Norwegian University of Life Sciences
Faculty of Environmental Sciences
and Natural Resource Management

Philosophiae Doctor (PhD)
Thesis 2021:74

Energetics in seasonal environments: reindeer as a case study

Energetikk i sesongmessige miljøer: reinsdyr som fokusart

Liv Monica Trondrud

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Monica
July 16, 2021

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English summary

How endothermic animals manage and allocate energy to critical processes (maintenance, reproduction, thermoregulation and activity) can determine their success and survival. The balance in energy expenditure related to these processes can also influence their ability to respond to disturbances in their environment. In seasonal environments, the predictable annual changes in resources (food, nutrients and water) have led to a large range of adaptations in animals, including seasonal adjustments in energy expenditure, body temperature and activity levels. The aim of my thesis is to evaluate the relative importance of central drivers of energy expenditure under different seasonal and reproductive contexts in reindeer. Reindeer provide an excellent example of a highly adapted species to the seasonal changes in food availability, temperature and light conditions of the Arctic and sub-Arctic regions. Studying the interplay between energy expenditure, environmental variation and physiological states in this species can shed light on how non-hibernating animals balance energy expenditure in seasonal environments.

My thesis consists of three papers. The data collected comes from two different reindeer populations, in Svalbard (Svalbard reindeer, Papers I and II) and in Northern Finland (domestic reindeer, Paper III). In both systems I use two common methods for measuring energetics in free-living animals, the doubly labelled water (DLW) method to quantify daily energy expenditure (DEE) over a given time period (1-2 weeks), and the heart rate method, in which heart rate serves as an indicator of energy expenditure over longer time periods (>1 month). In all three papers, I use biologgers to monitor behaviour (activity levels) and physiology (subcutaneous body temperature; T_{sc}) in relation to DEE (Papers I and III) or heart rate (Papers II and III).

The role of body mass for winter energetics and fasting endurance (how long animals can survive on their internal body reserves) is the main focus of Paper I. In Paper II, I explored determinants of heart rate to identify constraints on energy management strategies within summer and winter. Finally, in Paper III, I investigate drivers of energy expenditure during peak lactation in domestic reindeer and their physiological and

behavioural responses to extreme warm weather.

In winter, the most important determinant for energy expenditure was fat-free body mass (likely reflecting maintenance requirements), and to a lesser extent T_{sc} and activity levels (Paper I). Lactating females have overall higher energy demands (respiration + energy exported through milk), but lactation was not a driver of seasonal variation in energy expenditure, as there were only small differences in energy expenditure (respiration) between lactating and non-lactating females within summer (Papers II and III). Adaptations to seasonality in their environment has resulted in summer and winter phenotypes, in which there is likely less room for additional variation in energy expenditure (Paper II). Yet this variation appears to be greater in summer, when food is abundant (Papers II and III), than in winter, when food is scarce.

Monitoring animal behaviour and physiology with the use of biologgers allow us to study energetics in free-living animals, and models of mammalian energetics can be important tools for predicting responses to climate change. For instance, I show that reindeer do not elevate heart rates in response to hot environmental conditions, which suggests that other mechanisms are involved to alleviate heat stress (Paper III). I also show that both activity and T_{sc} are important components of energy expenditure, but adjustments in either of these are not as important as the size of autumn fat stores (Paper I), which can influence survival in warm and icy winters. The findings from my thesis demonstrate that the relative contribution of body mass and body composition, temperature (ambient and body), reproduction and activity as drivers of energy expenditure depended on seasonal, individual and reproductive contexts. Future research should therefore consider how individual variation, thermoregulation and body mass can be incorporated into models to predict long-term fitness consequences of different strategies for energy management.

Norsk sammendrag

Endoterme (varmblodige) dyrs overlevelsesevne påvirkes i stor grad av hvordan dyra tilpasser sitt energiforbruk i forhold til viktige prosesser som overlevelse, reproduksjon, regulering av kroppstemperatur og aktivitetsnivå. Balansen i energiforbruk mellom disse prosessene vil også påvirke dyras evne til å respondere på forstyrrelser i miljøet. Mange arter har utviklet tilpasninger til forutsigbare sesongmessige endringene i tilgang på ressurser som mat, næringsstoffer og vann. Slike tilpasninger kan være endringer i energiforbruk, kroppstemperatur og aktivitetsnivå. Målet for denne avhandlingen er å vurdere det relative bidraget av prosessene som påvirker energiforbruk hos reinsdyr i forskjellige sesonger og under forskjellige stadier av reproduksjon. Reinsdyr er et ypperlig eksempel på en art som er godt tilpasset sesongvariasjonene i mattilgang, temperatur og lysforhold i Arktis og sub-arktiske strøk. Å studere samspillet mellom energiforbruk og endringer i både miljøet og fysiologiske tilstander kan hjelpe oss med å forstå hvordan dyr kan balansere energiforbruket sitt i sesongmessige miljø.

