Living at the Extremes – Feeding and Fasting, Resting and Exercise in the Arctic

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In Erinnerung an Sheila, Quino und Luigi

Qaanaaq, 2008

There may be larger dogs than these,
there may be handsomer dogs; but I doubt it.
other dogs may work as well or travel
as fast and far when fully fed;
but there is no dog in the world that can work so long
in the lowest temperatures on practically nothing to eat

Robert Peary, 1910
The North Pole, p.74

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Summary

Phenotypic plasticity, the ability of a given genotype to produce different phenotypes and thus flexibly adjust to changing environmental conditions, is one strategy to survive in habitats that undergo strong seasonal changes. The Arctic with its relatively mild summers with 24 h of daylight and cold, harsh winters without sunlight is an example of such an environment. This thesis explores how animals that live in the Arctic and remain active throughout the year cope with the changing conditions. The Inuit sled dog, a breed selectively bred for more than thousand years to live and work in Greenland serves as model organism. During winter the dogs are used as draft animals whereas they rest during summer. Only while working the dogs are fed adequately. In summer, however, the dogs are fed just once or twice per week. The quality of the food provided varies locally. Two groups of Inuit sled dogs that differed in husbandry and climatic conditions were investigated and compared with each other. One group received sufficient food only during winter (low energy intake dogs; referred to as LO-dogs) and was used for recreational dog sledding in the mild West Greenlandic winter. The other group was well nourished throughout the year (high energy intake dogs; referred to as HI-dogs) and was used for hunting and transportation on a regular basis throughout the harsh winter of North Greenland. The investigations focused at changes in energy metabolism, adaptations of the cardiovascular system, and the locomotor muscles in response to the seasonally changing food supply, exercise and environmental temperature.

Chapter 2 is devoted to partitioning the energy budget of HI- and LO-dogs during summer and winter, i.e., while resting, exercising, and digesting in different temperature ranges. Constant resting metabolic rates (RMR) of HI-dogs within a wide temperature range showed the exceptionally wide thermoneutral zone of these dogs with a lower critical temperature at about −25°C. This minimizes energy investments spent for thermoregulation and enables them to cope with the extreme climate in the Arctic.

During summer while resting, digesting dogs doubled oxygen consumption compared to fasting dogs. Metabolic rate while resting and digesting did not differ between seasons. While HI-dogs maintained a constant body mass throughout the year, the LO-dogs lost up to 30% of their body mass in summer. The negative energy balance of these dogs was characterized by a rise in energy metabolism accompanied by a lowered heart rate. Working HI-dogs maintained the highest sustained metabolic rates (susMR) measured in a mammal so far (12.2 times their RMR during summer).

Seasonal changes in ambient temperature, exercise and food supply led to size changes of locomotor muscles of the dogs as investigated in **Chapter 3**. Examination of the ultrastructure of skeletal muscle biopsies revealed a normal functional appearance of the tissue during

winter in both dog groups. During summer, however, muscle fibers were atrophied, the sarcoplasmatic space was dilated, and the sarcomeres were depleted of myofilaments. Additionally, the subsarcolemmal and interfibrillar mitochondria were reduced and less lipid droplets were stored within the fibers. These changes were more pronounced in LO-dogs than in HI-dogs. However, the capillary network within muscles remained stable in both dog groups. These findings illustrate the differential adjustments of the muscle components in response to changing conditions; some parts are flexibly adjusted to the actual demand while others remain stable.

In contrast to the capillaries within muscles, the large blood vessels showed seasonal adaptations to changing conditions as investigated in **Chapter 4**. Ultrasonographs confirmed a larger aortic root diameter during winter as compared to summer in all dogs. The dimensions of the left ventricle in systole and diastole remained unchanged in LO-dogs while HI-dogs had reduced inner diameters during winter as compared to summer. However, the thickness of the interventricular septum did not change throughout the seasons in both dog groups. Additionally to the morphological measurements, Chapter 4 also addresses functional short-term and long-term adaptations of the cardiovascular system of the dogs in response to different modes of activity. In both dog groups, cardiac output did not change between seasons in resting digesting dogs, but it was reduced by 50% when the dogs fasted. Other parameters of cardiac function showed differential seasonal adaptations in the different dog groups: in winter, stroke volume, left ventricular ejection fraction, and fractional shortening of the ventricle increased in LO-dogs while heart rate did not change. In contrast, HI-dogs had increased heart rate but unchanged stroke volume, left ventricular ejection fraction, and fractional shortening of the ventricle.

The ability to maintain high susMR over extended periods must have been an important criterion for employing dogs as working animals. We have shown that Inuit sled dogs respond differentially to seasonal changing demands on different levels of the cardio-vascular system and the muscle apparatus. While some parts (e.g. capillaries between muscle fibers, ventricular septum) are kept in a condition that meets the demands in the working season, other parts (e.g. muscle fibers, aortic root diameter) are flexibly adjusted to the demands of the present season.

Zusammenfassung

Phänotypische Plastizität, die Fähigkeit eines Genotypes verschiedene Phänotypen hervorzubringen und so zur flexiblen Anpassung an sich verändernde Umweltbedingungen beizutragen, ist eine der möglichen Überlebensstrategien in Habitaten mit jahreszeitlich stark schwankenden Umweltbedingungen. Die Arktis, die sich durch relativ milde Sommer mit 24 h Tageslicht und kalte, unwirtliche Winter ohne jegliche Sonneneinstrahlung auszeichnet, ist beispielhaft für ein solches Habitat.

Die vorliegende Arbeit untersucht, wie sich in dieser Region lebende und ganzjährig aktive Tiere an solche sich ändernden Umweltbedingungen anpassen. Als Modellorganismus fungiert der Grönlandhund; eine Rasse, die seit mehr als 1000 Jahren selektiert wurde in Grönland zu leben und zu arbeiten. Im Winter werden die Hunde als Schlittenhunde genutzt, während sie im Sommer ruhen. Nur während der Arbeitsphase werden die Hunde hinreichend ernährt. Im Sommer hingegen werden die Hunde nur ein- bis zweimal pro Woche gefüttert, wobei die Qualität des Futters von Ort zu Ort variiert. Zwei verschiedene Hundegruppen, die sich durch ihre Haltungsbedingungen und das Winterklima, dem sie ausgesetzt sind, voneinander unterscheiden, wurden untersucht und miteinander verglichen. Die eine Gruppe (LO-Hunde) erhält nur im Winter ausreichend Nahrung und wird für touristische Schlittenfahrten im milden westgrönländischen Winter eingesetzt. Bei der anderen Gruppe (HI-Hunde) handelt es sich um ganzjährig gut ernährte Hunde, die regelmäßig zu Jagd- und Transportfahrten im harschen nordgrönländischen Winter eingesetzt werden. Die im Rahmen dieser Arbeit durchgeführten Untersuchungen konzentrierten sich auf die Veränderungen des Energiehaushaltes sowie die Anpassungen des Herz-Kreislauf-Systems und der Muskeln des Bewegungsapparates in Reaktion auf jahreszeitliche Schwankungen der Nahrungszufuhr, der Bewegung und der Umgebungstemperatur.

Das **zweite Kapitel** ist der Erfassung des Energiehaushaltes der HI- und LO-Hunde beim Ruhen, Training und Verdauen innerhalb verschiedener Temperaturbereiche (Sommer vs.Winter) gewidmet. Gleichbleibende Ruhestoffwechelraten (RMR) der HI-Hunde innerhalb eines weit ausgedehnten Temperaturbereiches zeigten deren große Thermoneutralzone mit einer unteren kritischen Temperatur bei circa −25°C auf. Diese große Thermoneutralzone ermöglicht den Hunden, die Energieaufwendungen für die Thermoregulation zu minimieren und befähigt sie somit, das extreme Klima der Arktis zu verkraften. Während der Ruhephase im Sommer verdoppelten verdauende Hunde ihren Sauerstoffverbrauch im Vergleich zu fastenden Hunden. Die Verdauungsstoffwechselraten ruhender Hunde unterschieden sich nicht zwischen den Jahreszeiten. Während die HI-Hunde eine ganzjährig gleich bleibende Körpermasse aufwiesen, verloren die LO-Hunde im Sommer bis zu 30% ihres

Körpergewichts. Die negative Energiebilanz dieser Hunde führte zu einem Anstieg des Stoffwechsels bei gleichzeitiger Absenkung der Herzfrequenz. Arbeitende HI-Hunde erreichten die höchste bisher bei Säugetieren gemessene Leistungsstoffwechselrate (12.2 mal höher als die RMR im Sommer).

Die Untersuchungen des **dritten Kapitels** zeigen, dass jahreszeitliche Veränderungen der Umgebungstemperatur, der Aktivität der Hunde und der Nahrungszufuhr zu Größenveränderungen der Bewegungsmuskulatur führen. Untersuchungen der Ultrastruktur von Skelettmuskelbioptaten beider Hundegruppen zeigten im Winter ein normales funktionsfähiges Erscheinungsbild des Gewebes. Im Sommer hingegen waren die Muskelfasern atrophiert, der Sarkoplasmaraum war erweitert und die Sarkomeren enthielten weniger Myofilamente. Des Weiteren war die Zahl der subsarkolemmalen und interfibrillären Mitochondrien reduziert und innerhalb der Fasern befanden sich weniger gespeicherte Fetttröpfchen. Diese Veränderungen waren bei den LO-Hunden stärker ausgeprägt als bei den HI-Hunden. Bei beiden Hundegruppen blieb jedoch das Kapillarnetzwerk innerhalb der Muskeln unverändert erhalten. Diese Ergebnisse illustrieren die differentielle Anpassung der Muskelbestandteile an sich verändernde Bedingungen: einige Komponenten werden flexibel an die aktuellen Anforderungen angepasst, während andere unverändert erhalten bleiben.

Die Untersuchungen, die in **Kapitel 4** beschrieben werden, zeigen, dass sich die großen Blutgefäße im Gegensatz zu den intramuskulären Kapillaren durch die jahreszeitlich schwankenden Umweltbedingungen verändern. Ultraschalluntersuchungen bestätigten bei allen Hunden im Winter einen größeren Durchmesser der Aortenwurzel als im Sommer. Die Dimensionen des linken Ventrikels in Systole und Diastole blieben bei den LO-Hunden ganzjährig unverändert, während der Innendurchmesser des linken Ventrikels bei den HI-Hunden im Winter kleiner war als im Sommer. Die Dicke des interventrikulären Septums blieb jedoch bei beiden Hundegruppen ganzjährig unverändert. Neben den morphologischen Messungen werden in Kapitel 4 auch funktionelle Kurzzeit- und Langzeitanpassungen des Herz-Kreislauf-Systems der Hunde als Antwort auf verschiedene Aktivitätsmodi beschrieben. Während des Ruhens und Verdauens wiesen beide Hundegruppen ein ganzjährig gleich bleibendes Herzminutenvolumen auf. Dieses wurde jedoch um 50% reduziert, wenn die Hunde fasteten. Andere Funktionsparameter des Herzens zeigten differentiell unterschiedliche jahreszeitliche Anpassungen bei den verschiedenen Hundegruppen. Im Winter erhöhten sich Schlagvolumen, linksventrikuläre Auswurffraktion und die partielle Verkürzung des Ventrikels der LO-Hunde, wohingegen sich die Herzfrequenz nicht veränderte. Im Gegensatz dazu hatten die HI-Hunde eine erhöhte Herzfrequenz bei gleich bleibendem Schlagvolumen, linksventrikulärer Auswurffraktion und partieller Verkürzung des Ventrikels.

Die Fähigkeit, eine sehr hohe Arbeitsleistung über längere Zeiträume hinweg aufrecht erhalten zu können, muss ein wichtiger Grund für die Nutzung von Hunden als Arbeitstiere gewesen sein. Wir konnten zeigen, dass Grönlandhunde auf verschiedenen Ebenen des Herz-Kreislauf-Systems und des Muskelapparates differentielle Anpassungen an jahreszeitlich unterschiedliche Anforderungen zeigen. Während einige Komponenten (z.B. Kapillaren zwischen den Muskelfasern und ventrikuläres Septum) in einem Zustand gehalten werden, der den Anforderungen der Arbeitssaison entspricht, werden andere Komponenten (z.B. Muskelfasern und Aortenwurzel) flexibel an die gerade bestehenden Anforderungen angepasst.

Chapter 1

Introduction

1.1. Flexible phenotypes

For numerous species, the conditions of living change seasonally because their habitats undergo considerable seasonal changes in abiotic ecological parameters. In particular day-length, temperature, and humidity may exhibit considerable seasonal changes. Consequently, a plethora of biotic parameters is affected ultimately resulting in sometimes dramatic fluctuations of the availability of food (and other resources) for animals. Migration, hibernation and tolerance are three strategies that mammals evolved to cope with such fluctuations of the environment. This study deals with the third strategy, i.e., “tolerance”. Animals that remain active within changing environments either tolerate a wide range of environmental conditions maintaining a stable phenotype or they flexibly adjust to the changing conditions. The ability of organisms to reversibly adjust their phenotype to changing environmental conditions has been defined as phenotypic flexibility (Piersma and Lindstrom, 1997).The evolutionary conditions that either lead to the stable or the flexible phenotype have never been explored in mammals. One may assume that it is ultimately the energetic cost invested in maintaining a stable phenotype versus the costs involved in flexibly restructuring a phenotype that determines whether a phenotype is stable or flexible. Applying the optimization principle of evolution one will assume that the energetically optimized phenotype will be favored by evolution.

1.2. Evolutionary optimization and the concept of symmorphosis

Evolutionary optimization has been conceptualized as “symmorphosis” (Weibel et al., 1981). Symmorphosis is built on three basic ideas: (1) maintenance of an organ system incurs energetic costs to an organism; (2) therefore the functional capacities of an organ (system) are adjusted to its functional demands to avoid costly excess capacity, and consequently (3) different organ systems of an organism match quantitatively in design and functional parameters to optimize energy expenditure for maintenance of an organism. As a corollary, varying functional demands on the system will elicit a concerted adaptive response of different organ systems so that the organism adjusts to the changed condition. This would result in an optimization of structures, i.e., “economic design”. The concept of symmorphosis has been challenged at different occasions and various concerns have been presented that symmorphosis is not testable in a scientific context (Garland, 1998; Gordon, 1998). For example, one problem is that an organ system may perform multiple functions simultaneously which probably creates constraints that prevent reaching an optimal solution for all processes. Thus, components in a system that seem to contradict the symmorphosis concept may have other dominant functions that require a different design. Therefore, the establishment of the dominant function of a given component in a system would be necessary in order to evaluate whether it follows the principles of economic design. Another problem is the possible implementation of safety factors (Diamond, 1998). For example, limb bones have a strength that exceeds the maximum expected load imposed on them, so that the bones will not break when maximally loaded. If structures were designed so that their functional capacity would exactly match the average expected demand, than the system would be in danger of exceeding the capacities beyond the limit in situations of excess demands. However, if we consider that resources and space are limited within an organism, excess capacity devoted to one component of the system comes at the expense of another. Therefore, excess capacity might be helpful as safety factor but it is difficult to decide whether or not it is crucial for survival or simply a wasteful use of resources (McNeill Alexander, 1998).

Studies that have tested the upper and the lower ceilings of "animal systems" have shown that there are limitations to the performance and to the physiological adaptation of an animal (Hammond and Diamond, 1997; Wu et al., 2009). Sustained metabolic rates (susMR) are defined as the time-averaged energy budget that an animal is able to maintain over extended periods of high physical activity without changes in body mass, so that energy intake equals energy expenditure. The ratio of susMR/RMR, where RMR is resting metabolic rate, i.e., sustained metabolic scope, describes an animal's capacity to up-regulate energy expenditure and to endure sustained energetic load of a certain quality. Hammond and Diamond (1997) compared sustained metabolic scopes of 50 species of wild animals. All measured values of sustained metabolic scope fall in the range from 1.3 to 7. To explore the existence of upper metabolic ceilings, these authors exposed laboratory animals to different modes of energy expenditure in the presence of excess amounts of food. If an upper limit existed, the excess load would push the experimental animals to the maximal sustained metabolic scope. Any upper limit would be recognized by the failure of the animals to process more food to gain the necessary amount of energy (central limitation). For example, if subjected to low ambient temperatures, laboratory mice reacted with increased heat production by non-shivering thermogenesis. They were able to maintain body mass by increasing food intake to fuel the increasing metabolic rate down to ambient temperatures of −15°C. If temperatures were reduced further these mice lost body mass and ultimately died after several days in the presence of excess food. The results of these experiments were interpreted as an example of exceeding the ceilings of low temperature tolerance, because the mice were not able to maintain body mass in the presence of sufficient food; i.e., central limits of processing, absorbing and metabolizing food constrained the maximum amount of energy absorption to fuel the response to low temperatures. Interestingly, when subjected to a different mode of energy expenditure, the mice display different ceiling values. While sustained metabolic scope peaked at 4.8 for heat production at low ambient temperatures, the ceiling was 6.5 when pushed to maximum susMR while producing milk for experimentally increased litters. When applying two stresses (cold temperatures and lactation) to the mice they increased food intake above levels seen when applying only one stressor alone. Although susMR of such mice rose even higher, RMR increased too, thus keeping sustained metabolic scope constant.

In contrast to laboratory experiments, animals living in natural settings are usually subjected to more than one stress factor. For example, animals living in the Arctic have to cope with environmental factors like cold temperature, absence of daylight, and aridity during winter. Because many prey animals of the Arctic migrate away by the end of the productive and mild summer or hibernate through the winter, the stationary and active carnivores have to cope with the lack of food and temperature stress.

1.3. Experimental framework

While the arctic summer is the time of abundant food and favorable climatic conditions that helps building up energy depots, winter is the period of famine. An exception to this rule is the seasonal life of the Inuit sled dogs which live in semi-natural conditions of dog husbandry by the Inuit in Greenland. Under these conditions, winter is the active period and the period when enough food is available to the dogs. For probably more than 10000 years, dogs were the only domesticated animals of the inhabitants of the Arctic. The dogs were company in daily life and during hunting; they guarded the camps and cleaned the settlements by scavenging for food leftovers. They provided fur for clothing, warmth during nights, and were a welcome source of food in times of famine. Although dog remains found in West Greenland date back to ca. 4000 B.P. (Fitzhugh, 1984), the intense use of dogs as draft animals is thought to have started little more than 1000 years ago with the emergence of the Thule culture (Morey and Aaris-Sorensen, 2002). Since then, working as draft animal has become the most important task of Inuit sled dogs. These dogs have been selectively bred to withstand extreme arctic winter conditions, to perform sustained work in front of the sled, and to endure long periods of famine. The combination of these characters and their ability to perform hard work even in times of famine ensured the survival of the Inuit.

1.4. Traditional dog husbandry in the Arctic

Inuit sled dogs were free to roam around during arctic summer living a more or less independent life from man. Sometimes they were brought to small and uninhabited Islands to keep marauding packs of scavenging dogs away from human settlements. Several authors (Hawkes, 1916; Quervain and Stolberg, 1911; Rikli and Heim, 1911) describe how the dogs freely feasted on millions of small fish (*Mallotus arcticus*) that come to the shallow water in June to spawn. Grenfell (1913) describes the hunting habits of the dogs: *"The Eskimo dog takes kindly to the water in summer. He will go fearlessly after fish. When the caplin run ashore, the dogs half starved after the winter (like most other animals), almost live in the water, eating their fill till they are like ambulatory barrels."* Visitors of Inuit camps in West Greenland repeatedly pointed out to the important role of the free ranging dogs to keep the Inuit camps clean by eating any hunting leftovers as well as human feces (Rikli and Heim, 1911; Trebitsch, 1910; Whittaker, 1937). Whittaker (1937) wrote about dog husbandry: *"While a dog is working, either in summer or winter, he is carefully fed….if idle in summer, however, they may not be fed at all, being turned loose to fend for themselves."* Rikli and Heim (1911) reported that dogs kept in Northern Greenland play a vital role in Inuit live for transportation and hunting, whereas the dogs kept further south in Western Greenland are mainly kept as pelt provider for clothing and are infrequently used as sled dog. The authors discussed the missing solid sea ice during winter further south compared to the thick ice cover in North Greenland as reason for the different use of dogs. Gilberg (1984) stated that among the Inuit of the Thule District, the dog sled was the most important means of transportation used up to ten months of the year. The original number of dogs in a team was 4−5 dogs. With the invention of the gun during the early 20th century it increased to 6−8 dogs because increased hunting success required higher transportation capacity and allowed the maintenance of larger dog teams, usually one female among male dogs. In recent years and with the advent of an intensive trade economy, the team size increased to 10−15 animals (Peplinski, 1996).

