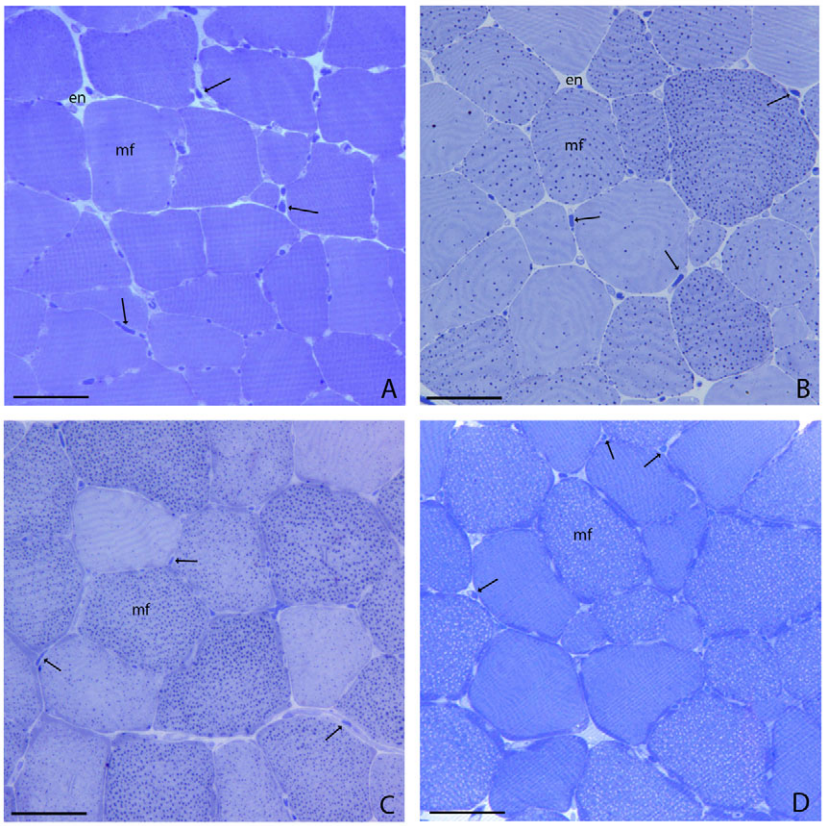

Fig. 3. Semithin cross-sections of m. adductor magnus (A,B) in summer, and (C,D) in winter condition. A and C are samples of dogs from Qeqertarsuaq, B and D are samples from Qaanaaq. Myofibers (mf) in winter condition contain more lipid droplets (dark dots in cross-sections A–C, lights dots in D) than myofibers of summer condition. Capillaries (black arrows) are visible between the myofibers. Note the expanded endomysium (en) in summer in sample (A) of a dog from Qeqertarsuaq. Dark blue edges of fibers from the Qaanaaq dog in winter condition(D) are aggregations of mitochondria. Micrographs are average representations of muscle histology of the different dogs. Scale bars, 50 μm.

In summer, the ultrastructure of myofibrils of dogs from Qeqertarsuaq looked strikingly different (Fig. 4A). Many sarcomeres had a concave shape, so that the sarcoplasmatic compartment appeared dilated. The myofilaments were less dense and in disorganized arrangement within the sarcomeres. Many myofibrils were oriented oblique to the long axis of the sarcomere. Also, ramifications of sarcomeres were observed which did not occur during winter. The sarcomere structure of dogs from Qaanaaq (Fig. 4B) resembled the winter conditions, but myofilaments appeared to be less densely packed although in orderly arrangement.

In winter, 20% of the sarcomeres of dogs from Qeqertarsuaq but none from the dogs in Qaanaaq were classified as concave (Table 3) whereas in summer this increased to 71% and 20%, respectively. In winter, the myofilaments within the sarcomeres were aligned in parallel in 93% of the samples from Qeqertarsuaq and all samples from Qaanaaq. In summer in dogs from Qeqertarsuaq, the majority of sarcomeres (57%) contained myofilaments obliquely arranged to the long axis of the sarcomere. By contrast, in summer 90% of the sarcomeres of dogs from Qaanaaq contained myofilaments arranged parallel to the long axis of the sarcomere. We observed a dilatation of the sarcoplasmatic compartment in 13% of the TEM images of tissue samples from Qeqertarsuaq and none in the samples from Qaanaaq in winter condition. In summer, 50% of the samples from Qeqertarsuaq and 30% of the samples from Qaanaaq showed extended sarcoplasmatic compartments.