Denne avhandlingen består av tre artikler. Innsamlet data kommer fra to forskjellige studiesystemer, på Svalbard (Svalbardrein, artikkel I og II) og i Nord-Finland (tamrein, artikkel III). I begge systemene bruker jeg to vanlige metoder for å måle energetikk i frittlevende dyr. Dette er dobbeltmerket vannmetoden for å måle daglig energiforbruk over en gitt tidsperiode (1-2 uker), og hjertefrekvensmetoden, der dyrets puls fungerer som en indikator på energiforbruk over lengre tidsperioder (> 1 måned). I alle artiklene bruker jeg biologgere til å overvåke atferd (aktivitetsnivå) og fysiologi (subkutan kroppstemperatur; T_{sc}) i forhold til daglig energiforbruk (artikler I og III) eller hjertefrekvens (artikler II og III).

Fokuset i artikkel I er hvordan kroppsvekt og kroppssammensetning (fett og fettfri masse) påvirker energiforbruket om vinteren, og hvor lenge reinsdyr kan overleve på sine indre kroppsreserver. I artikkel II utforsker jeg hva som påvirker hjertefrekvens for å identifisere strategier og begrensninger av energiforbruk og -balanse sommer og vinter. I artikkel III undersøker jeg hva som påvirker energiforbruket hos lakterende tamrein når melkeproduksjonen er høyest, videre også dyras fysiologiske og atferdsmessige

reaksjoner på ekstremt høye temperaturer (varmebølge). Om vinteren var det dyras fettfrie kroppsmasse som hadde størst påvirkning på energiforbruket, som sannsynligvis gjenspeiler vedlikeholdskrav, etterfulgt av T_{sc} og aktivitetsnivå (artikkel I). Simler som diet hadde høyere energibehov (respirasjon + energi eksportert i melka), men laktasjon i seg selv var ikke pådriveren for sesongmessige variasjoner i energiforbruk (respirasjon), da det bare var små forskjeller mellom simler med kalv (diende) og uten kalv om sommeren (artikler II og III).

Overvåking av dyrs atferd og fysiologi ved bruk av biologgere tillater oss å studere energetikk hos frittlevende dyr. Modellering av energetikk kan være viktige verktøy for å forutsi pattedyrs responser til klimaendringer. Jeg viser for eksempel at reinsdyr ikke øker hjertefrekvensen når det er varmt om sommeren, noe som antyder at andre fysiologiske mekanismer er involvert for å lindre dyras varmestress (artikkel III). Økende temperaturer i Arktis er en utfordring som vil bli større med klimaendringene. Jeg viser også at både aktivitet og T_{sc} er viktige komponenter i reinsdyrs energiforbruk. Dyras nedjustering av disse er derimot ikke like viktig som størrelsen på deres indre fettlagre (artikkel I), for å overleve milde og isete vintre. Resultatene fra min forskning viser at det relative bidraget av kroppsvekt og kroppssammensetning, temperatur (omgivelses- og kropps-), reproduksjon og aktivitet som påvirkere av energiforbruk avhenger av sesongmessige, individuelle og reproduktive sammenhenger. Fremtidig forskning bør derfor vurdere hvordan individuell variasjon, termoregulering og kroppsvekt kan innarbeides i modeller for å forutsi langsiktige konsekvenser av variasjoner i energiforbruk.

Synopsis

Energy sustains life. The rate at which energy and materials are being taken up, transformed and expended by an organism is represented by the metabolic rate (Schmidt-Nielsen 1972b). Metabolic rates inform us of how much energy (i.e.: food) an animal needs and how this impacts the ecosystem it inhabits (by consuming food; McNab 2002). Quantifying energy expenditure of animals therefore improves our understanding of critical processes, such as resource demands (Nagy 2005), growth rates (Brown et al. 2004; Anderson and Jetz 2005), and life history parameters, including lifespan and reproduction (Speakman 2005; Speakman 2008). The field metabolic rate, also referred to as daily energy expenditure (DEE), reflects the organism's energy expenditure under natural conditions and is therefore a key variable in ecological physiology (Nagy 2005; Anderson and Jetz 2005; Hudson et al. 2013). The main energetic components that account for an animal's DEE are basal metabolism (maintenance processes), growth, digestion (heat increment of feeding), thermoregulation, reproduction, and activity (McNab 2002). How animals manage and allocate energy to these different pathways at different life history stages and environmental conditions (Halsey et al. 2019), ultimately determine their success and survival. It can also inform us of their ability to respond to both short-term and long-term perturbations in their environment (Auer et al. 2015; Norin and Metcalfe 2019).