1.5. Modern Inuit sled dog husbandry

The population of Inuit sled dogs in Greenland declined from 40000–70000 in 1910 to about 21000 in 2005. Dog sledding takes place mainly on frozen sea ice. Inuit sled dogs are always working in a fan hitch formation which is different from western style where dogs are grouped in pairs on a long line in front of the sled (Fig. 1). Each dog is tied individually to the sled, thus the entire team is running next to each other spreading over a wide area. Running in a fan hitch formation is possible only on sea ice but has several advantages, e.g., if one animal breaks through the ice the other team members are not dragged into the crevasse and the sled is not lost.

Since the 1960s Danish laws prohibit the import of any other dog breed north of the Arctic Circle, thus keeping the Inuit sled dogs genetically isolated. As already noticed by Rikli and Heim (1911), dog use differs within Greenland. While dog sledding is merely a recreational activity or tourist attraction in West Greenland, the dogs in North Greenland are important helpers used for transportation and hunting during winter (Fig. 2). Because dog sledding takes mainly place on frozen sea ice the working period of dogs gets shorter with global warming

Fig. 1. Inuit sled dogs working in a fan hitch formation. The picture was taken in February 2008 during a research trip in the Thule district of Northern Greenland.

Fig 2. Map of the Arctic region. Red arrows show the study sites in West Greenland (Qeqertarsuaq on Disko Island) and North Greenland (Qaanaaq). URL: http://www.world-maps.co.uk/maps/600-arctic.jpg

and the shortening of the ice cover period of the sea. Within the last 30 years, the sledding period was reduced from eight or ten months to no more than six months per year in Northern Greenland.

Today, it is prohibited to have dogs older than six months roaming freely in a settlement and each village employs a dog shooter to enforce this law. At all times when not performing work in front of a sled, the dogs have to be chained to rocks. While these rules ensure safety for humans and prevent fighting among dog teams, it makes the dogs fully dependent on the continued care by their owners. This creates new problems because traditionally, the Inuit fed only working dogs. During summer when the dogs were not working they had to find their own food by hunting small mammals, birds or fish, or by scavenging through the human settlements. Today, depending on fishing and hunting success of their masters, the dogs are fed once or twice per week during summer with either energy rich (e.g. seal meat in North Greenland) or energy poor (e.g. cod in West Greenland) food. This leads to potential undernourishment during summer, especially in the population in West Greenland. With the confinement the dogs are forced to a lazy life without exercise from spring to fall. Other problems arise from chaining the dogs permanently, e.g., no adequate hiding places from sun and rain, insufficient availability of water, increased aggression among dogs, injuries caused by entangled chains. Therefore, Greenlandic dog husbandry increasingly gets into the focus of international animal welfare organizations.

1.6. Aims of this thesis

The objective of this dissertation work was to study structural and/or functional adaptations of a mid sized mammal to changing environmental conditions. Structural and functional adaptations were investigated in different organ systems. We started by examining seasonal changes of daily energy partitioning and studying size changes and structural changes of peripheral organs like the locomotor muscles that define the energetic demand. We then continued by studying the central supply machinery, i.e. the heart and large blood vessels.

The study reported in **Chapter 2** focuses on the question how Inuit sled dogs adjust their energy metabolism to the changing living conditions. One key question was whether an increase in RMR was induced during winter because of low temperature, elevated work load, and higher food intake. Conversely we were asking if the mild summers spent resting and often fasting would induce a down-regulation of RMR, thus reducing energy expenditure. Determining RMR requires the knowledge of the thermoneutral zone of an individual. When measuring metabolic rate in Inuit sled dogs over a broad range of temperatures from 15°C during summer down to −25°C during winter we gathered data on the thermoneutral zone of the Inuit sled dog under field conditions. After establishing the baseline of energy consumption in the field, RMR, the investigations focused on the metabolic scope of the dogs. To define the sustained metabolic scope, energy expenditure during maximal work had to be defined. Measurements were done during winter when the dogs are pushed to maximum sustained metabolic rates when performing sustained work in front of the sled.

Metabolic rate is also elevated in postprandial animals, a process termed specific dynamic action (SDA). The first systematic examination of the increase in metabolic rate in response to food intake was conducted in dogs by Max Rubner (1902). The original German phrasing was "Die spezifische dynamische Wirkung der Nahrungsstoffe" which was translated into the distorted English term "specific dynamic action", but, specific dynamic action has since then become established in the literature. The energetic costs associated with SDA are the result of numerous preabsorptive, absorptive, and postabsorptive processes and many studies have been conducted to characterize SDA in animals (review: McCue, 2006). The increase of energy expenditure due to SDA can be calculated by integrating energy expenditure over the period of digestion minus the RMR. During summer, the Inuit sled dogs are fed intermittently, but when they are fed they usually ingest large amounts of food (approximately 15% of their body mass). This feeding regime provided a good experimental framework to measure the increment of energy spent on SDA in the dogs.

If energy metabolism increases with rising work load during winter, one could ask how the machinery that performs the work is adjusted to the increased activity. **Chapter 3** deals with the question whether and how muscle tissue of locomotor muscles is morphologically adjusted to the seasonally changing work load and food supply. If muscles were designed to exactly meet the demands imposed on them as predicted by the concept of symmorphosis, one would expect to find morphological changes in the muscles of dogs performing intensive work during winter when compared to muscles of resting dogs during summer. Moreover, one would expect muscles to be morphologically adjusted to the degree of work intensity performed during winter. Dogs that are used infrequently for dog sledding should then possess muscles that differ from muscles of dogs performing work on a more intensive level. To explore this hypothesis, examinations of muscle tissue from dogs were conducted at two study sites differing in work load during winter. Another question was whether adjustments to certain levels of exercise were found to the same extend in all components of the muscle apparatus. An alternative would be that some components of locomotor muscles maintain a stable phenotype throughout the year while other components were adjusted flexibly to the changing demands of the different seasons. If the reconstruction of a structure is energetically more costly than its maintenance than it would be of advantage to maintain the structure throughout the year. However, if energy costs for maintaining a given component of a system exceed the costs for reconstructing that component, than flexible up-and-down regulation would be more economic. These questions were addressed by comparing muscle tissue of the same dogs during the inactive summer and working winter season. The nutritional state of an animal also influences the composition of organs such as the muscles (Boonyarom and Inui, 2006). A comparison of muscle tissue between the LO-dogs that lost up to 30% of their body mass during summer and the HI-dogs, that had a stable body mass throughout the year allowed the evaluation of the impact of nutrition on the ultrastructure of locomotor muscles.

During winter when working, the dogs' muscles need more oxygen than when resting during summer. One could ask how the supply machinery, the cardiovascular system is coping with the changing demand. In **Chapter 4**, investigations focus on structural and functional adaptations of the cardiovascular system of Inuit sled dogs to the seasonal changes in environmental conditions. Again the comparison between costs spent for maintaining structures and costs invested in morphological adjustments to actual demands is the key for understanding the pattern of change in the system. Taking a portable ultrasonography unit out to the dogs' places in the field enabled a repeated evaluation of the dimensions of the heart in systole and in diastole and the measurement of the diameter of the aortic root in winter and in summer condition. The continuous use of heart rate monitors added the possibility to evaluate functional responses of the heart to the different modes of activity. Literature data of physiological and morphological responses of the heart of dogs to exercise are fairly inconsistent. For example training resulted in decreased resting heart rate in a study conducted by Stepien et al. (1998) while Mackintosh et al. (1983) report no changes, and Pape (1985) even documented higher resting heart rates in trained versus untrained dogs of the same breed. A possible explanation of these contrasting results is that the studies applied different training regimes to different dog breeds. With respect to Inuit dogs and the multi-factorial changes in their living conditions we expected cyclic restructuring of the heart and the large blood vessels but were not explicit about the directionality of these processes.

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Chapter 2

Energy metabolism of Inuit sled dogs

2.1. Abstract

We explored how seasonal changes in temperature, exercise and food supply affected energy metabolism and heart rate of Inuit dogs in Greenland. Using open flow respirometry, doubly labeled water, and heart rate recording, we measured metabolic rates of the same dogs at two different locations: at one location the dogs were fed with high energy food throughout the year while at the other location they were fed with low energy food during summer. Our key questions were: is resting metabolic rate (RMR) increased during the winter season when dogs are working? Does feeding regime affect RMR during summer? What is the proportion of metabolic rate (MR) devoted to specific dynamic action (SDA), and what is the metabolic scope of working Inuit sled dogs? The Inuit dogs had an extremely wide thermoneutral zone extending down to −25°C. Temperature changes between summer and winter did not affect RMR, thus summer fasting periods were defined as baseline RMR. Relative to this baseline, summer MR was up-regulated in the group of dogs receiving low energy food, whereas heart rate was down-regulated. However, during food digestion, both MR and HR were twice their respective baseline values. A continuously elevated MR was observed during winter. Because temperature effects were excluded and because there were also no effects of training, we attribute winter elevated MR to SDA because of the continuous food supply. Working MR during winter was 7.9 times the MR of resting dogs in winter, or 12.2 times baseline MR.

2.2. Introduction

Mammals living in the Arctic experience extensive seasonal fluctuations in their living conditions. During the arctic summer, abundant food, mild climate, and 24 hour sunlight provide a thriving environment. In contrast, during winter food is scarce, temperatures can be extremely low, and permanent darkness restricts activities. To avoid such adverse winter conditions, some mammalian species migrate to areas where the environmental conditions are more favorable (e.g., caribou *Rangifer tarandus* L.) and others rest in dens (e.g., bears *Ursus arctos* L., *Ursus americanus* P., *Ursus maritimus* P.). However, some mammals are active throughout the year, adjusting their morphology and physiology to the fluctuations in their environment (e.g., the polar fox *Alopex lagopus* L., musk ox *Ovibos moschatus* Z., and wolf *Canis lupus* L.). We are particularly interested in how mammals adjust to their fluctuating environment while being active throughout the year. We have chosen Inuit sled dogs (*Canis lupus familiaris* L.) as a model system to study such seasonal adjustments because of their easy accessibility, uniform living conditions within each team, and willingness to cooperate in non-invasive physiological studies.

When kept under local husbandry conditions in Greenland, Inuit sled dogs experience the combined challenges of seasonally changing temperature, work load, and food supply. This combination may be unique because the dogs do not have control over whether they work or not. In contrast to all other mammals living in the Arctic, Inuit dogs are active mainly during winter, pulling sledges on hunting trips. During this period, they receive sufficient food and their energy budget is balanced. During summer, when temperatures are relatively mild and no sledding is possible, dogs remain chained to rocks and are fed only every 3–4 days. Depending on local husbandry tradition and hunting success, during summer the dogs either receive high energy food (e.g., seal *Phoca hispida* S., walrus *Odobenus rosmarus* L., and whale *Monodon monoceros* L.) and remain in positive or neutral energy balance despite long feeding intervals, or they receive low energy food (e.g. fish) and are in negative energy balance. Thus, both the amount and quality of food they receive changes seasonally.

Surprisingly, not much is known about the thermoneutral zone of Arctic canids (Korhonen et al., 1985; Remmert, 1980; Scholander et al., 1950a). Often cited data of the thermoneutral zone reaching to −25°C are inappropriate extrapolations from an early paper by Scholander (1950b). The thermoneutral zone observed for other domestic dog breeds cannot be equated with that of Inuit dogs, because of substantial differences in the thickness of insulating fur (Mussa and Prola, 2005).

Exercise increases energy expenditure. For racing Alaskan sled dogs, Hinchcliff and Reinhart (1997) measured a daily energy expenditure (DEE) of 47100 kJ per day. This would be 8–9 times above the average daily energy intake of untrained Siberian huskies in temperate climate conditions (Finke, 1991). Although differences in conditions and breeding lines between Alaskan and Siberian dogs mean that this composite factor may be an overestimation, it nonetheless suggests that working sled dogs likely display among the highest values reported for Sustained Metabolic Scope (SusMS, DEE/BMR, *sensu* Hammond and Diamond 1997) of any mammal. Measurements of both Resting Metabolic Rate (RMR) and DEE on the same dogs would confirm this. The instantaneous costs of hunting of African wild dogs *(Lycaon pictus)* are 25 times BMR, and the measured DEE represented a SusMS of about 5.2×BMR for this species (Gorman et al., 1998). The highest sustained energy budgets in humans are 4.3 times their BMR, achieved by Tour de France cyclists (Westerterp et al., 1986). Although instantaneous energy expenditure in endothermic animals may be very high, Hammond and Diamond (1997) reported a maximum SusMS of seven times RMR for lactating mice in the cold, confirmed by Johnson et al. (2001) the same species in excessive reproductive mode (pregnancy and lactation). Speakman et al. (2004) reported that, in laboratory mice, lactation in the cold gives a scope of approximately 8.9 times RMR.

Food processing requires energy to initiate the active processes of enzyme secretion, nutrient absorption of nutrients, and to fuel follow-up protein synthesis (specific dynamic action, SDA). While these processes certainly have only a minor effect on daily energy expenditure of exercising animals, they may well comprise a more substantial fraction of metabolic rate in resting animals. Specific dynamic action is the summed increase in metabolic rate that accompanies the processes of digestion, absorption, transport and assimilation, and accounts for approximately 10% of total DEE in humans (Westerterp, 2001). In contrast, fasting may cause RMR to decline (Fuglei and Øritsland, 1999; Ostrowski et al., 2006; Rosen and Trites, 2002).

We aimed to understand how the combined effects of seasonal changes in temperature, food supply, and exercise affected RMR in Inuit sled dogs. Specifically, we ask the following questions: (1) is resting metabolic rate (RMR) increased during the winter season when the dogs are working? We predict that increased exercise would result in an elevation of RMR. (2) Does feeding regime affect RMR during summer? While working in Greenland we realized that local feeding habits may affect the dogs' metabolic state. Therefore, we hypothesized that intermittent feeding during summer may result in a depression of metabolic rate (saving energy relative to dogs that were in a balanced energy budget throughout the year. (3) What is the proportion of metabolic rate (MR) devoted to specific dynamic action (SDA)? The exact knowledge of SDA is crucial when partitioning DEE. (4) Finally, what is the metabolic scope of working Inuit sled dogs?

Working under field conditions in the high Arctic, the experimental framework did not permit a full cross-over study design because the dogs were needed for transportation and hunting in winter and because physiological constraints (overheating) prohibited work in summer. Also, temperatures below the lower critical temperature may have confounding effects on energy metabolism. Characterization of the thermoneutral zone was therefore required.

Besides oxygen consumption, heart rate (HR) is used as an alternative measure of energy expenditure of an animal (Butler et al., 2004). The heart rate in mammals increases in immediate response to various factors, e.g., sustained work (Sanders et al., 1977), low temperature (Korhonen et al., 1985), presumably digestion, and behavioral and emotional stress (Beerda et al., 1998; Palestrini et al., 2005). Heart rate may also show longer term responses. Prolonged training results in a reduction of both resting and exercising HR in dogs (Wyatt and Mitchell, 1974). Also, undernutrition results in reduced HR in dogs (Alden et al., 1987) and in humans (Romano et al., 2003). To establish a relationship between heart rate and energy expenditure, all measurements of metabolic rates in this study are accompanied by HR measurements.

2.3. Materials and methods

2.3.1. Research site

In summer 2005 and winter 2006, research was conducted in Qeqertarsuaq on Disko Island at 69°15′N, 53°32′W. There, according to local husbandry traditions, the dogs are fed with low energy food during summer. In summer 2007 and winter 2008, we worked in Qaanaaq at 77°28′N, 69°20′W, where the dogs are fed with high energy food year-round.

2.3.2. Dogs

We studied male dogs from three teams kept in local husbandry conditions. A team of 12 dogs was studied in July/August 2005 and in February/March 2006 in Qeqertarsuaq. During winter, these dogs were used as sled dogs once or twice per week, but remained chained to their places at all other times. Because of poor feeding in the summer, they had a significantly lower body mass during summer (19.1±1.6 kg) than in winter (27.3±2.7 kg). Repeated veterinary examinations revealed signs of chronic malnutrition (see also Gerth et al. 2009). During summer, all these dogs were underweight and infested by intestinal parasites. We labeled this group of dogs as “low energy intake (LO)-dogs”. In Qaanaaq, we studied two teams of six dogs each during July/August 2007 and during February/March 2008. These dogs were used frequently for hunting and transportation in winter. The dog teams from Qaanaaq were in good condition and healthy throughout the year. Their mean body mass was 33.7±2.7 kg and 33.2±3.0 kg, during summer and winter respectively. This group of dogs was labeled “high energy intake (HI)-dogs”.

2.3.3. Feeding

Traditionally, Inuit dogs are fed every second day during winter but only once or twice per week during summer. However, their energy balance is ultimately determined by the quantity and the quality of their food. The HI-dogs received high energy food (whale: 100 g=464 kJ, 27 g protein, 0 g fat; walrus: 100 g=1180 kJ, 16.3 g protein, 24.1 g fat; seal: 100 g=596 kJ, 28.4 g protein, 3.2 g fat; USDA, 2009) throughout the year while the LO-dogs received low energy food, i.e., fish (cod: 100 g=343 kJ, 17.8 g protein, 0.6 g fat; USDA, 2009) during summer, but high energy food during winter. This enabled us to make three comparisons: summer versus winter within each feeding group, and high versus low energy food during summer, i.e., between groups. We recorded precisely the amount of food offered to the dogs by the hunters using an electronic balance (VMC VB-301-6000 portable digital scale balance, 6000−1 g, Precision Weighing Balances, Bradford, MA, USA). In the LO-dog group, we applied temporarily a standardized feeding regime that mimicked the local conditions. During these feeding trials, a meal was offered every fourth day. One meal consisted of 15% of the dog's body mass of fresh fish (2.5−3.6 kg fish per dog). In winter, the LO-dogs received dried fish or frozen seal meat daily (approximately 150–700 g per dog, i.e., 0.5–2.5% of body mass). While the total amount of food received per 4-day period did not differ much between winter and summer, the energy content of the food differed between the seasons.

The HI-dogs were fed every second or third day with seal, walrus or whale meat during summer. Meal size was 1–2 kg meat per dog. In winter, these dogs received melted and heated chunks of seal or walrus meat every other day (meal size: approximately 2 kg per dog). Only during hunting trips in winter, dog food was supplemented with commercial pelleted food (Nukik Polar, A/S Arovit Petfood, Esbjerg, Denmark, energy content 100 g=2100 kJ; 34 g protein, 30 g fat).

2.3.4. Working conditions

For the HI-group, working energy expenditure was measured during two three-day long sled trips on the sea ice (see below). The dogs were running on average 8 h per day on smooth sea ice with little snow, and no elevation. Two breaks were made during each day. Daily running distances were 60–80 km at an average speed of 9 km/h. Running speed, distances, and duration were recorded using GPS (GPSMAP 60CSx, Garmin International Inc, Olathe, KS, USA). One-hour averages of environmental temperature ranged between −33°C and −14°C for tour 1, and between −26°C and −2°C for tour 2.

2.3.5. Temperature recording

We recorded environmental temperature using temperature data loggers with an On-Chip Direct-to-Digital temperature converter with 11-Bit (0.0625°C) resolution (i-button DS2422, Maxim Integrated Products, Inc., USA). The loggers have an operating range from –40°C to +85°C at 0.0625°C resolution. Environmental temperature was recorded every 10 min at the dogs' home place. From these 10-min intervals we calculated 1-h averages.

2.3.6. Respirometry

We used a portable, open flow respirometry system originally designed for human exercise physiology (MetaMax III-X, Cortex Biomedical GmbH, Germany). We adjusted the system for use with dogs. It consists of an air-tight face-mask with the spirometry turbine, a small unit containing the pump for sub-sampling air from the mask, the O_2- and CO_2 sensors, and a radio transmitter for wireless data transmission to a remote PC. The data recording software was adjusted to the low tidal volume (minimum value 200 ml) of resting dogs. O_2 consumption $(\dot{V}O_2)$ of each dog was measured 1–3 times for 30–45 min while fasting (i.e. 2nd, 3rd, 4th day post-feeding), while digesting (i.e., 1st day post-feeding) and while walking. The lowest 5-min averages of each trial were extracted for analyses. Sample sizes are given with the data in Section 2.4. Measurements were limited by temperatures below −20°C by the operating temperature of the equipment.