An M/Z ratio <1, which is indicative of concave sarcomeres, was only found in summer in dogs from Qeqertarsuaq (0.87±0.11, N=9). M/Z ratios of 1 and higher (=straight or convex sarcomeres) were found in dogs from Qeqertarsuaq during winter (1.00±0.10, N=8), Qaanaaq during summer (1.03±0.12, N=10) and Qaanaaq during winter (1.10±0.06, N=8). When tested with a two-way ANOVA the M/Z ratio differed significantly between seasons (d.f.=1, F=8.9, P=0.006) and between locations (d.f.=1, F=14.4, P<0.001). No interactions between season and location were detected.

Table 2. Morphometry of skeletal muscle of Inuit dogs in summer and winter condition

	Qeqertarsuaq				Qaanaaq			
	Winter 2006	N	Summer 2005	N	Winter 2008	N	Summer 2007	N
Myofiber smallest diameter (μm)	52.63±6.54	11	42.84±8.98	11	57.02±5.35	10	51.96±4.38	10
Myofiber density (per mm^2)	278.78±51.30	8	318.34±64.39	9	241.35±25.06	10	322.56±44.82	10
Distance between myofibers (μm)	1.85±0.94	11	4.30±2.65	12	0.80±0.17	10	0.87±0.24	10
Number of capillaries surrounding each myofiber	4.46±0.40	8	5.06±0.94	9	6.57±0.94	10	6.05±0.76	10
Capillary density (per mm^2)	624.33±80.59	8	803.46±190.94	9	855.75±143.01	10	1041.16±151.64	10
Capillary-to-fiber ratio	2.29±0.31	8	2.58±0.57	9	3.57±0.64	10	3.25±0.39	10
Distance between neighboring capillaries (μm)	45.28±3.73	9	37.90±1.55	8	45.46±2.76	7	40.19±4.02	9
Area supplied by each capillary (μm^2)	1621±259	9	1129±91	8	1628±198	7	1280±246	9

Values are means ± s.d.