Drivers of energy expenditure

Because maintaining a larger body requires more energy than a smaller body (in absolute terms), body size (and hence mass) is an important organismal trait and one of central drivers of metabolic rates (Anderson and Jetz 2005; Nagy 2005; Speakman and Król 2010). Across species and taxa, metabolic rate scales positively with body mass on the form

$$aM^b$$

where M is body mass, a is a normalization constant and b is the allometric exponent. Hence large animals have higher overall energy demands (Kleiber 1961). The relationship between energy expenditure and body size and/or mass can determine whether animals are in energy deficit (lose mass) or in surplus (gain mass/export energy). Body condition, which reflects the nutritional status of an animal (often as ratio between mass/size or fatness), is linked to other functions like regulation of body temperature (reviewed in Hetem et al. 2016) and reproductive output (e.g. Festa-Bianchet 1998). Body mass (and condition) can therefore influence when and why animals employ different energy management strategies which in turn affects fitness and survival.

All mammals and birds are endotherms, producing their own body heat as a by-product of metabolic processes (Angilletta et al. 2010). Mammals typically maintain normothermic core body temperatures between 35 and 38°C (Clarke and Rothery 2008). When ambient temperatures decline, the temperature gradient between the body and the ambient air increases, leading to increased heat loss (Scholander et al. 1950). Hence it is more costly to maintain high body temperature when ambient temperatures are low (Schmidt-Nielsen 1972b). Energy expenditure therefore typically scales negatively with both ambient and body temperature across species (Gillooly et al. 2001; Speakman and Król 2010). Furthermore, the relationship between body size and temperature is important since, all else being equal, a small body has a greater surface-to-volume ratio than a large body, and hence small bodies lose heat more quickly (Speakman and Król 2010). Yet, many endotherms can modulate body temperature to varying degrees ("heterothermy"; Levesque et al. 2016; Hetem et al. 2016). The relationship between metabolic rates and body temperature not only reflects the relative costs of maintaining high body temperature, but also the potential benefit of heterothermy in response to environmental conditions (Arnold et al. 2004; Humphries et al. 2005) and physiological states like reproduction (Schmidt et al. 2020).

Reproduction is an energy demanding process (Gittleman and Thompson 1988). During reproduction, animals must acquire energy to their offspring and their own metabolic requirements. For mammals, lactation is the most energetically costly period of the reproductive cycle (Oftedal 1985; Gittleman and Thompson 1988; Speakman 2008). Energy demands during reproduction, and lactation in particular, are therefore much higher than non-breeding levels, and how animals balance these against the remaining energetic components can impact their reproductive success. Some animals increase food intake during reproduction (‘income breeding’, Drent and Daan 1980), typical for small mammals (Speakman 2008), while others mobilise stored energy reserves (‘capital breeding’), characteristic of true seals (e.g. Reilly et al. 1996) and denning bears (e.g. Atkinson and Ramsay 1995). Most species however, fall along a continuum of which income and capital breeding represent opposite ends (Stephens et al. 2009). There is also evidence that some lactating mammals downregulate other metabolic processes, for instance activity levels (Shuert et al. 2020), to compensate for the elevated energetic costs of reproduction (McLean and Speakman 1999; Mellish et al. 2000; Krockenberger 2003). Quantifying the influence of reproductive states on energy expenditure, can inform us of potential trade-offs between reproduction and other energetic processes (Speakman 2008).

Exercise and other forms of locomotion (collectively referred to as "activity") is facilitated by the mechanical work of the muscles and hence requires energy. Although the energetic cost of moving depends on the type of locomotion (walking, running, flying, swimming), moving a body demands more energy than resting (Schmidt-Nielsen 1972a). The intensity of activity is also inversely proportional to the duration it can be sustained (Piersma 2011). For a given type of locomotion, mass-specific costs per unit distance scales negatively with body mass (Alexander 2006), meaning that the relative cost of moving is lower for a large body than for a small body. Ground conditions can also impact costs of locomotion, for instance it is heavier to walk through snow than on barren ground (Parker et al. 1984), hence there may be seasonal variations in activity-related costs. Linking energy expenditure to activity can help us understand how environmental and physiological conditions impact behaviour (e.g. Pagano et al. 2018; Thiel et al. 2019; Shuert et al. 2020).