2.3.7. Energy budget

Measurement of metabolizable energy requires input-output comparisons (input=food intake; output=urine and feces). To collect feces quantitatively, we designed carry-on devices for the dogs and collected feces immediately when they were delivered, but did not collect urine, all data are "apparent metabolizable fecal energy". Data were obtained from three feeding trials in 2005, each with 12 dogs.

The analysis of food samples and feces followed standard laboratory protocols. Total wet mass of all food and fecal samples were obtained in the field. Also in Greenland, complete samples (3–4 whole fish per species, and complete fecal samples for each dogs) were dried to constant weight at 45–55°C (3–4 days was sufficient). These were re-weighed to determine water content, homogenized using a commercially available coffee grinder, and stored dry in sealed packets until return to the laboratory in Stellenbosch. There, complete samples were re-homogenized before sub-sampling for determination of energy content. Duplicate 0.3–0.5 g sub-samples were burned in a CP 500 dry bomb calorimeter (D'Amico Sistemas and Digital Data Systems, Buenos Aires, Argentina). If values differed by more than 2%, a third duplicate was run. Daily energy intake was extrapolated from the energy content of one meal over a 4-day feeding trial in summer 2005, and energy content of one meal (average 1.5 kg) over an average period of 2.5 days in summer 2007. Energy content of one meal per day was calculated to obtain daily energy intake in winter 2006 and 2008.

2.3.8. Doubly labeled water

To supplement metabolic data obtained using open-flow respirometry, we used the doubly-labeled water technique (Butler et al., 2004) to measure daily energy expenditure (DEE) of the dogs while resting in summer 2007, and while resting and running in winter 2008. The DLW technique has been found to provide an accurate estimate of energy metabolism for domestic dogs (Speakman et al., 2001). Background samples of blood were collected prior to isotope injection (Speakman and Racey, 1987). Exact amounts ($0.3\ \mathrm{ml\,kg^{-1}}$), of doubly labeled water ($D_2{}^{18}O$; ^{18}O=68%) measured with a balance (Pocket Pro PP-62, VWR International GmbH, Germany, 60 g×0.01 g) were injected subcutaneously. Initial samples were collected six hours post injection as recommended by Speakman et al., (2001). During summer 2007, blood was sampled every other day. Measurements spanning several days minimize the large day to day variability in DEE estimates (Berteaux et al., 1996; Speakman et al., 1994). During winter 2008 blood samples were taken at days 4 and 7 after injection; the dogs were pulling sledges on hunting trips between days 1 and 3 after injection, and resting the remaining time. All blood samples were taken from the cephalic vein of the left leg of the standing dog using injection needles (19 g Sterican, B. Braun Petzold GmbH, Germany). The blood was collected in heparinized 2 ml plastic tubes (Eppendorf, Germany), and then sealed in 100 µl glass micropipettes (intraMARK BLAUBRAND, Brand GmbH + Co KG, Germany; n=4 per blood sample), and stored at room temperature. Blood samples were vacuum distilled (Nagy, 1983), and the resulting distillate was used to produce CO_2 (Speakman et al., 1990) and H_2 (Speakman and Krol, 2005). The isotope ratios ^{18}O:^{16}O and ^{2}H:^{1}H were analyzed using gas source isotope ratio mass spectrometry (Optima, Micromass IRMS and Isochrom µG, Manchester, UK). Samples were run alongside three lab standards for each isotope (calibrated to International standards) to correct delta values to ppm. Previous validation work in dogs has suggested that two pool models work best (Speakman et al., 2001), as is generally the case for this size of animal. In the validation, the best equation was from Speakman (1993), however, this still gave an estimate of energy expenditure 6% greater than the reference method. This slight overestimate was probably because of the assumptions about evaporation used in the Speakman 1993 equation.

There are several approaches for the treatment of evaporative water loss in the DLW calculations (Visser and Schekkerman, 1999). In the present study we used an updated version of the two-pool equation from Speakman (1993) [equation 7.43 from Speakman 1997 with an RQ of 0.73] which assumed evaporation of 25% of the water flux (Speakman, 1997). This assumption has been shown to minimize error in a range of conditions (van Trigt et al., 2002; Visser and Schekkerman, 1999). During the labeling periods we also measured continuously activity and heart rate of the labeled dogs. By partitioning the DLW data according to activity and by comparing those values with RMR obtained by respirometry we present estimates of energy expenditure during different activities of the dogs. All methods applied to measure metabolic rates of dogs in different conditions, seasons and locations are summarized in Table 1.

Table 1. Methods applied to measure MR of dogs in different conditions.

	Qeqertarsuaq (LO-dog group)		Qaanaaq (HI-dog group)	
Condition	Summer 2005	Winter 2006	Summer 2007	Winter 2008
Resting	OFR, HR	OFR, HR	OFR, HR, DLW	HR, DLW
Digesting (SDA)	OFR, HR	OFR, HR	OFR, HR, DLW	HR, DLW
Walking on a leash	–	OFR	–	–
Running in front of the sled	–	–	–	HR,DLW

ORF open flow respirometry, *HR* heart rate recording, *DLW* doubly labeled water method

2.3.9. Activity monitoring

We recorded the activity of HI-dogs using activity monitors (ActiTrac, IM Systems Inc., Baltimore) fixed to the dogs' collars. Movements trigger a piezoelectric accelerometer that records acceleration in two planes at 0.012 g precision. Acceleration is sampled 40 times per second and integrated over 2 min to quantify activity during that period.

2.3.10. Heart rate

Heart rate monitors (Polar S610i, Polar Electro GmbH, Germany) originally designed for human athletes were used to record heart rate continuously. The heart rate monitors were set to 5 s recording intervals, resulting in a maximum recording period of 23 h 20 min. Data were downloaded daily. After downloading, the data logger was started again, thus recording was interrupted for approximately 30 min only. The flexible belt with the electrodes was placed around the dogs' chest immediately behind the front legs. To ensure signal transmission to the electrodes, the dogs' fur was moistened with a 0.5% aqueous solution of a sodium polymer (Gelbildner PNC 400, OMIKRON GmbH Naturwaren, Germany). During winter a layer of neoprene (7 mm) was added onto the belts to keep the animal warm and to stop the wet belt from freezing. For later analysis, the data were averaged as 1-h intervals. We recorded up to 23 days continuous heart rates of 12 dogs.

2.3.11. Statistics

Before analysis, all data were tested for normal distribution and equality of variances. When data failed these tests we used a Kruskal−Wallis one-way ANOVA for ranks or a Mann−Whitney *U* test to test for differences between groups. When data were normally distributed, we used ANOVA or t-test as stated in the text.

2.4. Results

2.4.1. Temperature

Temperatures differed significantly between seasons and locations (Kruskal−Wallis one-way ANOVA: $H=119.014$ $df=3$, $p<0.001$; Fig. 1). Winter temperatures in Qeqertarsuaq (LO-dog group) were significantly milder than winter temperatures in Qaanaaq (HI-dog group; Dunn's pairwise multiple comparisons, difference of ranks=32.4, $q=3.3$, $p<0.05$). Summer temperatures did not differ between locations. Of course, summer temperatures were significantly higher than winter temperatures in Qeqertarsuaq: (difference of ranks=64.6, $q=6.5$, $p<0.05$) and Qaanaaq, respectively (difference of ranks=75.1, $Q=8$, $p<0.05$).

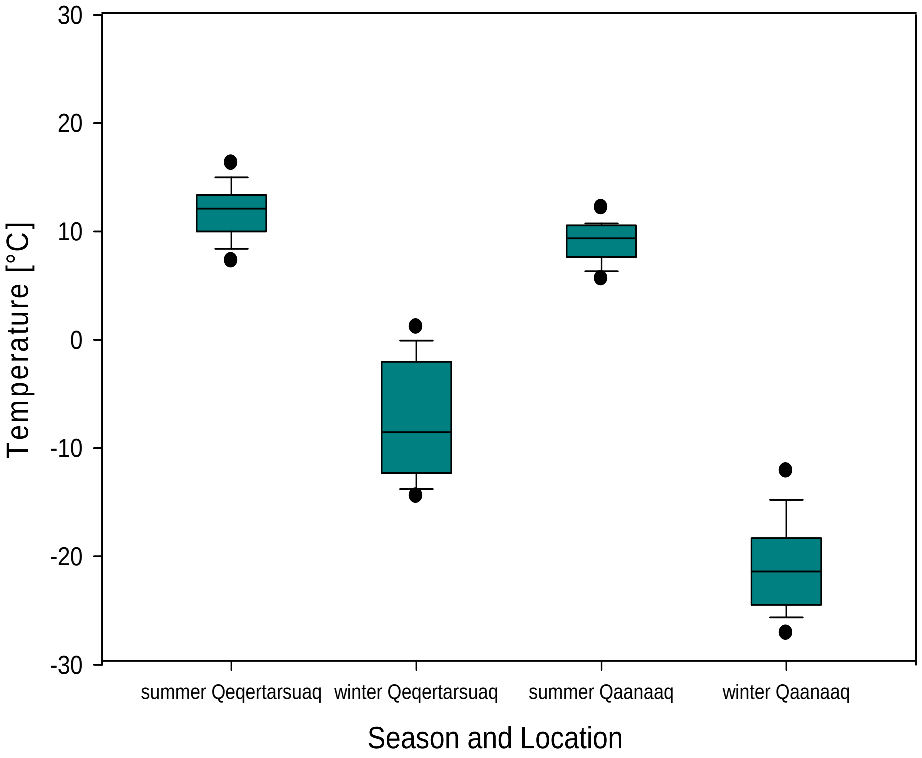

Fig. 1. Environmental temperatures at the study locations during field work in summer and winter. *Box plots* are daily temperature created from daily means of 32 days (26.07.2005–26.08.2005) in summer and 33 days (27.02.2006–31.03.2006) in winter in Qeqertarsuaq where the LO-dogs were located. In Quaanaaq, the location of the HI-dogs, we recorded temperatures for 39 days (27.07.2007–03.09.2007) in summer and for 34 days (28.02.2008–01.04.2008) in winter.

2.4.2. Energy expenditure

Respirometry

Intergroup comparisons of mass-specific values for MR may have been biased by weight differences between groups (Packard and Boardman, 1999). Therefore, we first tested for a possible effect of differences in body mass using an ANCOVA with resting oxygen consumption as dependent variable, HI- and LO-groups and season as main factors, and body mass as covariate. The general linear model was highly significant, but body mass was not a significant covariate (GLM: df=4, F=13.8; $p<0.0001$; body mass: df=1, F=2.9, p=n.s.). Therefore, we assumed that calculation of mass-specific oxygen consumption rates appropriately accounts for the effect for body mass within the range of dog sizes in our study, and we used these rates for all further analyses.

During summer, mass-specific oxygen consumption of LO-dogs (n=5) averaged at 6.9±1.2 ml O_2 kg^{-1} min^{-1}. Oxygen consumption of HI-dogs averaged at 4.6±0.7 ml O_2 kg^{-1} min^{-1} (n=11 dogs). During winter, we repeatedly measured oxygen consumption between 0°C and −10°C, and could not detect increased consumption with decreasing temperatures (t-test, df=10, t=0.696, p=n.s.). Therefore, all measured values within this temperature range were pooled. Average oxygen consumption of LO-dogs (n=7) was 8.2±1.1 ml O_2 kg^{-1} min^{-1}. Extrapolating from the measured proportions of summer DEE attributable to RMR, and to MR during activity and digestion, we calculated RMR in HI-dogs as a fraction of DEE obtained from DLW measurements during resting at −25°C (chained to the rocks). Resting MR was estimated to average at 8.1±0.3 ml O_2 kg^{-1} min^{-1} (n=8), indistinguishable from the winter RMR of LO-dogs.

Following a meal, metabolic rate increased as a result of SDA. 24 h after feeding $\dot{V}O_2$ was 11.2±3.7 ml kg^{-1} min^{-1} and 9.6±2.6 ml kg^{-1} min^{-1} for LO-dogs (n=5) and HI-dogs (n=7), respectively. Oxygen consumption during SDA did not differ between both groups (t-test), therefore data were pooled. 48h after feeding $\dot{V}O_2$ had declined to values not different from $\dot{V}O_2$ in post-absorptive state (Fig. 2). Oxygen consumption during SDA was significantly higher (one-way ANOVA, df=4, F=11.4, $p<0.001$) than $\dot{V}O_2$ while resting in LO-dogs in summer (pairwise comparisons Holm–Sidak method, difference of means=3.5, t=2.7, p=0.01) and in HI-dogs (difference of means=5.8, t=5.7, $p<0.001$), but was not different from $\dot{V}O_2$ while resting during winter at both locations.

For all further comparisons, we chose RMR of HI-dogs during summer as the baseline because temperatures were mild (thermoneutral conditions), the dogs were in a balanced energy budget (dogs maintained body mass), and the dogs were resting (chained to the rocks). Baseline MR is close or identical to basal metabolic rate (BMR), because it was measured from resting (often sleeping), postabsorptive dogs within their thermoneutral zone. We could not account for circadian patterns, but generally these are only weakly expressed in the Arctic. RMR of LO-dogs during summer was significantly higher than baseline MR (t-test, df=14, t=5.0, $p<0.001$). Because summer temperature and level of activity did not differ between both locations we attribute this difference to differences in feeding regime. During winter, RMR for both HI-dogs (t-test, df=17, t=4.1, $p<0.001$) and LO-dogs (t-test, df=16, t=8.7, $p<0.001$) was significantly higher than respective baseline MR values. While walking on a leash, LO-dogs consumed 5.3 times more oxygen (24.4±4.4 ml kg^{-1} min^{-1}) than baseline MR.

Apparent metabolizable energy

To see if dogs were in positive, negative or neutral energy balance, we measured the apparent metabolizable energy intake in LO-dogs. The average daily energy intake during feeding trials was 3603±388 kJ per dog during summer, and 4134±934 kJ per dog during winter. The mean extraction efficiency for overall energy in three feeding trials was 90.1±4.8% and the mean retention time was 20:25 h (Fig. 3).

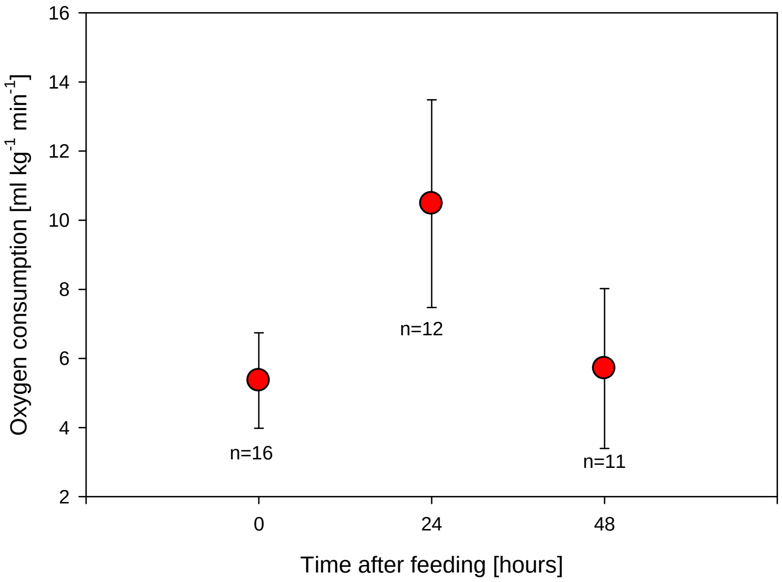

Fig. 2. Mass-specific oxygen consumption before, 24 and 48 h after feeding. The specific dynamic action (SDA) was characterized by a twofold increase in mass-speficic oxygen consumption within 24 h after feeding. At 48 h after feeding, oxygen consumption had returned to fasting values. Values (mean±SD; n=dogs) were pooled from four feeding trials conducted with LO-dogs and four feeding trials conducted with HI-dogs.

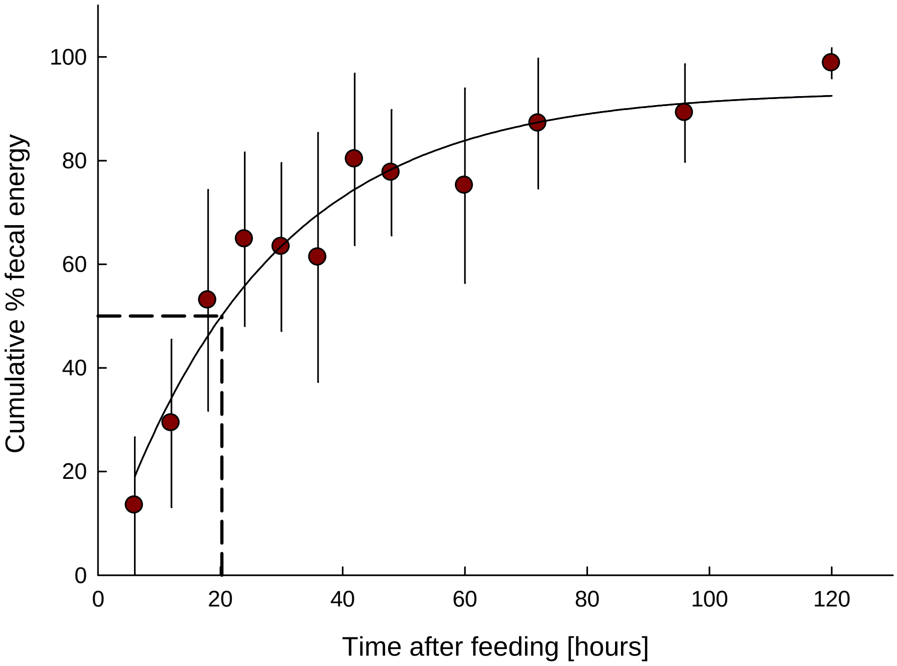

Fig. 3. Cumulative percent digestive efficiency over time for apparent metabolizable energy. *Dashed lines* indicate 50% digestion time point. Values are mean±SD of three feeding trials, n=12 LO-dogs.

Daily energy expenditure

During summer, the DEE of resting HI-dogs was 195.6±39.1 kJ kg^{-1} day^{-1} (6647 kJ/dog). During winter, their DEE during resting was 311.7±79.5 kJ kg^{-1} day^{-1} (11484 kJ/dog), which is 1.7-times the summer DEE. During sledding trips in winter, their DEE increased still further to 776.4±68.8 kJ kg^{-1} day^{-1} (25662 kJ/dog) which is almost fourfold compared to DEE while resting in summer and 2.2 fold compared to DEE on a non-working winter day (Fig. 4). Significant differences were detected between the three conditions (Kruskal−Wallis one-way ANOVA, df=2, H=23.3, $p<0.001$). Post-hoc pairwise comparisons showed that, in winter, DEE during work was significantly higher than DEE during resting (Dunn’s Method, difference of ranks=10.7, q=2.8, $p<0.05$). Daily energy expenditure while resting did not differ significantly between summer and winter (difference of ranks=8.1, q=2.1, p=n.s.).