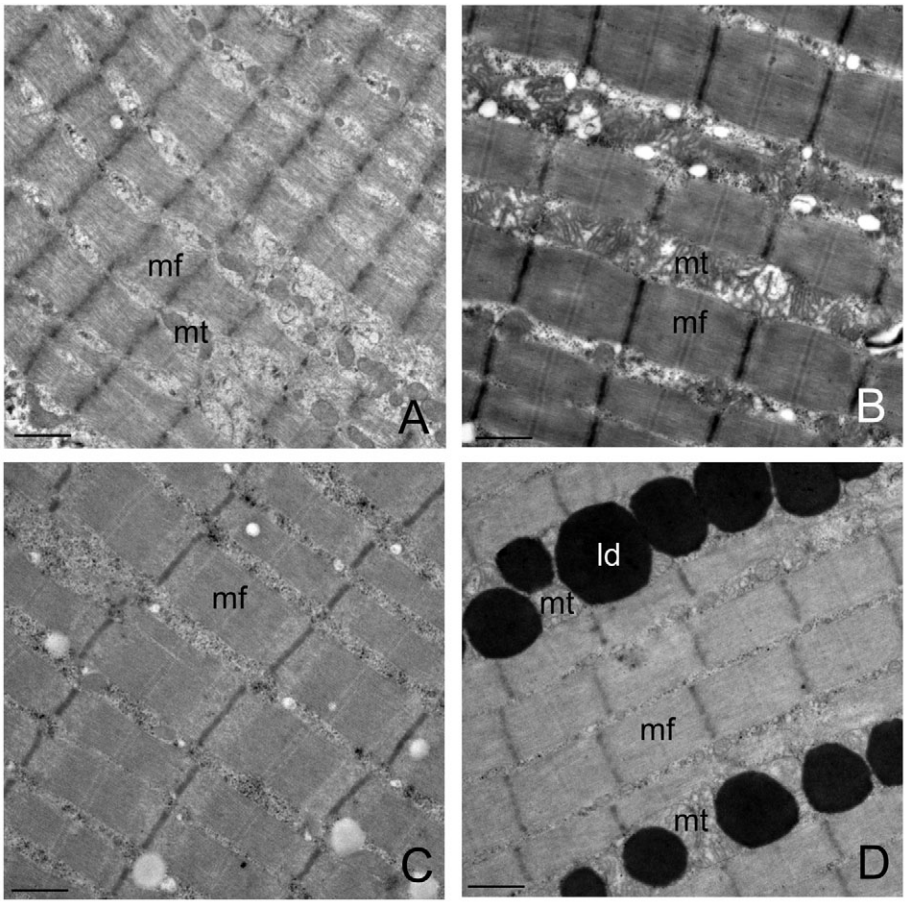

Fig. 4. Transmission electron micrographs of m. adductor magnus of dogs (A,B) in summer condition and (C,D) in winter condition. A and C are samples of dogs from Qeqertarsuaq, B and D are samples from Qaanaaq. Mitochondria (mt) are located next to lipid droplets (ld) between the myofibrils (mf). Scale bars, 1 μm. Transmission electron micrographs are average representations of muscle ultrastructure taken from different dogs.

Blood plasma parameters

Most blood plasma values of dogs located in Qeqertarsuaq were within a range that would not indicate pathologies. Only one dog showed permanently and seriously elevated values indicative of hepatic dysfunction, i.e. elevated values for ALT (measured: 2173 i.u. l^{-1} in summer, 2689 i.u. l^{-1} in winter, reference: 16–91 i.u. l^{-1}l), AST (only measured in winter: 243 i.u. l^{-1}, reference: 19–51 i.u. l^{-1}), and AP (measured: 1769 i.u. l^{-1} in summer, 201 i.u. l^{-1} in winter, reference: 11–225 i.u. l^{-1}). Five out of 12 dogs showed elevated levels of creatine kinase (measured: 653±579 units; reference: 33–351 units) in winter samples. In Qaanaaq, blood plasma parameters of all dogs were within a tolerance range for healthy dogs, whereas AP was above the reference value in seven dogs during winter (measured: 653±579 i.u. l^{-1}; reference: 11–225 i.u. l^{-1}). Also, four dogs showed elevated urea levels during winter (measured: 13.6±2.6 mmol l^{-1}; reference: 3.3–8.3 mmol l^{-1}).

DISCUSSION

Size measurements

This study aimed at understanding plasticity of skeletal muscle in Inuit sled dogs in response to seasonal changes of environmental temperature, exercise and food supply. We have studied Inuit dogs living under different husbandry conditions at different locations to distinguish between effects of activity and food supply on muscle tissue.

The observed fluctuations in body mass of Inuit sled dogs located in Qeqertarsuaq compared with the constant body mass of dogs living in Qaanaaq show that the main factor for changes in muscle size during resting in summer is food supply and food quality. Other parameters, like temperature and activity level were similar at both locations. Because the dogs located in Qeqertarsuaq were lean throughout the year, and because all investigated muscles were significantly thicker in winter than in summer, we conclude that overall body mass differences of these dogs are mainly based on changes in muscle bulk.

Although Inuit sled dogs are a morphologically rather diverse breed, we found no difference in the length of the head of the dogs from the two study sites. Thus we feel confident that the dogs represent similar size classes, even though body mass and of height of the withers differ. Certainly, the breed is uniform throughout Greenland because it is protected by a strict prohibition of any other dog breed north of the Arctic Circle.

In Qeqertarsuaq, the average daily energy intake of dogs was 3603±388 kJ during summer and 4134±934 kJ during winter for dogs

Table 3. Quantitative description of ultrastructural characteristics of myofibrils

	Qeqertarsuaq		Qaanaaq	
	Winter 2006 (*N*=11)	Summer 2005 (*N*=12)	Winter 2008 (*N*=10)	Summer 2007 (*N*=10)
Concave sarcomeres	20%	71%	0%	20%
Myofilaments aligned in parallel	93%	43%	100%	90%
Sarcoplasmatic compartment dilated	13%	50%	0%	30%

in thermoneutral condition (N.G. and J.M.S., unpublished data). The summer values are considerably below the values reported for Siberian Huskies (5021 kJ) and Labrador retrievers (5611 kJ) under thermoneutral conditions (Finke, 1991). The dogs located in Qaanaaq were in a balanced energy budget and maintained their weight during summer, receiving about 5900 kJ day^{-1}.