Seasonal environments

Interspecific relationships of metabolic rates have been used to predict large-scale ecological processes such as species distributions (e.g. Kearney and Porter 2009) and biomass/abundance (e.g. Clarke and Gaston 2006). Also variation in metabolic rates within a species, both between and within individuals, are important because morphological, physiological and behavioural adjustments can facilitate changes in metabolic rate (and hence energy requirements) in response to changes in the environment (Humphries et al. 2005; Careau et al. 2013; Blix 2016; Arnold 2020). Seasonal environments are particularly interesting in this context, because they are found in most parts of the world and host a wide range of species that have adapted to a predictable change in resource availability, temperatures and light conditions. Key adaptations involve the timing of reproduction and growth, processes that enhance fitness and survival (Stearns 1992), typically matched to the period when resources (e.g. food, nutrients, water) are abundant (Varpe 2017). In the unproductive season, resources are scarce or of poor quality, and key adaptations involve either migration (Alerstam and Bäckman 2018), hibernation (Geiser and Ruf 1995), energy conservation and/or energy storage (Blix 2016; Williams et al. 2016).

In the Northern Hemisphere endotherms face the challenge of defending high body temperatures during winter when ambient temperatures are low, and resources in most cases are scarce. To overcome this energetic challenge, many animals reduce energy requirements. Strategies to save energy in the cold involve increased insulation (fur or pelage) and physiological adaptations such as peripheral cooling, which is the process where the temperature of the outer extremities are much lower than that of the body core, thereby reducing heat loss (Scholander et al. 1950). Other important strategies include hibernation and torpor, which are periods of greatly reduced energy expenditure facilitated by substantial reductions in body temperature and activity levels (Geiser and Ruf 1995). With the exception of bears and badgers (e.g. Evans et al. 2016), hibernation and torpor are employed by small mammals and birds (Ruf and Geiser 2015). However, reductions in DEE during winter, although not to the same extent as hibernating animals, have been documented in several non-hibernating animals, including reindeer (*Rangifer tarandus;* Gotaas et al. 2000), American martens (*Martes americana;* Gilbert et al. 2009), red squirrels (*Tamiasciurus hudsonicus;* Fletcher et al. 2012), Shetland ponies (*Equus ferus caballus;* Brinkmann et al. 2014), and llamas (*Llama glama;* Riek et al. 2019). Furthermore, seasonal reductions in heart rates (as a proxy for energy expenditure) have been documented in a range of ungulate species, including reindeer (Arnold et al. 2018; Mesteig et al. 2000), red deer (*Cervus elaphus;* Arnold et al. 2004), Przewalski horses (*E. f. przewalskii;* Arnold et al. 2006), Alpine ibex (*Capra ibex;* Signer et al. 2011), moose (*Alces alces;* Græsli et al. 2020) and chamois (*Rupicapra rupicapra;* Arnold 2020). These reductions correspond with reductions in activity (Arnold 2020; Græsli et al. 2020), voluntary food intake (Arnold et al. 2006; Turbill et al. 2011; Mesteig et al. 2000) and reductions and/or variations in subcutaneous (Brinkmann et al. 2012) and core body temperatures (Arnold 2020; Græsli et al. 2020). Many species inhabiting other seasonal climates like sub-tropical and arid regions, also display reductions in DEE during the dry season when food and/or water is limited (Nagy and Knight 1994; Williams et al. 2001; Evans et al. 2003; Rimbach et al. 2018; Sha et al. 2018). In contrast, some species express seasonal variation in resting metabolic rates (RMR - reflecting maintenance requirements) but not DEE (e.g. Scantlebury et al. 2005), while others display no seasonality in RMR but elevated DEE in the good season (e.g. Zelová et al. 2011). Nonetheless, there are also species without any strong seasonal variation in energy expenditure, heart rate or body temperature (Corp et al. 1999; Quin et al. 2010; Reimoser 2012; Thiel et al. 2019).

Yet, in most ungulates, for whom vegetation is the primary food source, seasonal adjustments in energy expenditure are expressed even in captivity with ad libitium food access (Mesteig et al. 2000; Arnold et al. 2015). This suggests that reduced appetite is an adaptation to predictably low resource availability in winter (Tyler and Blix 1990). Indeed, seasonal food intake is modulated by hormones related to metabolism and growth (Rhind et al. 2002), which in turn are cued by photoperiod (Lincoln et al. 2001). Strong seasonality in life history traits, physiological traits (body composition and body temperature) and behavioural traits (activity) to accommodate seasonal regulation in energy expenditure, are evidently key adaptations to living in seasonal environments. Less information is known about how different energetic pathways (thermoregulation, reproduction, activity and maintenance) influence within-seasonal variations in energy expenditure (but see Arnold et al. 2004; Menzies et al. 2020; Schmidt et al. 2020, for examples). The aim of my thesis is to evaluate the relative importance of the central drivers of energy expenditure discussed above, in a species inhabiting one of the most seasonal environments on earth, reindeer in the Arctic.