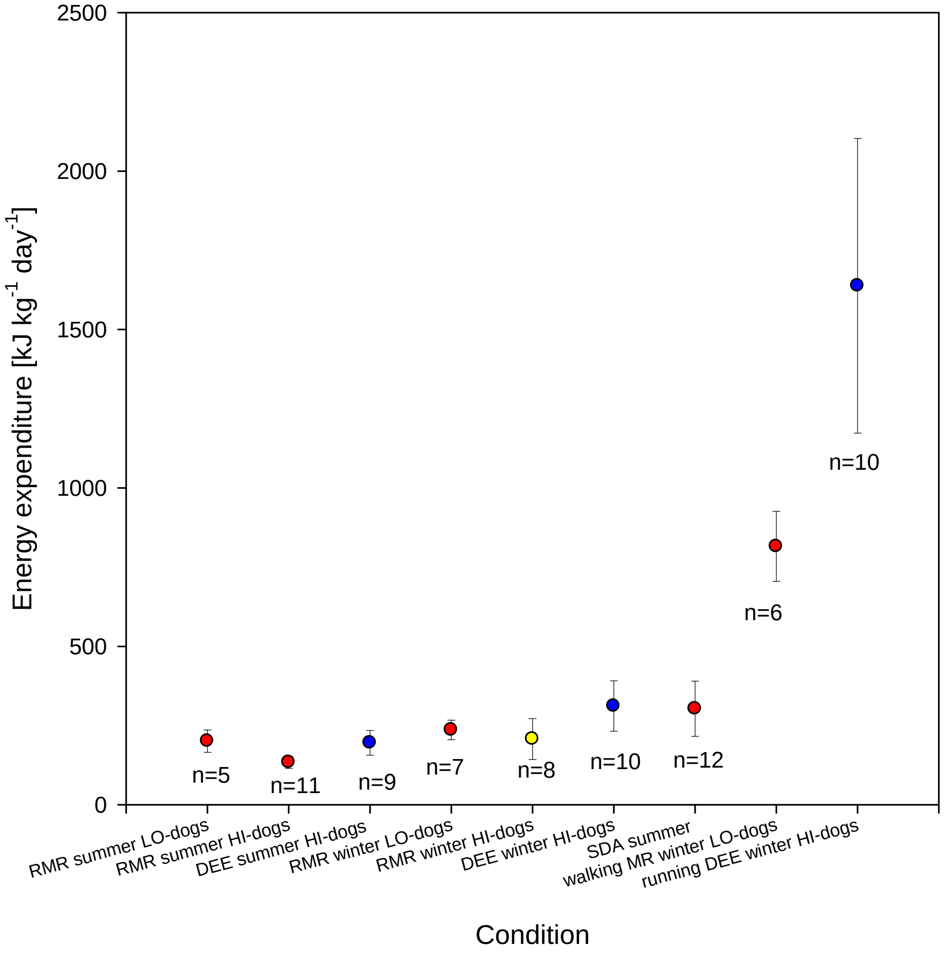

Fig. 4. Daily energy expenditure (mean±SD) calculated from oxygen consumption (*red symbols*), assuming an energy equivalent of 20.08 J ml^{-1} O$_2$, of dogs in different activity stages: resting LO-dogs in summer, resting HI-dogs in summer, resting LO-dogs in winter, both dog groups during SDA in summer, and while LO-dogs were walking in winter. Daily energy expenditure of HI-dogs calculated from doubly labeled water measurements (*blue symbols*), was measured in three different conditions: resting in summer, resting in winter, and running in winter. Estimates of RMR of HI-dogs during winter are indicated by the *yellow symbol*.

Estimation of working energy expenditure

However, a sledding trip comprises periods of sustained exercise, short breaks during the day, and extended resting periods during nights. DEE estimated using DLW gives only daily averages of total energy expenditure. Therefore, we used the activity measurements to partition DEE into working energy expenditure and resting energy expenditure. The energy expenditure during working was calculated for each dog as follows: (1) using data from activity monitors, total daily activity was partitioned into periods (hours) of running and resting for each day of a trip. (2) We used the DEE values from resting dogs to calculate an hourly average of energy expenditure during resting in winter; (3) the number of hours spent resting during a day on a trip was multiplied by the hourly average of energy expenditure to estimate the total amount of energy spent resting. (4) Finally, we subtracted this value from DEE and divided the result by the number of hours spent running to obtain a value of working energy expenditure per hour. (5) For comparative purposes we calculated working DEE (Fig. 4). When performing sustained work pulling sledges during winter, the HI-dogs reached $\dot{V}O_2$ values that were 12.2 times their resting $\dot{V}O_2$ during summer, and 7.9 times their resting $\dot{V}O_2$ during winter.

2.4.3. Heart rate

One-hour averages of heart rate (HR) in different metabolic states are presented in Fig. 5. During summer, median heart rate of resting LO-dogs (46.4±10.0 bpm) was 0.6 times that of resting HI-dogs (t-test, df=20, t=5.1, p<0.001). In winter, median HR of HI-dogs during resting (108.8±11.8 bpm) was 1.2 times of that of LO-dogs (90.8±16.0 bpm; t-test, df=21, t=3.1, p=0.006). The median HR during SDA did not differ between seasons, therefore data for this metabolic state were pooled for summer and winter. We calculated an average of 95.9±14.5 bpm for 24 h after feeding from n=21 dogs. Thereafter, HR declined to resting values. Again, we defined the median of the HR of resting HI-dogs in summer (73.6±15.1 bpm) as baseline for all further comparisons (Fig. 5A). We used a one-way ANOVA to test for the effects of feeding on heart rate during resting in both dog groups and seasons. The model was highly significant (df=4, F=37.3, p<0.001). Pairwise multiple comparisons using Tukey's Honest Significant Difference tests showed that in summer, median HR during SDA was significantly higher than resting values in both the LO-group (difference of means=49.5, q=14.0, p<0.001) and in the HI-group (=baseline; difference of means=22.3, q=6.0, p<0.001), but, it was not different from resting in winter for both dog groups.

Interseason comparisons within dog groups showed that for LO-dogs, median resting HR in summer was half that in winter (t-test, df=22, t=8.2, p<0.001), whereas the same comparison for HI-dogs showed that summer (baseline) values were three-fourth of the winter values (t-test, df=19, t=6.0, p<0.001).

To explore the lower margins of resting HR, we compared minima of 1-h averages between dog groups and between seasons (Fig. 5B). In summer, minimum HR of HI-dogs (58.3±12.5 bpm) was 1.7 times higher than that of LO-dogs (35.2±5.4 bpm; t-test, df=20, t=5.8, p<0.001). In winter, minimum resting HR of HI-dogs (70.0±12.7 bpm) was 1.6 times that of LO-dogs (44.5±12.2 bpm; t-test, df=21, t=4.9, p<0.001). For both dog groups, minimum HR was significantly higher in winter than in summer (t-test, LO-dogs: df=22, t=2.4, p=0.025; HI-dogs: df=19, t=2.1, p=0.047).

The upper margins of HR were likewise explored by comparing maxima of 1-h averages. Here, we compare resting, SDA and working HR. Maximum heart rate of resting in summer (87.2±24.4 bpm), resting in winter (156.5±26.1 bpm), and during SDA (126.3±24.5 bpm) did not differ between the two dog groups (Fig. 5C). Maximum HR of resting dogs during summer was three-fourth of that of digesting dogs during summer (t-test, df=41, t=5.2, p<0.001), approximately 50% of maximum resting winter HR (t-test, df=43, t=9.2, p<0.001), and less than half of maximum HR during running in winter (218.0±6.6 bpm, t-test, df=31, t=17.3, p<0.001).

2.5. Discussion

2.5.1. Resting MR and HR

We aimed to understand how the combined effects of seasonal changes in temperature, food quality and quantity, and exercise affected MR in Inuit sled dogs. Resting metabolic rate of HI-dogs during summer was defined as baseline for our comparisons, because this group of dogs did not experience metabolic challenges through temperature, exercise, or food supply. This condition was unusual because in most other situations Inuit dogs are either challenged by work and low temperatures in winter or by an imbalanced energy budget during summer.

2.5.2. Effects of environmental temperature on metabolic rate

Environmental temperature affects metabolic rate of endothermic animals; thus, knowledge of the thermoneutral zone is necessary for correct interpretation of MR, especially when working under extreme temperature conditions like in the Arctic. The RMR of dogs repeatedly measured with open flow respirometry between 0°C and −10°C did not differ, indicating that at −10°C they were still in the thermoneutral zone. Values of MR in resting HI-dogs calculated from DEE at −25°C were also not different from the values measured at higher temperatures (see above) and allow us to extend the thermoneutral zone of Inuit dogs down to −25°C. Personal (but not quantified) observations of resting dogs shivering at temperatures below −30°C indicate a lower critical temperature between −25°C and −30°C, whereas panting observed in resting dogs above 10°C suggests that this is the upper critical temperature.

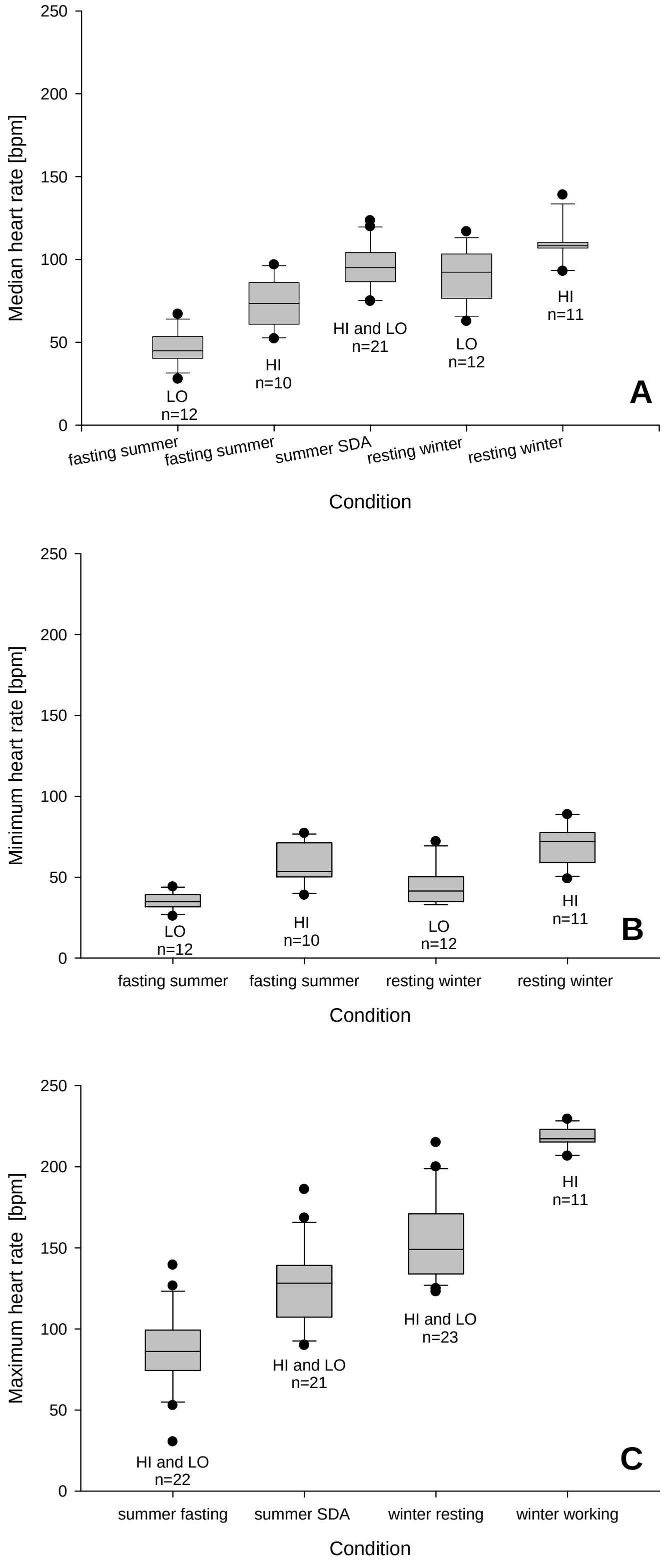

Fig. 5. One-hour average heart rate values of the different groups of Inuit dogs in different conditions: (A) median of all 1-h averages, (B) minima of 1-h averages, and (C) maxima of 1-h averages (for statistics see text, if LO- and HI-dog data did not differ in the particular condition then data were pooled).

Although we have not rigorously tested the upper and lower margins of the thermoneutral zone, we consider it likely that Inuit dogs' thermoneutral zone is from 10°C (resting in summer) to −25°C (resting in winter). Our results are in agreement with the lower limits of the thermoneutral zone (−25°C) given by Remmert (1980) for Inuit dogs. The upper limit of this zone is unlikely to be as high as the anecdotal value of 30°C reported for two immature dogs (Scholander et al., 1950a). For comparison, the NRC committee on cat and dog nutrition (National Research Council 2006) reported that the thermoneutral zone ranges from 20°C to 30°C in most domestic dogs, but Siberian huskies were reported to have a lower critical temperature of around 0°C. Given the enormous variability in the thickness and structure of the fur of domestic dogs this wide range of thermoneutral zones is not surprising. Certainly, the values reported here for Inuit sled dogs mark the lower edge of the currently known distribution.

2.5.3. Effects of fasting and/or starvation on metabolic rate

During summer, resting LO-dogs had a significantly higher RMR than baseline although they were in negative energy balance, having lost up to 30% of their winter body mass. Interestingly, the higher MR of these dogs was associated with a significantly reduced heart rate. Blood plasma parameters and muscle ultrastructure indicated a state of starvation in these same individuals (Gerth et al. 2009), during which muscle protein is utilized to fuel metabolism. Thus, in these dogs an elevated MR associated with a reduced heart rate seems indicate undernutrition. It is also possible that parasite infestation may have affected the MR of LO-dogs during summer. Seasonal cycling of parasite load is a common pattern in Greenlandic dog husbandry, and is not treated. The effect of parasitism on energy metabolism is not clear, and published data provide conflicting evidence for such effects on vertebrates (e.g. Delahay et al., 1995; Scantlebury et al., 2007).

To place our results and literature data in a correct relationship, a clear distinction must be made between fasting and starving. Short term food restriction (fasting) is associated with the mobilization and utilization of lipid from adipose tissue stores, along with reductions in BMR, resting HR, activity, and sometimes body temperature. Long term food deprivation (starvation), is characterized by protein catabolism, increased MR, and increased activity in search for food (Le Maho, 1984; Robin et al., 1988; Wang et al., 2006). Of course, a multitude of possible combinations in the degree and the duration of food deprivation may cause considerable variation in the animals' responses, making it sometimes difficult to distinguish between fasting and starving. However, we think that the distinction between fasting and starving as outlined by Le Maho (1984), Robin et al. (1988), and Wang et al (2006) provides a good framework for comparisons in our study. Fabry (1963) compared BMR in normal feeding, fasting, and starving rats, and found that fasting rats had a lower BMR than normal feeding rats, but, starving rats had a higher BMR. He suggested that a physiological response to fasting is suppression of BMR, while starvation resulted in increased tissue oxidation and thus increased MR. A reduced RMR after 9–14 days of fasting in Steller sea lions (*Eumetopias jubatus*) was shown by Rosen and Trites (2002). Metabolic rate was also significantly lower in Sand gazelles (*Gazella subgutturosa*) subjected to four months of progressive food and water restriction (fasting) compared to a control group with normal food intake (Ostrowski et al., 2006). Human volunteers responded with 40% reduction of BMR to 6 months of controlled severe food restriction (starvation) in the Minnesota Starvation Experiment (Keys et al., 1950). Because of the elevated MR of resting LO-dogs during summer, the concomitant loss of body mass, the reduced heart rate, and the degradation of muscle tissue as described in Gerth et al. (2009b) we conclude that these dogs were in starving condition. Down regulation of heart rate is a typical adjustment during periods of undernutrition and/or starvation in dogs (Alden et al., 1987) and humans (Keys et al., 1950; Romano et al., 2003). While up-regulation of MR and down-regulation of HR have been shown independently in the studies cited above, it has, to our knowledge, never been shown in dogs as a combined response to undernutrition. Because feeding intervals during summer did not differ between dog groups, the energy content of the food must have been the crucial factor leading to starvation of LO-dogs, while HI-dogs remained in a balanced energy budget.

2.5.4. Effects of feeding on metabolic rate

Metabolic rate is also affected by the response to feeding (SDA). Usually mammals ingest small amounts of food fairly frequently and are therefore in a continuous state of SDA, which makes this metabolic state notoriously difficult to quantify. In humans, approximately 10% of DEE is devoted to SDA (Westerterp, 2001). Inuit dogs are a particularly good model with which to study SDA, because they experience long post-absorptive periods as a result of infrequent feeding. With a mean retention time of 20 h we could be sure that they were in a post-absorptive (fasting) state 2–4 days after feeding. Because of the clear distinction between the fasting condition and the fed condition, we could show that $\dot{V}O_2$ was elevated for 24 h after feeding. The twofold rise of $\dot{V}O_2$ of dogs during SDA certainly was a result of the large amount of food ingested per meal. During SDA, we measured oxygen consumption at 24 h intervals. Assuming that $\dot{V}O_2$ measured during SDA increases and decreases at equal rates during the 48 h interval after feeding, we were able to estimate the portion of ingested energy attributable to SDA, because our measured values of SDA were BMR + energy invested in SDA. Using an average energy intake of 360 kJ kg^{-1} day^{-1} for the HI-dogs we calculated 9.5% of the energy ingested is spent in processes associated with SDA. Together with the increase in $\dot{V}O_2$, we recorded a twofold increase of HR during SDA. Thus, in resting dogs we observed significant changes of HR in opposing directions: an elevation of HR during digestion but a depression of HR during periods of undernutrition. Heart rate therefore was not a good predictor of energy expenditure of the resting dogs, except when the details of the physiological condition were known.

During winter, both dog groups were fed frequently, and, therefore, were continuously in a state of SDA while resting. Interestingly, $\dot{V}O_2$ while resting and digesting during winter was not different from $\dot{V}O_2$ in summer during SDA in both dog groups. As discussed above, the dogs were still within their thermoneutral zone at −25°C, thus a possible and straightforward interpretation is that the elevated rate of energy metabolism is due to concomitant SDA, rather than thermoregulation or the training effects of increased work load (see below).

2.5.5. Effects of exercise on metabolic rate

Exercise has repeatedly been shown to lead to long-term increases in RMR (Pinto and Shetty, 1995; Wilterdink et al., 1992). Thus, we predicted that Inuit dogs working extensively in winter would show RMRs elevated relative to that in summer, when they have no exercise. After accounting for possible effects of temperature and feeding, RMR of Inuit dogs in our study did not differ between summer and winter. The parasitic load during summer may have affected RMR, but, because veterinary parasite treatment is not available in Greenland we could not compare RMR of parasite free and parasite infested dogs during summer. Studies on the effects of parasitism on RMR generally indicate increasing metabolic rates and reduced body mass due to parasite infestation (Grenfell and Dobson, 1995), however, depending on phylogenetic relationship results can be contrasting; for example Scantlebury et al. (2007) found a suppression of RMR in infested Cape ground squirrels while Delahay (1995) reported an elevation of RMR in Red grouse due to parasite infestation. However, Speakman and Selman (2003) pointed out that most studies showing chronically increased RMR after exercise used forced running or swimming protocols, and that voluntary exercise did not elicit such effects. Voluntarily running short-tailed field voles (*Microtus agrestis*) had the same RMR as their sedentary controls (Speakman and Selman, 2003). For humans, some studies show long-term increases in RMR as a result of exercise (Alméras et al., 1991; e.g. Shvartz and Reibold, 1990; Tremblay et al., 1992),whereas others found no effect (e.g. Broeder et al., 1992), Sharp et al., (1992). A decreased RMR was reported by Westerterp et al. (1992) for subjects that underwent 44 weeks of training for a half-marathon. While data on humans remain somewhat conflicting (for a review see Speakman and Selman 2003), most studies on mammals indicate an increase in RMR in response to intensive and sustained training. However, occasional and voluntary exercise does not necessarily result in an elevation of RMR. Dog husbandry by Inuit does not involve sophisticated training. After the inactive summer break the dogs are used for increasingly longer hunting and fishing trips when the sea ice thickens and the daylight period elongates in early spring. This makes an important difference for the comparison of the traditionally kept Inuit dogs with other northern dog breeds (e.g., Siberian and Alaskan huskies) used in competitive dog sledding. Such sport athletes are selected and carefully trained, often all year long to meet the exceptional demands imposed on them during races. For example, Hinchcliff et al. (1997) reported a total daily energy expenditure of 47100 kJ per Alaskan sled dog during a 3-day race, and resting dogs had total daily energy expenditures of 10500 kJ. The HI-dogs in our study spent half this amount of energy during a three day sledding trip in comparable temperatures, and spent roughly the same energy when resting as did the Alaskan dogs, which however weighed only two thirds of the Inuit sled dogs. Wyatt (1963) estimated daily energy requirements of working Inuit sled dogs of 14665–19275 kJ, while tethered dogs require more than 9889 kJ per day to maintain body mass in Antarctic conditions (Orr, 1966). This author reported that 10500 kJ per day was sufficient to maintain body masses of idle Inuit sled dogs in the Antarctic, but that working dogs needed twice this. The energy requirements of working and resting HI-dogs match these data on Inuit sled dog nutrition in the Antarctic.