During winter, the daily energy budget of the dogs in Qaanaaq (11,800 kJ; N.G. and J.M.S., unpublished data) was balanced, but, it was at the lower margins of the range of values given by Orr (Orr, 1966), i.e. between 10,500 kJ day^{-1} for a non-working dog and 21,000 kJ day^{-1} for working sled dogs.

Racing sled dogs are known to have the highest sustained metabolic rates (47,100 kJ day^{-1}) of any mammal measured so far (Hammond and Diamond, 1997; Hinchcliff et al., 1997).

Histological morphometry and sarcomere ultrastructure

Many studies have analyzed ultrastructural changes of muscles in response to endurance training, inactivity or fasting (Flück, 2006; Hamilton and Booth, 2000). Lindboe and Prestus (Lindboe and Prestus, 1985) found that immobilization and food deprivation had different effects on the size of different histochemical fiber types in the tibial muscles of rats. Food deprivation resulted in atrophy of all fiber types, but, immobilization resulted in a differential size change of different histochemical fiber types.

The dogs in Qeqertarsuaq experienced the combined effects of inactivity and undernourishment during summer, whereas the dogs in Qaanaaq experienced only inactivity but were well fed. Thus, by comparing these two groups, we can partition change of muscle size and structure for effects of undernourishment and inactivity.

The average diameter of muscle fibers of dogs during winter at both locations, and of dogs in Qaanaaq during summer is within the range of 50–64 μm reported for domestic dogs (Z'Berg and Augsburger, 2002). The diameter of muscle fibers of dogs during summer in Qeqertarsuaq is much smaller, indicating atrophy of muscle fibers. Based on a qualitative or semi-quantitative analysis, Lindboe et al. (Lindboe et al., 1982) reported that anorectic human patients also have muscle fiber diameters that are significantly below average. McLoughlin at al. (McLoughlin at al., 1998) found that the muscle fibers of anorectic patients showed separation and segmental loss of myofibrils in the m. vastus lateralis, thus indicating severe atrophy. All these patients were physically active, some even over-exercising, walking or jogging up to 6 h daily, so the skeletal muscle atrophy with underlying ultrastructural changes was clearly a result of undernutrition and not of inactivity. These findings are identical to what we see in dogs from Qeqertarsuaq, where dogs were undernourished and inactive. In Qaanaaq, where dogs were well nourished and inactive, we did not find any of these changes. Based on the similarity of ultrastructural findings, we can safely conclude that depletion of myofilaments and segmental ramification of sarcomeres is a result of undernourishment. Comparing a large number of TEM images, the effects of undernourishment appear to be unevenly distributed within a myofiber, i.e. some myofibrils are more affected than others, resulting in differential depletion of sarcomeres.

We introduced the M/Z ratio as a semi-quantitative measure to support the observed sarcomere changes. The key to interpreting changes of the M/Z ratio is that the Z-line remains constant while the M-line changes with an increasing or decreasing size of the contractile filament compartment in a sarcomere. Thus, an M/Z ratio smaller than 1 indicates a reduction/depletion of myofilaments from a sarcomere whereas an M/Z ratio of 1 or higher suggests normal sarcomere structure. The M/Z ratio of Qeqertarsuaq dogs during summer suggests a serious depletion of sarcomeres, whereas Qeqertarsuaq dogs during winter and all dogs from Qaanaaq had normal sarcomeres. However, many of our TEM images showed that for example in dogs from Qeqertarsuaq in summer even within one myofibril the sarcomere structure varies between full and depleted. We suggest that this sketchy pattern is caused by differential depletion of myofibrils. Because we applied random sampling for M/Z-ratio measurements we are confident that we have identified average differences between muscle samples from different locations and different seasons. Because we have taken that measurement only on clearly defined longitudinal sections of muscles we excluded stereological artifacts. Differential depletion of myofibers explains the patchy pattern of concave sarcomeres and the rather proportional changes of depleted sarcomeres observed between the different groups.