Reindeer as a study species

Reindeer (*Rangifer tarandus*) are a Holarctic ruminant species with circumpolar distribution (Figure 1). They are the only cervids with a long history of domestication and today, both domesticated and wild subspecies exist. The Arctic is a strongly seasonal environment. Winters are typically long (6–9 months), dark and cold with scarce vegetation, and the period of plant growth in summer is relatively short (2–4 months). Reindeer are well adapted to this seasonality: heat loss in winter is strongly reduced because of a thick winter coat, and counter-current heat exchange of the blood vessels in the limbs and the nasal cavities (Blix and Johnsen 1983; Johnsen et al. 1985). Reindeer are also known to accumulate relatively large fat stores prior to winter, and the voluntary food intake, resting metabolic rate and activity levels in winter are considerably lower than in summer, even when fed *ad libitum* (Nilssen et al. 1984b; Mesteig et al. 2000). Reindeer mate in autumn (late September–early October) and give birth to a single calf (twin births are rare) in late spring/early summer (late May–early June), often before the start of vegetation growth (Åhman and White 2018). Body fat and protein reserves are typically mobilized to support energy demands in the late stages of gestation and early lactation (Barboza and Parker 2008), and up to 91% of nitrogen in milk protein is derived from the mother's body reserves (Taillon et al. 2013). Still, there is disagreement on the definition of reindeer as an income or capital breeder (e.g.: Kerby and Post 2013), although it appears that there may be different strategies among subspecies (e.g.: reindeer vs caribou; Barboza and Parker 2008).

One of the smallest and Northernmost subspecies is the Svalbard reindeer (*Rangifer tarandus platyrhynchus*) which are only found on the Svalbard archipelago (Figure 1). It is not known exactly when reindeer colonized Svalbard, but evidence suggests they have inhabited the archipelago for at least 5000 years (Knaap 1989). In contrast to most reindeer subspecies, Svalbard reindeer have no natural predators, there are no wolves, and predation by polar bears is rare (Derocher et al. 2000). Svalbard reindeer are not obligate migrants (Tyler and Øritsland 1989), but seasonal displacement can occur in response to poor winter conditions (Stien et al. 2010; Loe et al. 2016). The main cause of mortality is starvation in late winter (Reimers 1983) and population growth is mainly driven by late winter body mass of adult females (Albon et al. 2017), as this affects both