2.5.6. Effects of exercise on heart rate

Extensive exercise causes a long term decrease of resting HR in dogs (Wyatt and Mitchell, 1974) and other mammals including man (Scheuer and Tipton, 1977; Stein et al., 1999). Because of increased exercise during winter, we expected that winter resting HR would be lower than that during summer, but it was in fact 47% higher. Here again, we attribute the elevated resting winter HR with SDA, as dogs that were fed every second day in winter, thus never became post-absorptive. This is further suggested by the fact that resting HR in winter was indistinguishable from SDA HR in summer.

2.5.7. Comparative perspective

Any comparative discussion must consider the possible confounding effects of body mass, latitudinal adaptations, or phylogenetic relationship on BMR. When comparing BMR values of 12 wild canid species, Careau et al. (2007) found higher mass-specific BMR in canids from the arctic climate zone than species from intermediate and hot climates (desert). A comparison of MR of Inuit sled dogs with these data is difficult because seasonal challenges in exercise and food supply are reversed relative to those experienced by wild canids. Comparison with other domestic dog breeds (Kienzle and Rainbird, 1988; 1991) is also difficult, because domestication has resulted in a great diversity of dog breeds with differing fur quality, insulation, and body composition (Speakman et al., 2003), all contributing to differing energy demands.

In winter, the HI-dogs in our study reached working MRs that were 7.9 times RMR in the same season, a metabolic scope that exceeds the range given by Hammond and Diamond (1997). This is likely an underestimate, because winter RMR is probably chronically elevated above BMR as a result of SDA. As shown above, during winter the dogs were in a continuous state of SDA and what we measured was (RMR + energy consumption because of SDA). Without the increment of SDA, RMR during winter was not different from RMR during summer. Taking this into account, working metabolism during winter was 12.2 times RMR (equivalent to baseline MR as defined here). The working metabolic rate of 12.2 times RMR is not particularly spectacular if compared to African wild dogs, for which active metabolism during hunting was approximately 25 times BMR (Gorman et al 1998). However, African wild dogs were active for an average of 3.5 h daily while the Inuit dogs in this study were working for 8–10 h per day. As pointed out by Gorman et al. (1998) African wild dogs most certainly cannot prolong their hunting periods at that energy expenditure because of physiological constraints. The highest sustained (daily) energy expenditures by humans were 4.3 times BMR, achieved by Tour de France cyclists (Westerterp et al., 1986). These athletes probably experienced work durations (8–10 h) similar to those of the Inuit dogs in this study.

Because our estimates of sustained metabolic scope rely on RMR rather than BMR baselines, the above comparisons await refinement by measurements of BMR in Inuit dogs. We are also aware of the limitations of comparisons between dog athletes such as Alaskan sled dogs running the Iditarod race, and dogs in our study. These limitations notwithstanding, our data are the best currently available for this distinctive and long-isolated breed of dog, and support previous studies showing that active canids such as sled dogs and African wild dogs are capable of maintaining outstanding metabolic scopes (Gorman et al., 1998; Hinchcliff et al., 1997). High metabolic scopes in dogs are probably one of the attributes favoring their domestication as working animals under extreme environmental conditions and, in a more modern environment, making them suitable as long distance running athletes. High aerobic capacities probably evolved as part of the cursorial hunting strategies of dogs that require them to run for long periods to exhaust their prey. This is in contrast to the ambush hunting of felids, which have never become domesticated as working animals, but have been domesticated in different contexts (Driscoll et al., 2007).

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Chapter 3

Muscle plasticity of Inuit sled dogs in Greenland

3.1. Abstract

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. Sarcomers 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 fibres 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 depot size, and the number of contractile filaments all increase with increasing workload and food supply while the capillary network remains unchanged.

3.2. 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 who stay need to adjust to cycling changes in their environment. 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 (sarcomer 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 down-regulate muscle size in winter due to starvation (Josefsen et al., 2007), we expected that Inuit dogs to up-regulate their skeletal muscle size in response to increased work load, sufficient food supply, and cold acclimatization. Such up-regulation of muscle size is presumably based on changes in fiber size and architecture.

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.

Second, 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. (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.

3.3. Materials and methods

3.3.1. 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 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).

3.3.2. 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, Inc., 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.

3.3.3. 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 150–700g 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. While 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. Starck, N. Gerth, and S. Jackson, 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. Starck, S. Sum, S. Jackson and N. Gerth, unpubl. data). The dogs studied in Qaanaaq were in good condition in summer and in winter.

3.3.4. Size measurements

Dog body masses were measured using a hanging scale (Kern CH 50K100, Kern & 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.

3.3.5. 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 Inc., Chicago, IL, USA).

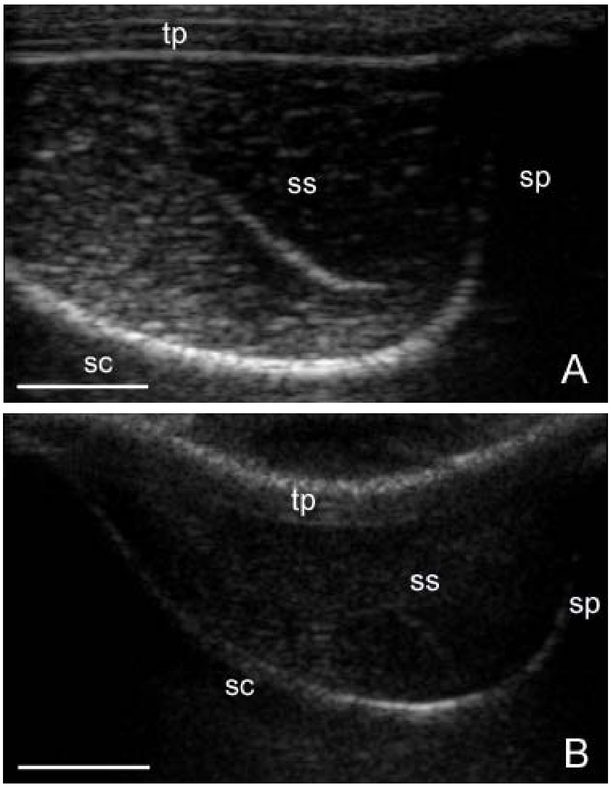

Fig. 1. Ultrasonographs of the supraspinatus muscle of Inuit dogs from Qeqertarsuaq in (A) winter and (B) in summer condition. The ultrasound probe was placed lateral and perpendicular to the scapula (sc), so that the spina scapulae (sp) is seen on the right side of the image. Top of the image shows 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 to a concave shape in summer. These are representative sonographs of the range from the different dogs. Scale bars, 1 cm.

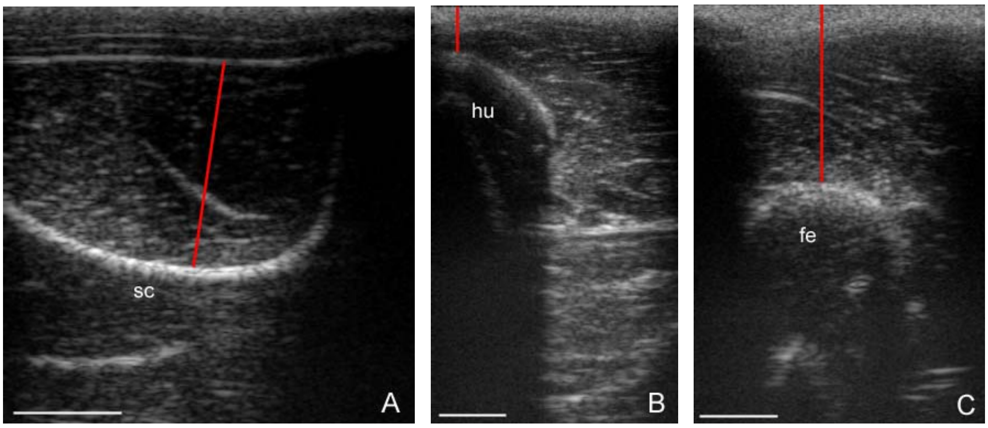

Fig. 2. Ultrasonographs of measurement sites and muscle thickness of (A) m. supraspinatus above the scapula (sc), (B) muscles (m. triceps brachii, m. brachialis) lateral of the humerus (hu), and (C) muscles (m. biceps femoris, m.vastus lateralis) lateral of the femur (fe); the measured distance from 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.

3.3.6. Biopsy sampling

A 14 gauge spring-loaded side-cutting needle with a sampling notch of 1.5 cm (Temno; Allegiance Healthcare, McGaw Park, IL) was used for needle biopsy sampling of the m. adductor magnus. Biopsies were taken from standing dogs in the field under local Lidocain 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 3 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 (2008) was neither possible nor intended. Care was taken to obtain cross-sectional samples in a 90° angle to the muscle fibers. After biopsy sampling all dogs received subcutaneous Carprofen injections for analgesia ($4\,\mathrm{mg\,kg^{-1}}$ Rimadyl, Pfizer GmbH, Karlsruhe, Germany). Bacterial inflammation was suppressed by a single subcutaneous dose of amoxicillin/clavulanic acid ($10\,\mathrm{mg\,kg^{-1}}$ Synulox RTU, Pfizer GmbH, Karlsruhe, Germany).

3.3.7. Histology

Muscle biopsies were preserved in 2.5% glutardialdehyde in $0.1\,\mathrm{mol\,l^{-1}}$ 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 $0.1\,\mathrm{mol\,l^{-1}}$ phosphate buffer (pH 7.4) for 2 h, and dehydrated in a series of ethanol and pure acetone. Following dehydration, samples were embedded in epoxy resin (Epon, Carl Roth GmbH & Co, 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 for studying the sections.

3.3.8. 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 lesser 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 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.

3.3.9. 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 reaching 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) Sarcomer shape: We assigned the outer line of the sarcomers to two different categories; either parallel/convex or concave shaped. All sarcomers, that were fully visible in the TEM images, were categorized and the percentage of sarcomers in each category was calculated. Furthermore, we measured the width of the sarcomers at the M-line and at the Z-

line and calculated the M/Z-ratio of individual sarcomers. The M/Z ratio is a simple estimate of sarcomer shape: a ratio <1 is present if the sarcomer has a concave shape which indicates a depletion of myofilaments in the sarcomers, while a ratio ≥ 1 is typical for straight or convex shaped sarcomers indicating normal structure of myofilaments. We measured the M/Z ratio of all fully visible sarcomers on each TEM image and then calculated the percentage of the sarcomers that were either normal or concave. Because sample size differs between years and locations it is always given with the results. We run a two-way ANOVA to test for effects of season and location as fixed factors on the measure of sarcomer 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 sarcomer is another character that can be used to qualitatively describe possible effects of workload and/or nutrition on sarcomer 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) sarcomer. The second category was characterized by myofilaments in a diffuse arrangement, usually consisting of sarcomers that are depleted of myofilaments. Transmission-electron micrographs were screened for the arrangement of myofilaments in each sarcomer. Sarcomers 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.

3.3.10. 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 4000 rpm 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, in winter and in Qaanaaq, this was 24 h after feeding. Measured parameters were those of 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).

3.4. Results

3.4.1. 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: $64.6_{\text{Qeqertarsuaq}}$, 42.6_{Qaanaaq}; q: $6.5_{\text{Qeqertarsuaq}}$, 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).

3.4.2. 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 weight (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: df=1, F=25.3, $p<0.001$; location: df=1, F=176.9, $p<0.001$). Interactions between the factors were significant (df=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; df=1, F=9.6, p=0. 01). However, the head length of the dogs did not differ between the study sites (Table 1).

3.4.3. Ultrasonography

Ultrasonography of dogs from Qeqertarsuaq showed that shoulder muscles were on average 19% thicker during winter than during summer (df=1, F=50.4, $p<0.0001$) (Fig. 1), foreleg muscles were 44% thicker during winter than during summer (df=1, F=18.1, p=0.0002), and hindleg muscles were 39% thicker during winter than during summer (df=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 (df=1, F=13.1, p=0.004).

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

	Qeqertarsuaq (n=12)		Qaanaaq (n=10)	
	Winter 2006	Summer 2005	Winter 2008	Summer 2007
Body mass (kg)	26.8±2.6	18.8±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)[a]	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)[b]	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)[c]	2.3±0.2	1.4±0.2	2.2±0.2	2.0±0.2

Values are mean±SD

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

[b] Combination of m. triceps brachii and m. brachialis

[c] Combination of m. biceps femoris and m. vastus lateralis

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 mean±SD

3.4.4. 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 which showed a widened space between the fibers. The margin of each myofiber showed no concentration of mitochondria and the intermyofibrillar space contained only few lipid droplets (Fig. 3A). In 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: df=1, F=15.2, $p<0.001$; location: df=1, F=12.6, $p<0.001$). At both locations, the myofiber density was significantly higher (df=1, F=11.1, p=0.002) in summer than in winter. No differences were found for the factor 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: df=1, F=12.7, p=0.001; location: df=1, F=35.5, $p<0.001$). However, we detected a significant interaction between factors, thus an interpretation of main factor will 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 (df=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: df=1, F=9.5, p=0.004; location: df=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 (df=1, F=39.1, $p<0.001$) in Qaanaaq than in Qeqertarsuaq. No interactions between the two factors were detected.

Significant seasonal differences (df=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 (df=1, F=30.5, p<0.001), but not between areas, and no interactions between the factors were observed.

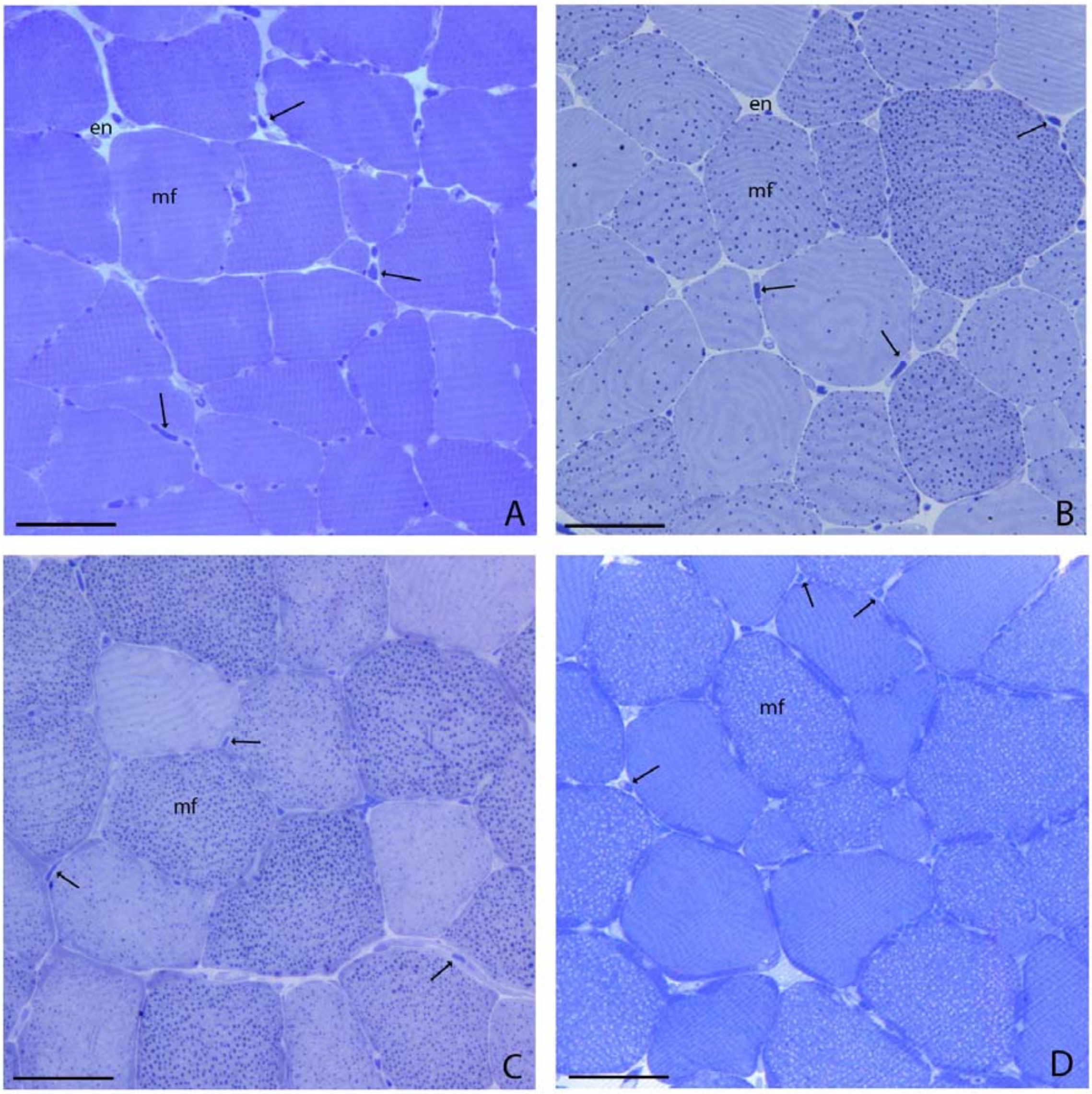

Fig. 3. Semithin cross-sections of m. adductor magnus (A,B) in summer, and (C,D) in winter condition. A and C are samples of dogs from Qeqertarsuaq, B and D are samples from Qaanaaq. Myofibers (mf) in winter condition contain more lipid droplets (dark dots in cross-sections A–C, light dots in D) than 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.

3.4.5. Sarcomer ultrastructure

In winter, myofibrils are characterized by full sarcomers 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 sarcomers. Between the myofilaments numerous lipid droplets were stored close to the intermyofibrillar mitochondria. This ultrastructural characterization of myofibrils holds for muscle samples from winter for both locations.

In summer, the ultrastructure of myofibrils of dogs from Qeqertarsuaq looked strikingly different (Fig. 4A). Many sarcomers had a concave shape, so that the sarcoplasmatic compartment appeared dilated. The myofilaments were less dense and in disorganized arrangement within the sarcomers. Many myofibrils were oriented oblique to the long axis of the sarcomer. Also, ramifications of sarcomers were observed which did not occur during winter. The sarcomer 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 sarcomers 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 sarcomers were aligned in parallel in 93% of the samples from Qeqertarsuaq and all samples from Qaanaaq. In summer and in dogs from Qeqertarsuaq, the majority of sarcomers (57%) contained myofilaments in oblique arrangement to the long axis of the sarcomer. In contrast, 90% of the sarcomers of dogs from Qaanaaq obtained during summer contained myofilaments with a parallel arrangement to the long axis of the sarcomer. We observed a dilatation of the sarcoplasmatic compartment in 13% of the TEM images from tissue samples from Qeqertarsuaq and none of 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 a concave shape of sarcomers, 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 sarcomers) 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 (df=1, F=8.9, p=0.006) and between locations (df=1, F=14.4, p<0.001). No interactions between season and location were detected.