The capillary network supposedly is an important determinant for oxygen transport to the muscles (Hoppeler and Kayar, 1988). The dogs in all four groups of our study maintained a stable capillary network throughout the year, as shown by the constant capillary-to-fiber ratio and the constant number of capillaries adjacent to one myofiber. However, dogs from Qaanaaq that were used more intensively for hunting always had a higher capillary-to-fiber ratio than dogs from Qeqertarsuaq. We also found significant differences in capillary density and the distance between neighboring capillaries between the seasons. Because in each of these groups the capillary density is higher in summer than in winter, we conclude that these changes are not affected by training but are related to the decreased fiber size in summer. When the fiber diameter decreases but the capillary network remains unchanged the capillary density per area will automatically increase. The same type of correlated changes was described by Deveci and Eggington (Deveci and Eggington, 2002) in a morphometric study of Syrian hamsters.

At a first glimpse, our results appear to be in partial contrast to those of Capric and James (Capric and James, 1983) who reported that the capillary-to-fiber ratio of untrained dogs increased after training on a treadmill for 6 weeks. However, in our study, the capillary-to-fiber ratio of the dogs from Qeqertarsuaq always was in the range of the values found in untrained dogs, whereas the dogs from Qaanaaq had a capillary-to-fiber ratio comparable to that of trained dogs, but we found no change in the capillary-to-fiber ratio in response to season. We suggest that training has a long-term effect on the capillary network and is not easily downregulated during periods of low activity. This would explain why the dogs from Qaanaaq that were more frequently used for sledding have a higher capillary-to-fiber ratio than dogs from Qeqertarsuaq that were used only on shorter and occasional trips. However, the capillary network may be affected by many more factors than just training, e.g. Hepple and Vogell (Hepple and Vogell, 2004) and Mathieu-Costello et al. (Mathieu-Costello et al., 2005) showed that the capillary network does not change with age. This suggests that once the capillary network has been established there is no flexibility for downregulation, ultimately supporting our interpretation of long-term effects on the capillary network and the lack of flexibility for downregulation.

Other mammalian model systems show similarly contrasting results. For example, capillary densities of heart and skeletal muscles of European woodmice (*Apodemus sylvaticus*) that were trained on a treadmill did not differ from activity-restrained woodmice (Hoppeler et al., 1984). But, in rats (Poole and Mathieu-Costello, 1989) and humans (Ingjer, 1979; Zumstein et al., 1983) endurance training resulted in an increased capillary network.

Blood plasma parameters

Blood plasma parameters must be discussed with caution. Blood sampling and in particular storage cannot always match standard conditions as requested by laboratory protocols. In our study, samples could not be kept at –25°C during transport back to the home laboratory thus potentially increasing error variance in the final analysis. Blood samples in general suggested a satisfying health status of the dogs, except for one dog in Qeqertarsuaq, which obviously suffered from some liver dysfunction. However, this dog in particular was very active and second in the hierarchy of the team, and did not show any signs of impaired health.

An elevated level of AP is difficult to interpret if it is not clearly associated with other blood plasma parameters. Elevation of AP can be associated with extreme exercise or over-exercising. For example, Hinchcliff (Hinchcliff, 1996) and Burr et al. (Burr et al., 1997) compared sled dogs finishing a long distance race with dogs that did not finish the race, and always found significantly higher levels of AP in the non-finishing group than in the finishing group.

Conclusion

The reported seasonal changes in skeletal muscle morphology of Inuit sled dogs are a result of the Greenlandic sled dog husbandry, that varies depending on the importance of the dogs in the daily life of the Inuit. The summer season is marked with movement restriction and severe undernutrition in parts of Greenland, where the dogs are no longer important in peoples' daily lives. During the working winter season the dogs receive sufficient food; especially where they are still used frequently for hunting and transportation, in the northernmost parts of Greenland. While other arctic mammals gain weight rapidly during summer and build up fat deposits for the upcoming winter season, the sled dogs keep or even lose weight in summer as a result of undernourishment and inactivity. In summer, skeletal muscle morphology of dogs kept in western Greenland is characterized by atrophied fibers with depleted and deranged myofilaments in the concave sarcomeres. The sarcoplasmatic compartment is dilated. These changes in muscle structure are reversible and the dogs quickly recover, their muscles gain full functionality and normal structural appearance when fed more and regularly during the working season. Muscle fibers of dogs kept in Northern Greenland are atrophied too, but packed densely during summer and the structure of the sarcomeres appears normal. By contrast, the capillary network remains unchanged throughout the year at both locations.

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