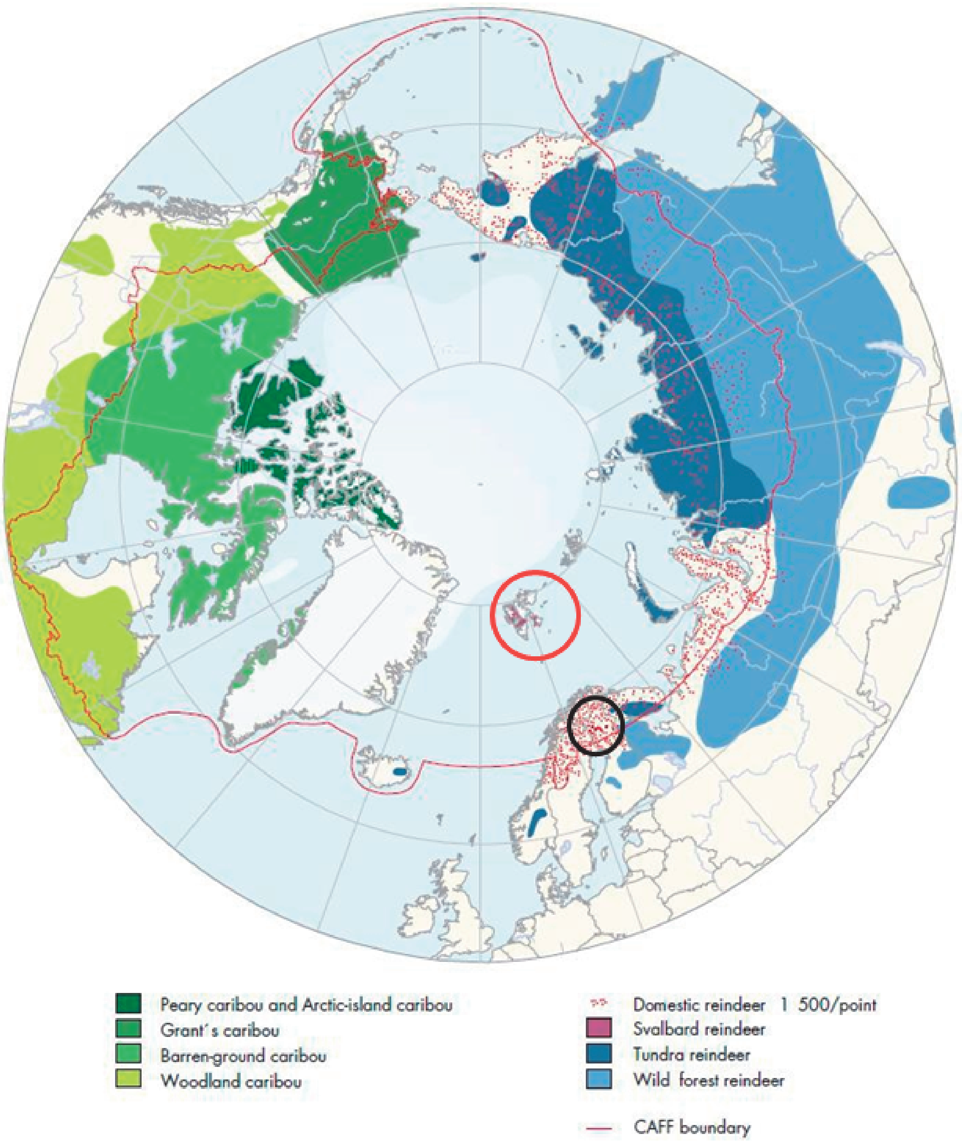

Figure 1: Distribution of extant subspecies of Rangifer tarandus (wild reindeer, caribou and domestic reindeer) in the Arctic. Source: Conservation of Arctic Flora and Fauna Working Group (2001). Two circles are inserted to highlight the location of Svalbard (red circle) and Northern Finland (black circle), the two study populations used in my thesis (Box 1).

survival and reproductive output. Because of its latitude (75–81°N), Svalbard undergoes drastic seasonal changes in light conditions, temperatures and vegetation (Van Der Wal et al. 2000). Svalbard reindeer are particularly well adapted to this extreme seasonality, being characteristically trunk-shaped with shorter limbs and noses (*platyrhynchus* = "flat nose") compared to domestic reindeer (Figure 2). Also, they have lower mass-specific resting metabolic rates in winter (Nilssen et al. 1984b), higher resting heart rates in summer (Arnold et al. 2018; Mesteig et al. 2000), thicker winter fur and larger fat stores compared to domestic reindeer (Blix 2016; Pedersen et al. 2019). Yet, the degree of plasticity in the balance and management of energy between individuals within the seasons has not been investigated. Because the Arctic is one of the regions where climate change is occurring the fastest (Meredith et al. 2019), plasticity in these traits may be key to the species' future survival (Boutin and Lane 2014).

Figure 2: Adult reindeer females in winter coats (Feb-Mar). Left: Svalbard reindeer (ca. 50 kg). Right: domestic reindeer in Finland (ca. 80 kg).

Box 1. Reindeer study systems in this thesis

The **Svalbard reindeer capture-mark recapture** project has been ongoing since 1994. Individual reindeer are marked as calves (thereby knowing their age) and followed throughout their lives. Because female body condition and reproduction rates are the main drivers of population dynamics in this system (Albon et al. 2017), only females are followed in detail. In late winter (March-April), reindeer are captured from snow mobiles using handheld nets. During handling they are weighed, checked for pregnancy status, and samples of blood, feces and hair are collected. In late summer (August), reindeer are re-sighted on foot to assess whether they reproduced successfully (calf-at-heel), and carcass information is gathered, providing data about mortality rates. All reindeer in Svalbard are wild. In this thesis, I use a wide range of data sets from this system (Papers I and II).

The Svalbard study system. From left to right: study site on map (©Norwegian Polar Institute), capturing reindeer from snow mobiles (© E. Ropstad), re-sighting of reindeer in autumn.

The **Kutuharju reindeer research station** is owned by the Finnish Reindeer herder's association and is located in Inari municipality, Northern Finland. There are around 170-200 reindeer before the autumn culling, after which around 100 females, 10–40 calves, and 10–20 males are left. Females give birth in a small calving paddock (~0.5 km^2) under close surveillance, so birth date, birth mass and ID are registered. From September to April, reindeer are gathered ca. once per month for weighing. Reindeer are not normally handled in summer after calving. The station has an indoor handling facility containing a floor scale the reindeer walk onto to be weighed, and a wall-mounted handling rack to restrain adults. In all paddocks, reindeer have access to water and natural forage, but are typically provided with pellets and lichen as supplementary food. These reindeer are semi-domestic. In Paper III, I use data collected between February to December 2018 from this system.

Kutuharju Reindeer research station. Left: map of summer enclosure (©Google) and location in northern Finland (inset ©Wikimedia). The blue area is the calving paddock and where animals were kept during daily handling, and the yellow area is one of two summer pastures which were used. The scale bar (red) is 200m. Middle: reindeer in coral prior to handling. Right: blood sampling of an adult reindeer under manual restraint.