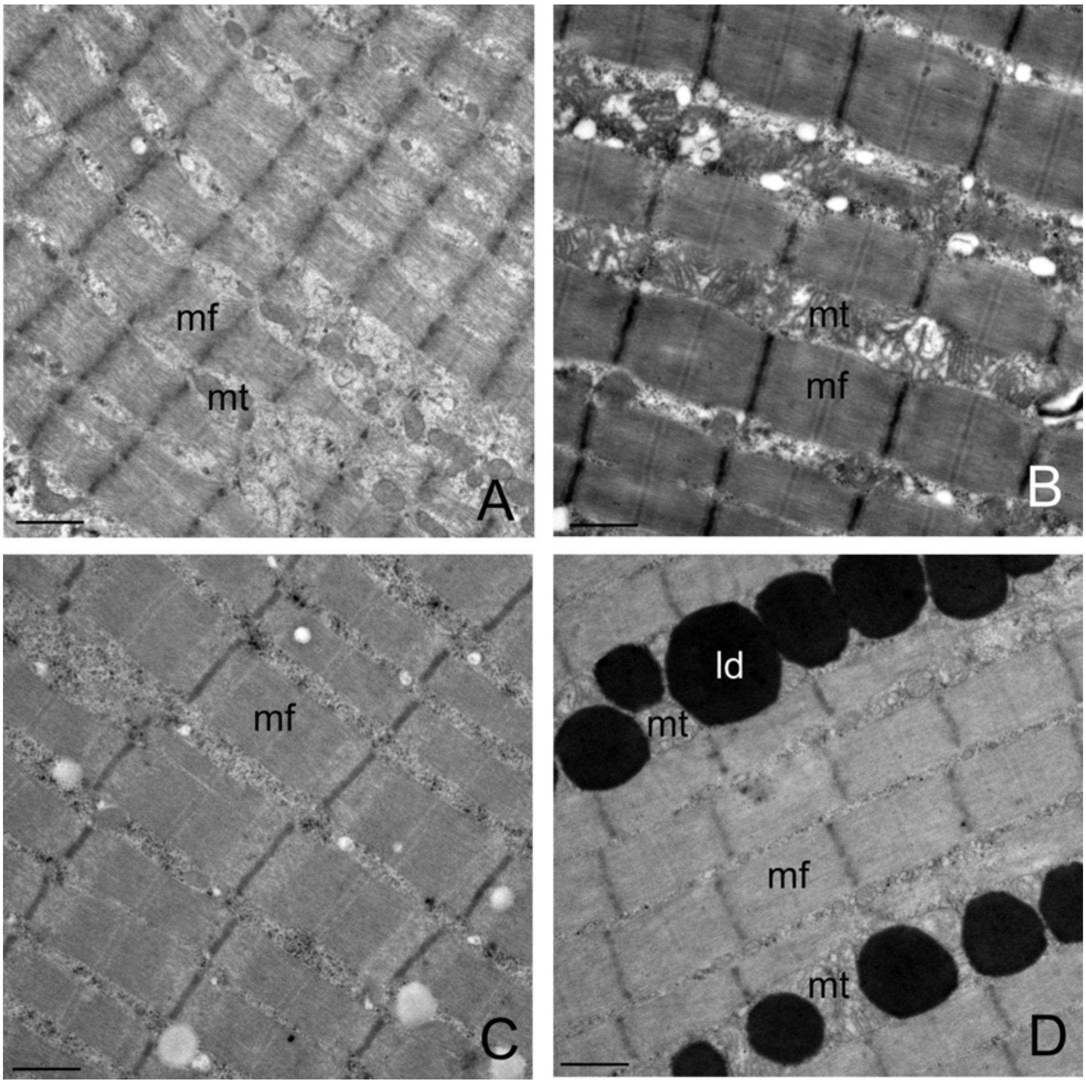

Figure 4. Transmission electron micrographs of m. adductor magnus of dogs (A,B) in summer condition and (C,D) in winter condition. A and C are samples of dogs from Qeqertarsuaq, B and D are samples from Qaanaaq. Mitochondria (mt) are located next to lipid droplets (ld) between the myofibrils (mf). Scale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

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%

3.4.6. 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}), 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, while 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}).

3.5. Discussion

3.5.1. 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 to 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 in thermoneutral condition (unpubl. 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 per day.

During winter, the daily energy budget of the dogs in Qaanaaq (11800 kJ; unpublished data) was balanced, but, it was at the lower margins of the range of values given by Orr (1966), i.e., between 10500 kJ day^{-1} for a non-working dog and 21000 kJ day^{-1} for working sled dogs.

Racing sled dogs are known to have the highest sustained metabolic rates (47100 kJ day^{-1}) of any mammal measured so far (Hammond and Diamond, 1997; Hinchcliff et al., 1997).

3.5.2. Histological morphometry and sarcomer 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 (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, while 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. (1982) reported that anorectic human patients also have muscle fiber diameters that are significantly below average. 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 sarcomers 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 sarcomers.

We introduced the M/Z-ratio as a semi-quantitative measure to support the observed sarcomer changes. The key to interpret 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 sarcomer. Thus, an M/Z ratio smaller than 1 indicates a reduction/ depletion of myofilaments from a sarcomer while an M/Z ratio of 1 or higher suggests normal sarcomer structure. The M/Z ratio of Qeqertarsuaq dogs during summer suggests a serious depletion of sarcomers, while Qeqertarsuaq dogs during winter and all dogs from Qaanaaq showed normal sarcomers. However, many of our TEM images showed that for example in dogs from Qeqertarsuaq in summer even within one myofibril the sarcomer structure varies between full to 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 fetched 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 exclude stereological artifacts. Differential depletion of myofibers explains the patchy pattern of concave sarcomers and the rather proportional changes of depleted sarcomers 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 rather correlated changes due 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 (2002) in a morphometric study of Syrian hamsters.

At a first glimpse, our results appear to be in partial contrast to 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 down-regulated 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 (2004) and 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 down-regulation, ultimately supporting our interpretation of long term effects on the capillary network and the lack of flexibility for down-regulation.

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.

3.5.3. 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 (1996) and Burr (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.

3.6. 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 due to 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 shaped sarcomers. 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 sarcomers appears normal. In contrast, the capillary network remains unchanged throughout the year at both locations.

3.7. Acknowledgements

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The experiments of this chapter were designed and conducted by J. Matthias Starck and myself. Morphometry and statistics were conducted by myself and the manuscript was written by J. Matthias Starck and myself.

Chapter 4

Cardiovascular adaptations of Inuit sled dogs to seasonal changes in work load, temperature, and feeding

4.1. Abstract

Using ultrasonography and external heart rate monitors we have measured morphological and functional parameters of the heart of Inuit sled dogs living in Greenland. We compared dogs from two sites differing in husbandry. Husbandry conditions at one site (LO-dogs) included intermittent feeding of low energy food during the inactive summer season, while at the second study site, the dogs were provided with sufficient food throughout the year (HI-dogs). During winter, the LO-dogs were used for recreational sledding, while the HI-dog group was used regularly for transportation and hunting. All dogs were fed regularly with high energy food during winter.

We explored adaptive long-term and short-term responses of the cardiovascular system to changes in exercise, temperature and food supply. We compared measurements of the same dogs in summer and winter when resting while fasting and digesting, and working. In winter, the diameter of the aortic root was larger than in summer, whereas the dimensions of the left ventricle remained unchanged. We found substantial functional adjustments during winter: stroke volume (SV) was 28% higher, left ventricular ejection fraction (LVEF) was 11% higher, and fractional shortening (FS) was 7% more intensive in the LO-dogs. We also explored short term responses to feeding and working in the dogs. In summer in resting LO-dogs heart rates and cardiac output doubled when digesting. Elevations were maintained for 4 h and slowly declined within 34 h to fasting levels. When pulling a sledge in winter HI-dogs maintained working heart rates of 200 bpm and cardiac output of 221.7 ml min^{-1} kg^{-1} compared to resting heart rates of 109 bpm and cardiac output of 114 ml min^{-1} kg^{-1}. The Greenlandic Inuit sled dogs adjust functionally rather than morphologically to the differing demands between the seasons and conditions.

4.2. Introduction

Domestication and artificial selection of the wolf (*Canis lupus* L.) by humans has created a most diverse taxon, the domestic dog *(Canis lupus familiaris* L.*)*. No other species is so divers in body size, morphology, temperament, climatic tolerance, and ecological adaptations like domestic dogs. Associated with differences in size and morphology, the functional parameters of inner organs differ between dwarfs like 0.5 kg Chihuahuas and giants like 100 kg Great Danes.

However, even within a single breed, heart morphology and functional parameters change in response to external conditions. For example, resting heart rate decreases with exercise (Stepien et al., 1998; Wyatt and Mitchell, 1974). Like in the human body, the heart of dogs may increase in size in response to exercise training or may decrease during periods of detraining (Stepien et al., 1998; Wyatt and Mitchell, 1974). In concert with morphological parameters, cardiac output and stroke volume increase in response to intensive exercise (Barnard et al., 1980). However, different breeds, different experimental training conditions, and different research interests resulted in data that, at the first glimpse, appear to be inconsistent or contrasting. For example, in contrast to the studies mentioned above, Mackintosh et al. (1983) reported no changes in resting heart rate after 7 weeks of endurance training in beagles, and, Pape (1985) measured even higher resting heart rate in trained Greyhounds as compared to untrained breed mates.

The nutritional status of an animal is an additional parameter affecting morphology and function of the heart in long term and short term perspective. Malnutrition, i.e., several weeks of undernourishment, may result in reduced heart weight (dogs: Alden et al., 1987), smaller cardiac output (dogs: Haxhe, 1967; humans: Romano et al., 2003), and bradycardia (dogs: Alden et al., 1987; humans: Gottdiener et al., 1978). However, while the heart is smaller, the ejection fraction increases in humans (Gottdiener et al., 1978; Romano et al., 2003) and in dogs (Alden et al., 1987; Haxhe, 1967). A short term, i.e., immediate response to feeding is a rise in heart rate, aortic blood pressure, and cardiac output (Gerth et al., 2009a; Rokowski and Spodick, 1989; Vatner et al., 1970a; Vatner et al., 1970b).

When studying functional adaptations of organs like the heart one faces the problem of choosing the right reference to differentiate between breed specific adaptations and condition related deviations of morphological and functional parameters. Therefore, various studies have been undertaken to establish reference data of functional parameters like heart rate of different dog breeds in different conditions (Boon et al., 1983; Della Torre et al., 2000; Hanton and Lodola, 1998).

Longitudinal studies of morphological and functional changes of inner organs in same individuals require non-invasive methods that do not impair the performance of the individual. Echocardiography and heart rate monitoring are such non-invasive tools to study cardiac function. Both methods have been applied in human medical diagnostics since decades, but, it was Mashiro et al. (1976) who introduced echocardiography to evaluate left ventricular function in awake domestic dogs. A validation study by Friedman et al. (1982) found excellent agreement in end-systolic dimensions of the left ventricle but overestimation of end-diastolic dimension in M-mode echocardiograms when M-mode echocardiography was simultaneously recorded with sonomicrometer crystal signals in open chest dogs. The data of that study support the recommendations of The American Society of Echocardiography to quantify left ventricular minor axis dimensions from M-mode echocardiograms (Sahn et al., 1978).

Inuit sled dogs are a unique model to study the effects of both, exercise training and nutritional state on the morphology and function of the heart. Since at least 1000–1500 years, Greenlandic Inuit use their dogs for transportation and hunting. The dogs were selected for their ability to withstand harsh conditions, a strong will to perform sustained work in front of the sled and to endure prolonged periods of famine (MacRury, 1991). Most certainly, there was no selection for morphological uniformity. In contrast to modern sled dog breeds used for racing sport, the Greenlandic dog husbandry does not involve line breeding or sophisticated training. Inuit sled dogs are an old aboriginal breed which is genetically isolated from more modern dog breeds (Morey and Aaris-Sorensen, 2002).

Traditionally, these dogs are fed every other day during the winter working season. In summer, when resting they were allowed to scavenge free or were fed only occasionally. Today, “resting” is “force resting” because the dogs are, by law, chained to rocks, but still they are fed only 2–3 times per week and may lose up to 30% of their body mass. Thus traditional husbandry incurs seasonal changes between strong physical work in winter and lazy periods during summer, and seasonal changes between feast in winter and fasting in summer. Both factors potentially affect cardiac morphology and functioning in the dogs.

In a previous study, we showed that seasonal changes in food supply and exercise have strong effects on the structure of locomotor muscles (Gerth et al., 2009b). The myofibrillar compartment of the myofibers was up- and down-regulated between seasons. The oxygen demand may change together with the adjustments of the muscles, ultimately resulting in an up- or down-regulation of the need for blood supply according to the changes in the muscle compartment. Consequently, one may hypothesize that the cardiovascular supply system adjusts to the changes in oxygen demand by increasing or decreasing the heart and the vascular network dimensions. However, in that study the muscle fibers adjusted flexibly to the actual requirements of winter and summer season, but, the capillary network in locomotor muscles was stable. Obviously, it was irreversibly adjusted to the peak demands during the active winter period. Here, we ask, if the cardiovascular system adjusts flexibly to the seasonally changing demands of peripheral organs (like musculature) or if it is permanently adjusted to a certain level of workload during winter (like the peripheral capillary network of the muscles). To test these ideas, we compare two groups of dogs differing in workload, feeding regime, and environmental temperatures during winter. The dog group studied in West Greenland was kept for short recreational sledding trips in relatively mild winter conditions and experienced periods of fasting during summer. In a previous publication (Gerth et al., 2009a) we labeled them "LO-dogs" (low energy intake dogs). The second group of dogs was used for long distance sled trips to hunting grounds during the challenging cold winter of Northern Greenland. Because these dogs were kept in a balanced energy budget throughout the year we called them "HI-dogs" (high energy intake dogs).

The key-question in this study is whether the peripheral adaptations of locomotor muscles to seasonally increasing work load and seasonally differing food supply are mirrored by central cardiovascular adaptations. Such adaptations could include long term morphological changes of the heart or changes in heart function as well as short term functional adjustments.

If the dogs' cardiovascular system flexibly adjusts to seasonal changes in work load we expect both groups of dogs to up- and down-regulate heart size, aortic root diameter, heart rate and functional parameters. If, however, dogs maintain an up-regulated supply apparatus, like the capillary system in the leg myofibers, then we hypothesize that the HI-dogs experiencing higher oxygen demand during winter show larger morphological changes and physiological adaptations which they maintain throughout the year. Then, LO-dogs will also be expected to have a stable phenotype but at a lower level than the high challenge group.

This study did not follow a common garden design because it is beyond the physiological capability of the dogs to perform intense work during the mild summer, or to fast during winter. Also, the study merged into the traditional life of Inuit hunters and the dogs were required for transportation during winter, thus a full cross over design (with dogs resting throughout the winter season) was not possible. Consequently, we miss some conditions for direct comparison. We intentionally choose to work with dog teams belonging and cared for by their Greenlandic owners and used traditional husbandry conditions as experimental framework to explore adaptations of the dogs to seasonal differences in environmental temperature, activity, and food supply.

4.3. Materials and methods

4.3.1. Research site

The study was conducted at two study sites. In summer 2005 and winter 2006, research was conducted in Qeqertarsuaq on Disko Island at 69°15′N, 53°32′W. In summer 2007 and winter 2008, we worked in Qaanaaq at 77°28′N, 69°20′W. During summer, temperatures were mild (median: 12.1°C in Qeqertarsuaq, 9.4°C in Qaanaaq). Winter temperatures were lower (median −21.4°C) in Qaanaaq than in Qeqertarsuaq (−8.5°C).

4.3.2. Dogs

We studied exclusively male dogs kept in local husbandry conditions. A team of 12 dogs (age between 2 and 4 years) was studied in Qeqertarsuaq. During winter, these dogs were used as sled dogs once or twice per week for relatively short trips. They were chained to their places at all other times. During winter, the dogs were fed with dried fish or frozen seal on a daily basis. During summer, the dogs were constantly chained to rocks and fed with fresh fish (meal size: about 15% of their body mass) every fourth day. Because of intermittent feeding of low energy food they had a significantly lower body mass in summer (average 19.1±1.6 kg) than in winter (average 27.3±2.7 kg). Repeated veterinary examinations revealed signs of chronic malnutrition during summer and all dogs were heavily infested by intestinal parasites (see also Gerth et al., 2009b). We labeled this group of dogs as "low energy intake (LO)-dogs".

In Qaanaaq, we studied two teams of 6 dogs (age between 2 and 10 years). These dogs were used frequently for hunting and transportation in winter. They were out on the sea ice for trips lasting between a few days and several weeks. During winter, the dogs were fed every other day with 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) or received their share of the hunt. During summer, while chained to the rocks, these dogs received walrus and seal meat from the previous hunting period every second to third day. 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. Starck, N. Gerth unpublished data).

The dog teams from Qaanaaq were in good condition and healthy throughout the year. Their body mass did not differ statistically between summer and winter and averaged at 34.1±2.8 kg throughout the year. This group of dogs was labeled "high energy intake (HI)-dogs".

4.3.3. Working conditions

During winter, the HI-dogs were used for transportation to the fishing and hunting grounds. Such journeys vary from day trips to excursions of several weeks. We joined these two dog teams during two three day trips to take measures of heart rate, activity, and energy metabolism (Gerth et al., 2009a). The HI-dogs were running on average 8 h per day on smooth sea ice with little snow, and no elevation. Two breaks were made during each day. Daily running distances were 60–80 km at an average speed of 9 $\mathrm{km\,h^{-1}}$. Running speed, distances, and duration were recorded using GPS (GPSMAP 60CSx, Garmin International Inc, Olathe, KS). One-hour averages of environmental temperature ranged between −33°C and −14°C for tour 1, and between −26°C and −2°C for tour 2.

4.3.4. Echocardiography

For non-invasive measurements of heart morphology, we used a portable ultrasonography machine equipped with an 11 mm convex-scanner head (C11/8-5MHz micro-curved array, Titan, SonoSite, Bothell, WA, USA). A 0.5% aqueous solution of a polyacrylic acid (sodium polymer PNC 430, Spinnrad, Norderstedt, Germany) was applied as acoustic coupling gel.

All dogs underwent an echocardiographic examination that included trans-thoracic two-dimensional M-mode echocardiograms (left parasternal long- and short-axis views). Examinations were performed with conscious standing dogs during a period of quiet respiration. The measured beats were selected on the basis of quality of the recording. We took three measurements per session, and each dog was scanned in at least four independently repeated sessions. Recordings were analyzed later by the operator blinded to the dog's identity. The daily averages of each session were pooled to obtain averages of all values for each dog, and compared within each location between seasons using T-tests, if values failed the equal variance test or were not normally distributed a Mann-Whitney Rank Sum test was performed.

The following cardiac measurements were taken from two-dimensional M-mode cross-sectional views at the level of the chordae tendineae (left parasternal short axis view): left ventricular internal diameter in diastole (LVIDd in mm) and systole (LVIDs in mm), left ventricle wall thickness in diastole (LVWd in mm) and systole (LVWs in mm). The aortic root diameter in systole (AO in mm) was measured using the left parasternal long axis view.

From the echocardiographic measurements we calculated left ventricular end-diastolic and end-systolic volumes (LVEDV and LVESV in ml) using the Teicholz formula: $V=7D^3/(2.4+D)$ with D=LVIDd or LVIDs respectively for LVEDV and LVESV (Teichholz et al., 1976). Stroke volume (SV in ml) was calculated as SV=LVEDV-LVESV, cardiac output (CO in $\mathrm{ml\,min^{-1}}$) as SV×HR (HR=heart rate in bpm). Fractional shortening (FS %) was calculated as [(LVIDd–LVIDs)×100]/LVIDd, and ejection fraction LVEF(%)=(SV/LVEDV)×100.

4.3.5. Temperature recording

We recorded environmental temperature using temperature data loggers with an On-Chip Direct-to-Digital temperature converter (i-button DS2422, Maxim Integrated Products, Inc., USA). The loggers have an operating range from –40°C to +85°C at 0.065°C resolution. Environmental temperature was recorded every 10 min at the dogs' home place. From these 10-min intervals we calculated 1-h averages.

4.3.6. Activity monitoring

We recorded the activity of the HI-dogs using piezoelectric accelerometers that record acceleration in two planes at 0.012g precision (ActiTrac, IM Systems Inc., Baltimore). Acceleration was sampled 40 times per second and integrated over 2 min to quantify activity during that period. ActiTracs were fixed to the dogs' collars.

4.3.7. Heart rate monitoring

Heart rate monitors (Polar S610i, Polar Electro GmbH, Germany) originally designed for human athletes, were used to continuously record heart rate. The heart rate monitors were set to 5 sec recording intervals resulting in a maximum recording period of 23 h 20 min. Data were downloaded daily. After downloading, the data logger was started again, thus recording was interrupted for ca. 30 min only. The flexible belt with the electrodes was placed around the dogs' chest immediately behind the front legs. To ensure signal transmission to the electrodes, the dogs' fur was wetted with a 0.5% aqueous solution of a sodium polymer (PNC 430, Spinnrad, Norderstedt, Germany). During winter a layer of neoprene (7 mm) was placed over the belts to keep the animal warm and to prevent the wet belt from freezing. For later analysis, the data were averaged as 1-h intervals. We recorded continuously heart rates of 12 dogs up to 23 days. Heart rate during digestion was defined as the heart rate within 24 h after ingestion of a meal; fasting heart rates include values from 24 h after ingestion of a meal until the next feeding event. For heart rate during exercise, 1-h averages during the periods spent running of each individual dog were extracted and pooled from each of the three sledding days to obtain a running heart rate value for each dog and these values were pooled to obtain an average for all dogs.

4.4. Results

4.4.1. Echocardiographic measurements

During winter, dogs of both groups had a significantly enlarged aortic root diameter in systole as compared to summer (Table 1). Comparisons between groups of dogs within season showed that the HI-dogs always had a larger aortic root diameter than the LO-dogs (one-way ANOVA, df=3, F=32.289, $p<0.001$). In the LO-dogs, the inner diameter of the left ventricle (LVIDd and LVIDs) and the thickness of the septum did not change between seasons although the body mass of the dogs differed by 30% between summer and winter. In the HI-dog group we found inner diameters of the left ventricle to be smaller in winter than in summer and no changes in the thickness of the septum between seasons.

4.4.2. Resting heart rate

During summer, when resting and fasting, the heart rates of the LO-dogs were the lowest HR we ever measured. Minimum 1-h averages of HR of individual dogs were as low as 24.6±3.8 bmp; on average the HR during resting and fasting was 46.4±10.0 bpm (Table 1). However, when resting and digesting a meal, the HR of the LO-dogs averaged at 99.8±16.3 bpm during summer and 90.8±16.0 bpm during winter. The HI-dogs during summer had resting and fasting heart rates of 73.6±15.1 bpm. When resting and digesting during summer the same dogs' heart rate averaged at 90.7±10.4 bpm. Winter heart rates of HI-dogs during resting and digesting were on average 108.8±11.8 bpm (Table 1).

When comparing heart rates between seasons one should keep in mind that winter has resting periods but no fasting periods. Therefore, comparisons can be made only between summer resting and digesting and winter resting and digesting. Possible differences account for differences in temperature and long term effects of work load between seasons and dog groups. A one-way ANOVA, comparing HI- and LO-dogs between seasons was significant (df=3, F=4.036, p=0.013). A subsequent post-hoc test (Holm−Sidak method) revealed significant differences between summer and winter in HR of HI-dogs (difference of means=18.118, t=2.85, p=0.007). In contrast, the HR of LO-dogs did not differ between summer and winter. We further compared heart rates of HI-dogs with those of LO-dogs during winter and found a significant difference (difference of means=18.007, t=3.05, p=0.004). Such difference was, however, not found during summer.

4.4.3. Short term effects of feeding

In summer, we continuously measured the heart rate of LO-dogs during feeding trials, when the dogs were fed every fourth day large amounts of food. At the end of a fast, heart rates were consistently low at around 46 bpm (Fig. 1). After ingesting a meal, the 1-h average of heart rate rose more than twofold to 118.6±11.2 bpm and reached peak values two hours after ingestion at 126.6±23.1 bpm. Certainly, part of the immediate increase in HR was because of excitement, but feeding was a rather short event of 2−5 min, and then excitement declined making place for a physiological response to digesting a large meal. The elevated HR was maintained for about four hours. Then, heart rate slowly and continuously declined reaching fasting values at thirty four hours after feeding; i.e., heart rates did not differ statistically from fasting values.

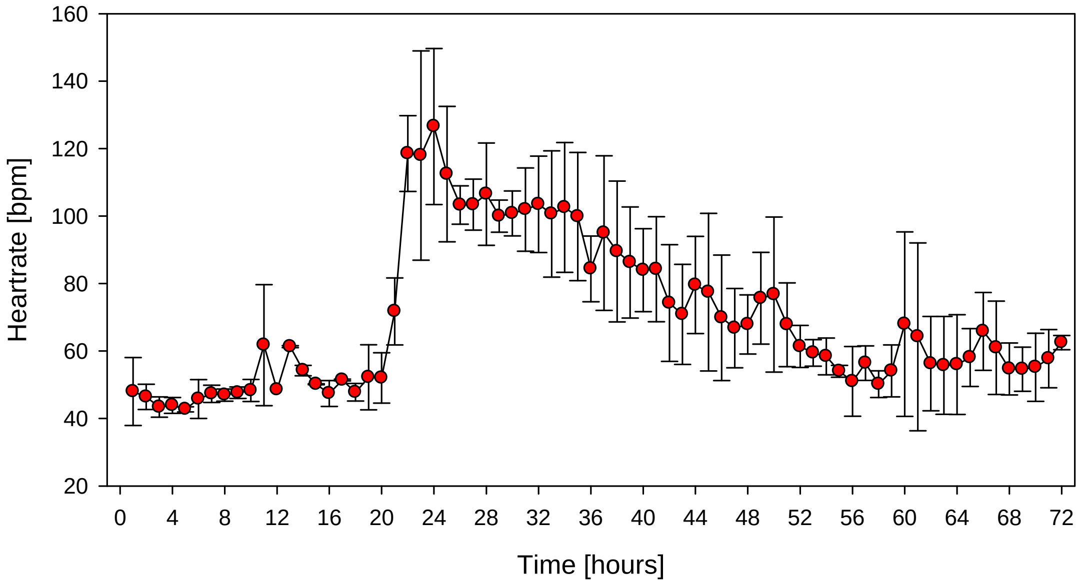

Fig 1. Heart rate over time of dogs (n=12) during fasting and digestion. Values are means±SD of four feeding trials. The actual feeding event was set to hour 22.

Table 1. Cardiac measures of HI- and LO-dogs during summer and winter. Because the left parasternal short axis view does not provide three landmarks for measurements in HI-dogs during winter, these data are italized.

	LO-dog group			HI-dog group			
Cardiac measure	Summer	Winter	Difference between seasons	Summer	Winter	Difference between seasons	Reference values
AO (mm)	19.3±1.4	20.6±1.3	T=106, U=116, p=0.012	22.8±1.2	23.9±1.2	t=2.361, df=24, p=0.027	13.7–31.1[a]
LVIDd (mm)	35.16±2.91	36.20±3.07	n.s.	41.48±3.53	*38.21±3.75*	T=189.5, U=32.5, p=0.024	27.6–44.5[a]
LVIDs (mm)	27.11±3.48	25.43±3.27	n.s.	31.76±3.56	*28.2±2.37*	t=3.224, df=24, p=0.004	15.7–29.5[a]
Diastolic septum thickness (mm)	8.27±1.22	8.43±1.74	n.s.	9.64±2.77	*9.50±1.45*	n.s.	7.1–9.5[a]
Systolic septum thickness (mm)	10.62±1.58	12.75±2.70	n.s.	12.79±3.55	*13.86±2.47*	n.s.	11.6–15.0[a]
HR resting+fasting (bpm)	46.4±10.0	n.m	–	73.6±15.1	n.m.	–	–
HR resting+digesting (bpm)	99.8±16.3	90.8±16.0	n.s.	90.7±10.4	108.8±11.8	T=53, U=91, p=0.002	133±24$_{pre}$ / 113±23$_{post}$[b]
HR working (bpm)	n.m.	n.m.	–	n.m.	200.4±10.9	–	142.3±26.6[c]

Values are mean±SD, n=12 dogs each season and location

[a] Values from domestic dog breeds of similar body mass (Boon, 2002)

[b] Values from Alaskan sled dogs before and after five months of training (Stepien et al., 1998)

[c] Treadmill running Siberian Huskies at 5 mph, 0% grade (Ready and Morgan, 1984)

AO aortic root diameter, *LVIDd* left ventricular inner diameter in diastole and *LVIDs* in systole, *n.m.* not measured, *n.s.* not significant

4.4.4. Response to work

The HI-dogs performed sustained work in front of the sled during winter. We recorded the heart rate and activity during two three-day sled trips (Fig. 2). 1-h average HR of working HI-dogs was 200.4±10.9 bpm, thus was almost twice as high as heart rates of these dogs when resting and digesting (108.8±11.8 bpm, Table 1). However, peak values of 1-h average HR while working reached 218.3±3.2 bpm at the third day of that trip. Heart rate and activity were perfectly matched only when running in front of the sled but not during the resting periods. Breaks during the daily working hours are visible in the activity curve as well as the heart rate curve in Fig. 2. During each break heart rate immediately declined and rose again to working values when the trip was continued. At the end of each day the dogs were separated in pairs and fed. Then, social activities and excitement about feeding caused substantial rise in heart rate, but within two to three hours, HR declined to values identical to resting and digesting. Because social activities between the dogs (including playing, fighting, mating) prolonged and overrode the actual recovery phase after the work period, it was not possible to record the recovery time in individual dogs.

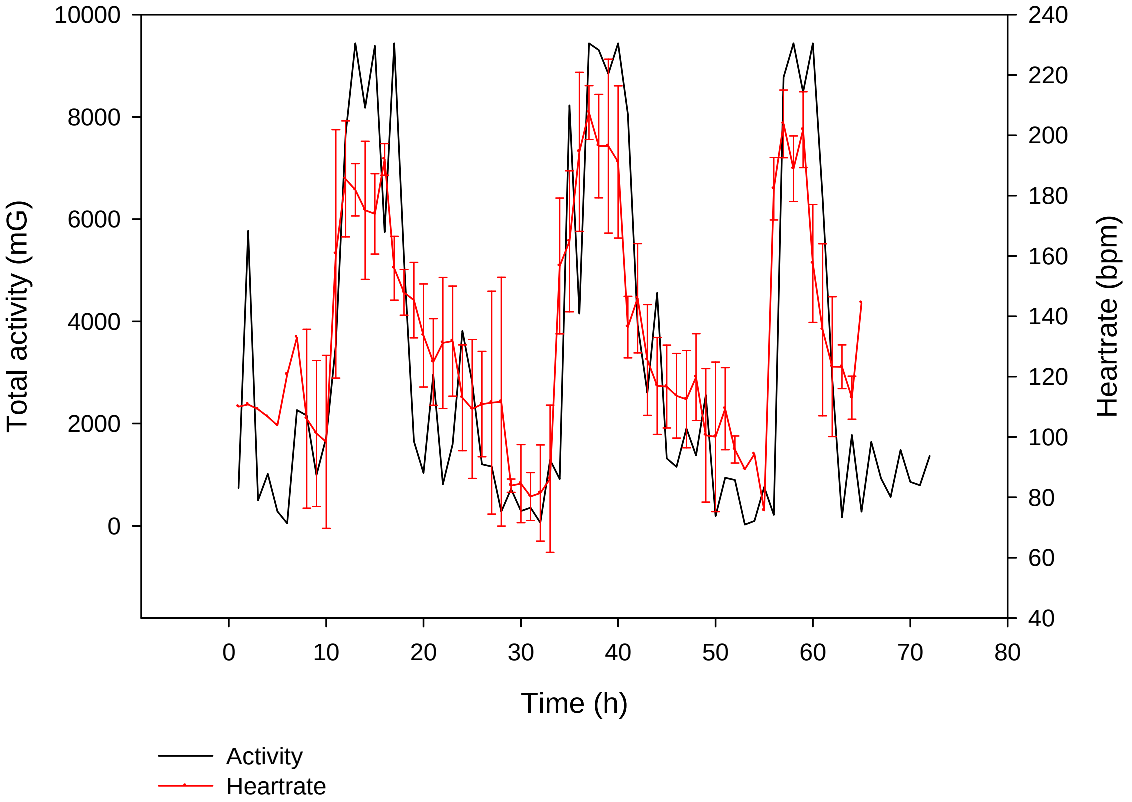

Fig. 2. Heart rate±SD and total activity of working dogs are closely correlated during a three-day trip (tour2). Data are 1-h averages of six dogs (heart rate) and one individual (activity) over 76 hours.

4.4.5. Functional cardiac parameters

We found no seasonal differences in LVEDV and LVESV in the LO-dogs (Table 2). However, SV during winter was 28% higher than during summer, LVEF was 11% higher, and FS was 7% more intensive. While resting in summer, digesting LO-dogs had more than twofold higher cardiac output than fasting LO-dogs. Likewise, digesting HI-dogs had a 1.4 times elevated cardiac output as compared to fasting during summer. Because HI- and LO-dogs differed so much in body mass, we calculated mass specific CO to compare the two dog groups. When testing mass specific CO while resting and digesting for seasonal and dog-group differences using one-way ANOVA, we found no differences between groups and seasons. The percentages of LVEF and FS are mass-independent parameters and did not differ between the two dog groups during summer. Size dependent variables like LVEDV, LVESV, and SV were higher in the larger HI-dogs than in the LO-dog group. Because we suggest that the reported changes in the dimensions of the hearts of the HI-dogs are due to an anterior-shift of the heart during winter, we only present values of functional parameters derived from morphological measurements during summer in these dogs.

Table 2. Echocardiographic parameter of HI- and LO-dogs. Because measurements of left ventricle dimensions presumably have been done at a more posterior section of the ventricle in HI-dogs during winter we excluded values derived from these measurements.

	LO-dog group			HI-dog group	
Echocardiographic measure	Summer	Winter	Difference between seasons	Summer	Reference values
LVEDV (ml)	53.05±9.92	55.71±11.36	n.s.	77.01±15.82	$67.9\pm4.9_{\text{pre}}$/ $68.3\pm7.0_{\text{post}}$[a]
LVESV (ml)	28.23±8.28	23.90±7.61	n.s.	40.98±10.91	$48.1\pm13.6_{\text{pre}}$/ $50.6\pm15.0_{\text{post}}$[b]
SV (ml)	24.82±7.46	31.82±6.35	t=2.475, df=22, p=0.022	36.07±14.21	$25.9\pm2.0_{\text{pre}}$/ $32.0\pm2.0_{\text{post}}$[c]
LVEF (%)	47±12	58±8	T=198, U=24, p=0.006	46±13	$45\pm12_{\text{pre}}$/ $47\pm12_{\text{post}}$[b]
FS (%)	23±8	30±5	T=200, U=22, p=0.004	23±8	$23\pm7_{\text{pre}}$/ $24\pm7_{\text{post}}$[b]
CO resting+fasting (ml min^{-1})	1159.2±432.9	–	–	2661.1±899.6	
CO resting+fasting (ml min^{-1} kg^{-1})	60.8±21.8	–	–	77.0±24.9	
CO resting+digesting (ml min^{-1})	2437.7±704.3	2886.6±771.8	n.s.	3829.2±1804.7	
CO resting+digesting (ml min^{-1} kg^{-1})	128.0±35.7	106.9±29.5	n.s.	111.1±51.0	

Values are mean±SD, n=12 dogs each season and category

[a]Values from dogs before and after 12 weeks of treadmill-training, 1 h/day, 5 days/week (Wyatt and Mitchell, 1974)
[b]Values from Alaskan sled dogs before and after five months of training (Stepien et al., 1998)
[c]Values from dogs before and after 12–18 weeks of treadmill-training (Barnard et al., 1980)

LVEDV and *LVESV* left ventricular end-diastolic and end-systolic volumes, *SV* stroke volume, *LVEF* left ventricular ejection fraction, *FS* fractional shortening, *CO* cardiac output, *n.s.* not significant

4.5. Discussion

The key question of this study was if seasonally changing work load and food supply elicit morphological and/or functional central cardiovascular adaptations. In a semi-natural setting, the Inuit sled dogs experience seasonal fluctuations between work and leisure, and between feast and famine. This is different from most experimental studies (including studies with domestic dogs) in which the animals' responses to a single training period or fasting period were investigated. Although Inuit sled dogs are exclusively bred and kept for sledding they are not easily comparable to the athletic sled dogs kept by western mushers, which are trained and fed regularly throughout the year.

In both groups of dogs of this study, measured parameters of cardiac morphology and function fall within the broad range of reference values for domestic dogs of same body size (Boon et al., 1983; Lombard, 1984; Mashiro et al., 1976). Nevertheless, values of some parameters differed between seasons. Morphologically, the aortic root was enlarged during working in winter; presumably to meet the higher demand of oxygenated blood to be transported to peripheral organs such as enlarged locomotor muscles. The size changes of the aortic root have been found to be similar in both, the LO-dog group and the HI-dog group. Therefore, we suggest that only the seasonal differences in activity/exercise trigger size adjustments of the aortic root. Difference in the food supply seem to have no effect on aortic root diameter. Our results are in contrast to Stepien et al. (1998) who did not find any changes in aortic root dimensions in Alaskan sled dogs after 5 months of training. No other sources for comparisons are available. Therefore it is possible that differences in training/work conditions and/or breed specific responses may account for the observed contrast between Inuit sled dogs and Alaskan Huskies.

4.5.1. Morphological and functional changes of the heart in the LO-dog group

The LO-dogs lost up to 30% of their body mass during summer which might have resulted in a reduction of heart mass and a thinning of ventricular walls and interventricular septum. During winter, adequate feeding and increased oxygen consumption of the peripheral organs might have caused an increase of the heart and a thickening of ventricular walls and septum. However, we did not observe morphological changes of the left ventricle. This is in straight contrast to Alaskan sled dogs in which the inter-ventricular septal thickness and the inner diameter of the left ventricle in diastole and systole increased significantly after 5 months of training (Stepien et al., 1998). Also, Wyatt and Mitchell (1974) reported increases in left ventricular end-diastolic wall thickness and estimated LV mass in dogs (unspecified breed) after 12 weeks of treadmill training but did not report about changes in inter-ventricular septal thickness or inner diameter of the left ventricle. Responses to training may differ between training regimes and intensity. The Inuit sled dogs do not receive a sophisticated training at all; they are used as draught animals whenever needed and remain chained to their places at all other times. Working periods vary in length and intensity but the Inuit sled dogs never race at same speed as the Alaskan sporting dogs do. Again, we can state only that heart size of Inuit sled dogs obviously responds differently to changes in work load as compared to Alaskan Huskies, but we cannot discriminate whether this is breed specific or depending on different working conditions.

Besides effects of training, nutrition affects heart morphology and functioning. Weight loss of 20–25% within three weeks of protein-calorie malnutrition led to ventricular wall thinning and a decreased cardiac mass in dogs of unspecified breed (Alden et al., 1987). In humans, self induced starvation leads to significantly reduced LVIDd and LVIDs (St. John Sutton et al., 1985) but it had been shown that left ventricular dimensions and aortic root dimensions normalize after refeeding and recovering body mass in Anorexia nervosa patients (Gottdiener et al., 1978). In our study, the heart of Inuit sled dogs loosing 30% body mass did not change, thus the response clearly differed from that in the aforementioned studies. However, the timing of the weight loss might be an important factor. A comparison between long-term voluntary undernutrition as in Anorexia nervosa, short-term malnutrition in the dogs studied by Alden et al. (1987), and the seasonal undernourishment of Inuit sled dogs may be inappropriate.

Two explanations are possible for the absence of morphological change of the heart in the Inuit sled dogs: (1) of course, there is always the possibility that the effects of fasting during summer and exercise during winter were not strong enough to cause any changes in the morphology of the left ventricle. However, we may safely exclude this possibility because we observed changes in aortic root diameter. (2) The dogs' heart size might permanently be adapted to the level of work load they experience. We reported previously non-reversible phenotypic adjustment to external conditions for the capillary network of the locomotor muscles of Inuit sled dogs (Gerth et al., 2009b). Support for this idea comes from the observation that those dogs that experience higher work load (HI-dogs in our study) generally have larger heart dimensions than the LO-dogs that have lower work load during winter. Although we found changes of ventricle dimensions in the HI-dog group, we assume that the reported smaller diameter of the left ventricle during winter is an artifact of the measurement method. If the diameter of the ventricle did change, it should have increased in size during winter due to the increased work load. Because measurements of heart dimensions in the short-axis view are only possible in a small area between the ribs we must have measured the ventricle diameters at a more posterior place of the ventricle. Despite the absence of morphological changes in the LO-dogs, functional cardiac parameters did change enabling the heart to work more efficient.

A higher SV was described in racing greyhounds compared to non-racing greyhounds (Pape et al., 1985), in beagles after a ten-week treadmill training (Ritzer et al., 1980), and in dogs that were progressively trained on a treadmill for 12–18 weeks (Barnard et al., 1980). An increase in SV was also found in undernourished dogs as response to 20–25% weight loss within three weeks of undernutrition (Alden et al., 1987). Surprisingly, Stepien (1998) did not find changes in the SV in Alaskan sled dogs even though he reported significant increases in inner diameters of the left ventricle in diastole and systole after a five-month training period. The different results in these studies show that, depending on dog breed, work intensities, and nutritional status of the dogs, SV-changes appear differentially depending on the stimulus tested and trainings- or feeding-protocol applied in the particular study.