Measuring energy expenditure

To understand how different energy pathways influence energy expenditure we must measure energy expenditure, accurately. In laboratory settings, where animals can be confined, energy expenditure can be measured directly by measuring heat generated by the body, or indirectly by estimating heat produced, via measurements of carbon dioxide (CO_2) production or oxygen (O_2) consumption (Kleiber 1961; Battley 1995). In the field, these options are challenging and the current gold standard is the doubly labelled water (DLW) method. The technique has been validated by indirect calorimetry (e.g. oxygen consumption) in a wide range of animals (Butler et al. 2004). Here, stable isotopes of hydrogen (^2H, deuterium) and oxygen (^{18}O) are used to trace water turnover of an organism (Speakman 1997). This method is useful because the isotopes occur in only small concentrations in nature (the most common isotopes are H and ^{16}O). Artificially elevating these concentrations in the body allow us to measure the rate at which they are eliminated (via exhaled CO_2 and water, H_2O) and hence the animals' metabolic rate (Figure 3) (Speakman 1997). The method requires a background sample (to establish baseline values for deuterium and ^{18}O) followed by administration of a dose of DLW, an equilibrium sample when DLW has mixed with the body water, and a final sample after some time, typically 1–3 weeks in large animals (Westerterp 2017). Metabolic rates are calculated by using the ratio of oxygen used in metabolism to CO_2 production (the "respiratory quotient"; Black et al. 1986), divided by the time between equilibrium and final samples. This results in an estimate of daily energy expenditure (DEE) (Schoeller 1988). The major limitations to this method are the logistical procedure of re-capturing wild animals over short time periods (but see Gotaas et al. 1997), and that oxygen-18 is relatively expensive. There are also other pathways of elimination (besides exhaled CO_2 and H_2O), that should be accounted for and different approaches exist to calculate DEE from the isotope elimination rates (Speakman and Hambly 2016). Since doses depend on body size and the animal's metabolic rate, financial constrains can also be a major determinant for sample size, especially in large animals (e.g. Gotaas et al. 2000; Nie et al. 2015; Pagano et al. 2018; Riek et al. 2019; Acquarone et al. 2006).

Another option to estimate energy expenditure is to use biotelemetry and biologging devices (Box 2) to measure heart rate, which reflects oxygen consumption (Brosh 2007;

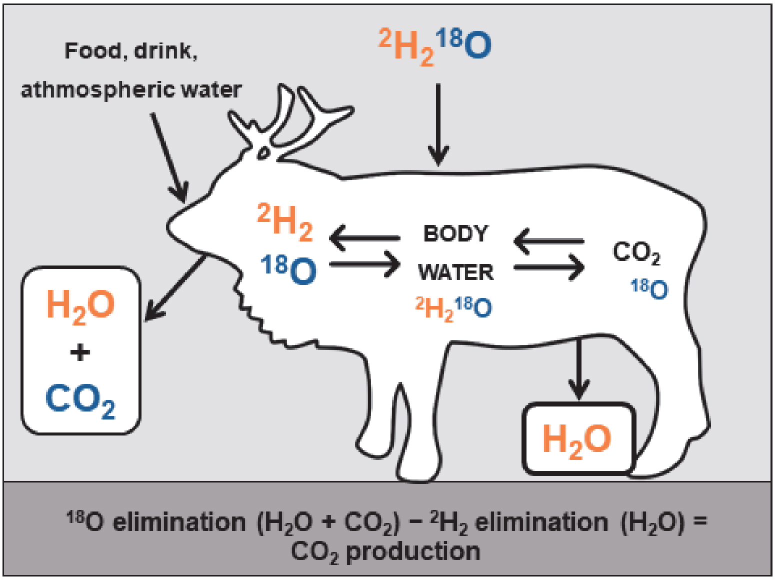

Figure 3: Diagram showing the main exchange pathways of oxygen and hydrogen in the body, which are the principles of the doubly labelled water technique.

Green 2011), and/or acceleration, which reflects whole-body movements, and hence activity (Green et al. 2009; Halsey et al. 2009). Measuring heart rate using biologging devices has the advantage that it provides high temporal resolution sampling over several months or years (Green 2011). However, in order to estimate energy expenditure directly from heart rates in an animal, heart rates should be calibrated for that species and preferably under different natural conditions (Halsey and Bryce 2021). Individual differences in the rate of oxygen uptake and heart rate (Aharoni et al. 2003), and other variables that influence heart rate (e.g.: emotional arousal; Wascher 2021) can further complicate these inferences. Brosh (2007) showed that for ruminants, the estimated oxygen pulse per heartbeat did not differ significantly with reproductive status, food intake or ambient temperature. Further, Nilssen et al. (1984a) showed a linear relationship between heart rate and oxygen consumption in reindeer while resting and running on treadmills (i.e.: unnatural conditions). While using heart rate as a proxy for energy expenditure can in many cases be useful, caution must be applied when inferring that a relative increase in heart rate reflects the same relative increase in energy expenditure (Halsey and Bryce 2021). Even without calibrations, heart rates can be used to evaluate energy expenditure qualitatively (e.g.: Halsey et al. 2019).