The values of LVEF in both, the resting LO-dogs as well as resting HI-dogs match the values found in the Alaskan sled dogs studied by Stepien et al. (1998). However, a rise in LVEF during the working period in winter as found in our LO-dogs was not found in Alaskan sled dogs (Stepien et al. 1998), nor in the dogs studied by Ritzer et al. (1980) or the Beagles studied by Mackintosh et al. (1983) after 7 weeks of endurance training.

Reported effects of malnourishment are similarly contrasting. Compared to winter, we measured decreasing LVEF in summer when the dogs progressively lost weight up to 30% of winter mass. In straight contrast to our results, Alden et al. (1987) reported increasing LVEF in dogs after calorie-reduced feeding that resulted in a 20–25% weight loss over a three-week period.

While Lombard (1984) reported a mass independent value of fractional shortening (FS) of the left ventricle in systole of 39±6% for dogs, our values for both resting dog groups during summer are lower and match the pretraining values of Alaskan sled dogs given by Stepien et al. (1998). Contrary to the constant FS of these Alaskan sled dogs after five months of training, our LO-dogs had a significant increase in FS during working season. When comparing trained with untrained Greyhounds, Lonsdale et al. (1998) also reported significantly higher percentages of FS in the trained dogs.

Like Wyatt and Mitchell (1974) in their 12-week treadmill-trained dogs, we found no differences in the end-diastolic and end-systolic volumes of the left ventricle between winter and summer condition. Contrary, Ritzer et al. (1980) report an increase in LVEDV of the treadmill-trained dogs after ten weeks. A possible explanation for such differing results is that dogs adapt to a certain degree of activity or training and no further changes in LVEDV occur (developmental plasticity). Then, Wyatt and Mitchell’s (1974) dogs and the dogs in our study had already reached the plateau value while the dogs studied by Ritzer et al. (1980) were still in the process of developing their set point in LVEDV. Also, the intensity of training protocols applied in the different studies could have differed so that results appear contrasting although they actually only reflect differences in duration and intensity of training protocols.

The lack of morphological differences of the left ventricle between the seasons and the observation of functional changes suggest that the morphology of the cardiovascular system of Inuit sled dogs is permanently set to peak values/upper ceiling. This would be described as stemming from irreversible plasticity (developmental plasticity of Piersma and Drent, 2003). Keeping the morphology stable, the dogs in our study respond by adjusting functional parameters to the seasonally changing demand. This pattern of response is in contrast to what has been described as long term adaptations to training in human or animal elite athletes, or, as morphological adaptations to undernourishment as found in humans with eating disorders. A possible explanation for such different responses might be the repetitive and short term pattern in which workload and feeding conditions change in Inuit sled dogs.

4.5.2. Changes in resting heart rate

Seasonal differences in exercise and food supply led to long-term and short-term effects on heart rate. When comparing resting heart rates of Inuit sled dogs with reference heart rates of dogs of same size, it is important to acknowledge, that resting heart rates of dogs kept under western husbandry conditions always include digesting heart rate due to regular feeding schedules. If we assume resting and digesting as “baseline” for further comparisons, then values of both dog teams in both seasons fall within the range of HRs measured for a variety of domestic dog breeds of same size. Even though the values of resting and digesting HI-dogs during winter were higher, they are still within the lower level of the range of domestic dogs of similar size (Noble et al., 1967; O'Rourke and Bishop, 1971). There position at the lower margin of the reference values is not surprising because the sled dogs in this study were adapted to higher activity than "normal" companion dogs of same size and a decrease in heart rate is a well known effect of endurance training in dogs (Stepien et al., 1998; Wyatt and Mitchell, 1974) and in man (Giada et al., 1998).

The pronounced bradycardia of fasting LO-dogs may be an adaptation to seasonal undernourishment. Such vast down-regulation to half the resting heart rate, presumably to spare energy, has been described in voluntarily starving humans (Keys et al., 1950; Romano et al., 2003) as well as in (involuntarily starving) dogs (Alden et al., 1987). Here, we may assume that a reduction of 23% in heart rate as found in fasting HI-dogs is a rather normal response of dogs to intermittent feeding. During the seasons these dogs did not loose weight.

4.5.3. Immediate effects of feeding

Feeding elicited an immediate increase in heart rate in the dogs that was maintained for several hours until heart rate slowly declined to fasting values. In both dog groups, the heart rate increased to same values. Clearly, the pronounced bradycardia found in fasting LO-dogs can be reversed quickly and heart rate returns to normal values when the dogs are fed. A possible explanation for the stronger increase of heart rate in digesting LO-dogs compared to HI-dogs is a graded response of HR increase to differing meal size, because the LO-dogs received meals comprising 15% their body weight while meal size in HI-dogs was about 6–9% of their body mass. Grollmann (1929) reported feeding induced increases in heart rate in man. He reported that the magnitude of this increase depends on the amount of food ingested, but one hour after ingestion of a meal the heart rate started to decrease. Kelkbaek et al. (1989) reported an 17% increase of heart rate in man 30 min after eating a standard meal (6300kJ). However, in dogs, Vatner et al. (1970b) found heart rates that had returned to control levels already 10–30 min after ingestion of a single meal but did not report about the amount of food ingested. The pronounced and prolonged elevation of heart rate that we documented in the Inuit sled dogs is probably due to the enormous amounts of food that were ingested by the dogs after a long period of fasting.

Cardiac output is highly variable depending on the actual state of the animal. It is calculated as stroke volume times heart rate and thus can change within seconds due to stress, physical exertion, or excitement (Beerda et al., 1998; Sanders et al., 1977). Additionally, stroke volume is higher at low heart rates and decreases with increasing heart rate (Bristow et al., 1963). A methodological drawback is that calculation of CO includes SV, a volume parameter derived from two dimensional measurements of ventricle diameter in diastole and systole. Therefore, data may vary over a wide range. In summer, the CO of LO-dogs during

SDA more than doubled as compared to the resting and fasting condition. We cannot assign HR-data to resting and fasting conditions in winter because it was neither possible nor intended to fast LO-dogs for several days during winter.

Even though the CO of LO-dogs during SDA did not differ between the seasons we suggest that a 28% increase of SV in winter would lead to a potentially higher CO in exactly similar circumstances, but the amount of food ingested per feeding was smaller in winter than in summer. Because SDA depends on the amount of food ingested we suggest that the increment of SDA may have been smaller in winter than in summer.

4.5.4. Response to working

Sustained winter working heart rate of HI-dogs doubled resting and digesting heart rate of these dogs. Wyatt and Mitchell (1974), who recorded heart rates of exercising dogs on a treadmill also reported sustained working heart rates that were approximately twice the resting heart rate of the individual dogs used in that study. But, the dogs in that study were just running one h per day without pull load as compared to our dogs that run 8 h per day with 2.3 kg pull load.

4.5.5. Discussion of the methods and experimental setup

While the increase in the aortic diameter measured by the left parasternal long axis view indicates an enlargement of the left ventricular outflow tract in the HI-dogs during winter, the other echocardiographic parameters we measured did not. However, enlargement of the heart has been shown in many previous studies, triggered by low temperatures (Rezende et al., 2009) and physical training (Lonsdale et al., 1998). While the dogs in our study encountered both stimuli we failed to measure ventricular size changes. Instead we measured a decreased inner diameter of the left ventricle during diastole and especially during systole in HI-dogs during winter while septum thickness remained stable. An interpretation of these results that are in contrast to current literature data must include a critical evaluation of the methods. Echocardiographic access to the standing dogs’ heart it possible only at one position between the fourth and the fifth rib and change of the topographic position of the heart may result in a systematic error. If the access window is fixed but the position of the heart changes seasonally, for example due to increased ventricles, echocardiographic measurements might be conducted at a slightly different positions of the heart resulting in measurements taken at a more distal part of the left ventricle. While the standard landmarks for cross-sectional echocardiographs, i.e., the chordea tendinae are still visible at that position, the chamber may already have narrowed down. We therefore suggest that while echocardiography unambiguously is a helpful non-invasive tool to measure cardiac parameters, it appears to be difficult to use this method in longitudinal studies where treatments possibly lead to organ size changes or changes of the topographical position of inner organs. If repeated measurements are conducted, the usage of precise landmarks is crucial to avoid systemic measurement errors or high variability in the data.

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Chapter 5

General discussion

Summer life in the Arctic is characterized by 24 h daylight, mild temperatures, and plenty of food. Winter is harsh, cold, without sunlight, and scarce food. Animals living in the Arctic evolved various strategies to cope with these seasonally changing conditions. Some wander away by the end of the summer migrating further south where they find more opulent living conditions. Others escape the harsh winter by entering dormancy, thus avoiding unfavorable conditions. However, some animals stay and remain active throughout the year. Those who stay may need to adjust to the changing conditions. Phenotypic plasticity, i.e., the ability of a given genotype to produce several different phenotypes and thus adjust flexibly to changing environmental conditions, is one strategy to adjust to fluctuations in habitats that undergo seasonal changes. However, adjustments of one organ system will cause others to follow, because demand, supply and delivery within an organism need to be fine tuned to match the functions of different organ systems. The concept of symmorphosis states that no structure is built in excess or even maintained unless it is required (and fine tuned) to satisfy the functional needs of the organism.

The studies presented in this thesis address different aspects of structural and functional adaptations to seasonal changes in ambient temperature, exercise, and food supply using the Inuit sled dog as model organism. During winter the dogs perform sustained work as draft animals under extreme climate conditions whereas during summer they rest. A more than 4000 years long history of dog husbandry in the Arctic has established the tradition that dogs are fed regularly and adequately only when they are working, i.e., during winter. During summer, when the dogs are resting, they are fed only once or twice per week. The quality of food provided varies between locations and depends on the hunting success of the dog owners. These human made living conditions differ from the living conditions of wild arctic carnivores, where summer is the most active and food-rich period of the year. — The sled dog project purposely merged into this traditional system of human-dog interrelationship, considering the Inuit sled dogs as model system for an Arctic carnivore. Of course, artificial selection had shaped and changed the seasonal life cycle of the dogs, but, as an arctic carnivore it was easily accessible, quite in contrast to others like polar bear, wolf or fox. The following questions were addressed: (1) Does resting metabolic rate (RMR) vary between seasons due to differences in exercise, food supply, and environmental temperature? (2) What is the sustained metabolic scope of working Inuit sled dogs? (3) What is the proportion of metabolic rate devoted to specific dynamic action (SDA)? — If the metabolism is flexibly adjusted to changing demands, how do the organs respond? In particular, (4), what kind of structural adjustments are found in the locomotor muscles? Structural changes in the locomotor apparatus may require subsequent adjustments in the supply system which leads to the question (5), does the cardiovascular system show adaptive long-term and short-term responses to the changing conditions, and, (6) are such changes realized by structural or functional adjustments to the changing demands?

To test these questions a repeated measures design was selected that allows to study repeatedly same individuals under different conditions. Such an approach has several statistical advantages but requires, of course, non-invasive methods of investigation. Each individual serves as its own control, which allows an intra-individual comparison of measured parameters. Also, sample size for each group can be reduced thus allowing elaborate measurements under challenging conditions. Two different groups of Inuit sled dogs, the LO-dogs (low energy intake dogs) from Qeqertarsuaq in West Greenland and the HI-dogs (high energy intake dogs) living in Qaanaaq in North Greenland were investigated and compared with each other. The LO-dogs received sufficient food only during winter and were used for recreational dog sledding in the mild West-Greenlandic winter. The HI-dogs were well nourished throughout the year and were used for hunting and transportation on a regular basis throughout the harsh winter of North Greenland.

Because energy is the universal currency of life, Chapter 2 was devoted to characterize and partition the overall energy budget of Inuit sled dogs at different periods of the year. We have compared HI- and LO-dogs as two different representatives of living conditions in Greenland during summer and winter, i.e., while resting, exercising, and digesting in different temperature ranges. The key question is how animals living under extreme conditions partition their energy budget to survive periods of extreme cold or periods of famine. We were interested to see if Inuit sled dogs were able to down-regulate their daily energy budget during periods of fasting and inactivity. The first surprising result was the widely constant RMR of HI-dogs within a wide temperature range. Although postulated in earlier studies, but never seriously measured, we determined the lower critical temperature at about –25°C, i.e. only when temperatures fall below –25°C energy is invested in thermogenesis.

We investigated other conditions that potentially affect energy metabolism; e.g., processing, digesting, and absorbing food are processes that require energy investment even in otherwise resting animals (SDA). Carefully controlled feeding trials during summer revealed that during SDA, the metabolic rate doubled as compared to resting and fasting. However. The metabolic rate of dogs during SDA in summer did not differ from RMR measured during winter. The rise of MR during SDA provides an explanation for the constantly higher metabolism of resting dogs during winter as compared to RMR values during summer. During summer, RMR was measured in dogs for 3–4 days. Because of the daily food supply during winter the dogs were in a condition of continuous SDA. Therefore, we suggested that the actually measured RMR during winter always includes a portion of SDA.

While HI-dogs maintained a constant body mass throughout the year, the LO-dogs lost up to 30% of their body mass in summer. The negative energy balance of these dogs was characterized by a lowered resting heart rate accompanied by a rise in energy metabolism as compared to the RMR of HI-dogs during summer. While the lowered heart rate might be an attempt to slow metabolic processes down to save energy, the rise in metabolic rate shows ongoing breakdown of muscle protein to fuel energy consuming processes within the organism.

Working in front of the sled is the most energy consuming activity of Inuit sled dogs. When compared to other mammals, including human athletes, working HI-dogs showed the highest sustained metabolic rates (susMR) measured so far. Their susMR reaches 12.2 times resting metabolic rate. This is two times the values measured for other mammals or three times that measured in humans athletes where highest susMR were reported in tour de France cyclists ranging at 4.7 times resting metabolic rate.

Certainly, such comparisons are made on a wide phylogenetic scale and using different methods. However, they provide evidence that canine carnivores are able to maintain exceptionally high susMR. Obviously, it was this ability that made them most suitable as working animal in extreme environments.

When working during winter, the functional demand on the locomotor muscles of the dogs is considerably higher than during the “lazy” summer. Therefore, we were interested (Chapter 3) to see if structure and size of muscles change throughout the year. Investigating muscle structure also provides an excellent system to study if the different components of the muscle, e.g., fibers, mitochondria, capillaries, energy stores (lipid droplets) and the intercellular compartment showed similar degrees of change (in the sense of symmorphosis) or if different compartments were responding differently. While working during winter, muscle fibers were in full functional condition at both study sites. In summer, when the dogs were resting the muscle fibers were atrophied. The ultrastructure of fibers of the atrophied muscles was characterized by sarcomeres that were depleted of myofilaments and a dilatation of the sarcoplasmatic space between myofibrils. Additionally, the subsarcolemmal and interfibrillar mitochondria were reduced and less lipid droplets were stored within the fibers. The myofibrils of muscles of LO-dogs showed a higher degree of degradation than the myofibrils of HI-dogs. This supports the interpretation that undernourishment was the main factor for the observed structural changes of muscle tissue. With the smaller diameter of muscle fibers the distances between neighboring capillaries became smaller so that the capillary density increased even tough the number of capillaries per fiber and capillary-fiber-ratio remained constant. While muscle fibers showed different degrees of degradation in the two dog groups the capillary network remained unchanged in both groups. These results make two important contributions to understanding flexible responses to fluctuating conditions: (1) even within one functional system like the locomotor muscles, different compartments show different responses. Here, the fiber architecture was flexible but the capillaries were stable. (2) The stable morphology of the capillaries is in clear contrast to the idea of symmorphosis because one functional component of the system (the contractile compartment) changes, while the other (supply system) does not match its capacities to the changing component.

The fourth chapter looked at the heart size and function. We hypothesized that the heart would exhibit major functional and structural changes in response to the seasonally fluctuating conditions (as known from human athletes). Indeed, the large blood vessels (aorta) exhibited seasonal adaptations to the changes in exercise, food supply and ambient temperature. Ultrasonographs confirmed a larger aortic root diameter in all dogs during winter than during summer. In the heart, the dimensions of the left ventricle in systole and diastole remained unchanged in LO-dogs while HI-dogs had reduced inner diameters of the left ventricle during winter.

These findings are in contrast to current literature where cold exposure and physical work always lead to an enlargement of the heart. Currently, we can offer only unresolved methodological problems to explain the contrasting results. One possible explanation is based on the echocardiographic access window to the heart. The heart can be imaged only if the scanner head is placed on a narrow window between the fourth and fifth rib with basically no space for adjusting the access position. When inner organs and the heart of working dogs change in size and position within the thorax but the access window remains the same, the morphometric measurements become confounded by the changing position of the heart. For example, if the heart moved anterior and measurements were conducted through the same access window as in summer, than we would have measured the inner dimensions of the left ventricle more posterior, closer to the tip of the ventricle where the ventricle walls already narrowed down.

In our measurements, the thickness of the interventricular septum did not change throughout the seasons at both study sites. Again this is in contrast to the expectations and may be confounded by an error in the measurements of the ventricle dimensions. Only a thickening of the ventricle wall would have been a coherent physiological explanation of the smaller inner diameters of ventricles of HI-dogs in winter condition as compared to the summer condition.

We also explored changes of functional parameters of the cardiovascular system like cardiac output. During SDA, cardiac output did not change between seasons in both dog groups. Fasting in summer led to a 50% reduction of cardiac output in LO-dogs. Other parameters of cardiac function showed different seasonal adaptations in both dog groups. Compared to summer values, stroke volume, left ventricular ejection fraction, and fractional shortening of the left ventricle increased during winter in LO-dogs but remained unchanged in HI-dogs. In contrast, when resting during winter the HI-dogs had elevated heart rates whereas the heart rates of LO-dogs did not differ from summer values.

5.1. General conclusions

Because of their ability to maintain very high susMR over extended periods and to withstand extremely low temperatures, Inuit sled dogs are perfect working animals under Arctic conditions. High aerobic capacities probably evolved as part of the cursorial hunting strategies of the ancestors of the dog that required them to run for long periods to exhaust their prey. This is in contrast to the ambush hunting of felids, which have never become domesticated as working animals.

We have shown that Inuit sled dogs respond differently to changing demands on different levels of the cardio-vascular system and the muscle apparatus. Some components (e.g. capillaries between muscle fibers, ventricular septum) are kept continuously in a condition that meets the demands of the working season. However, other components (e.g. muscle fibers, aortic root diameter) are flexibly up- and down-regulated meeting the demands of the present season, thus minimizing energy costs for maintenance of organ structures. Minimizing energy investments in thermoregulation by maintaining a wide thermoneutral zone and down-regulation of the entire metabolism by reducing heart rate during times of famine are also strategies that enable the Inuit sled dog to cope with the present husbandry conditions in Greenland.

While the physiology of the Inuit dogs makes them perfectly adapted to a life under extreme conditions, the Danish legislation, has recently created rules that conflict with dog physiology as well as with a long Inuit tradition of dog husbandry. The legislation requests that dog owners chain their dogs when they are not working. The original intention was reduce dog induced accidents and other problems caused by free roaming and scavenging dogs within settlements. However, the chaining of dogs results in injuries (and potential death through strangulation) because of entangled chains, undernourishment because Inuit do not feed dogs regularly, and dehydration because permanent water supply is almost impossible. Solutions to these relatively recent problems are possible only when all sides, dog physiology, Inuit traditions and Danish safety standards are considered.

Together with their human masters dogs have inhabited every continent and every climate zone on earth. This study revealed the remarkable ability of the dogs to perfectly adjust morphologically and functionally to changing demands in the Arctic. We would wish the unique interaction between Inuit and dog that is built on the outstanding physiology of the dogs will not be destroyed by cultural deprivation and legislation from a coalition of legislation, administration and economy that is alien to the life in the Arctic.

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Ehrenwörtliche Erklärung

Hiermit versichere ich, dass diese Arbeit von mir selbständig und nur unter Verwendung der angegebenen Hilfsmittel angefertigt wurde.

München, den 28.01.2010

Nadine Gerth