Box 2. Biologger data and equipment used in thesis

Biologgers are devices that are mounted on or implanted in an organism and stores data internally. A caveat is that the individual in most cases must be recaptured, and battery and memory size determine the amount of data that can be collected and the duration of data collection.

Activity sensors are used in a wide range of species (Wilson et al. 2020). Most sensors record gravitational acceleration in 2 or 3 axes at high frequency (4–128Hz) which allows for fine-scale assessment of movement. Acceleration data also provides information about overall activity levels and patterns. In Papers I and II, I use data collected with biaxial activity sensors integrated into a GPS collar (GPS PLUS, Vectronic Aerospace, Germany) where activity is averaged internally to 5-min periods. In Paper III, I use high-resolution tri-axial acceleration data (8 Hz) from small activity sensors (VERTEX Plus) to calculate overall dynamic body acceleration (ODBA). ODBA is an index of the total movement of the body within a given time frame and is useful because it removes the static acceleration caused by gravity.

Heart rate sensors typically record heart beats from an electrical signal generated by the heart muscle, but other methods exist (e.g. acceleration-derived; Arnold et al. 2018). I used data collected with DST Centi-HRT loggers (StarOddi, Iceland), which also contain a temperature sensor. These small (19g), leadless devices were programmed to record heart rate and temperature every 15 min alongside an index of signal quality. They also stored raw 4 sec electrocardiograms every 6 hours, which allowed for manual validations of the heart rate readings (Paper II). Validations allow filtering of erroneous readings which, if retained, could lead wrongful interpretations of the relationship between heart rate and other factors.

Activity sensors and axes recorded. Top: GPS collar with activity sensor on Svalbard reindeer (© L.E. Loe). Bottom: acceleration sensor attached to plastic collar on reindeer in Kutuharju, Finland.

Heart rate- and temperature loggers. From left to right: DST Centi-HRT logger with scale (© StarOddi), surgical implantation to the subcutaneous cavity, and post-implantation surgery site (©A. Evans). The red circle overlaid on the silhouette marks the position of the logger in the reindeer.

Thesis summary

In this thesis, I explore drivers of energy expenditure in reindeer under different seasonal contexts, reproductive stages and individual states. The aim of the thesis is to identify key physiological and environmental factors that influence individual variation in energy expenditure (summarised in Figure 4). The data collected come from two separate reindeer study systems (Box 1). The analyses throughout this thesis are based on two commonly used methods of measuring energetics in free-living animals, DLW and the heart rate method. In Paper I, I use the DLW method to estimate daily energy expenditure (DEE) over a two-week period in late winter. Paper II uses heart rate as a proxy for energy expenditure in both winter and summer, and Paper III uses both methods in summer. The role of body size and composition on winter energetics and fasting endurance is the main focus of Paper I. I combined estimates of DEE, activity and subcutaneous body temperature (T_{sc}) of Svalbard reindeer with historical data of body composition into a theoretical framework for modelling fasting endurance (Speakman and Westerterp 2013). I present the first-ever measurements of DEE in a wild arctic ungulate and show that late winter DEE is mostly determined by lean (fat-free) body mass, activity levels, and T_{sc}. The focus in Paper II is on within-seasonal variation in energetics in Svalbard reindeer. Here, I explored determinants of heart rate to identify constraints on energy management strategies within summer and winter, representing the ‘peak’ and ‘through’ in energy expenditure, respectively. I show that the relationship between heart rate and T_{sc} appears to be state-dependent, i.e. varying in its effect depending on reproductive status, body condition and age. Typical drivers of energy expenditure explained surprisingly little of the within-seasonal variation in heart rate, while seasonality itself explained as much as 75% of the variation throughout the year. These findings suggest that within-seasonal adjustments are constrained by the strong phenotypic adaptation to the prevailing season. Paper III focuses on drivers of DEE during peak lactation in domestic reindeer and their physiological and behavioural responses to extreme warm weather (heatwave). I show that DEE does not differ between the reproductive groups, despite lactating females having higher energetic demands. During the heatwave reindeer responded to high ambient temperatures via co-current reductions in activity levels and heart rate, while T_{sc} rose, but lactating reindeer did not reduce heart rates to the same extent as non-lactating reindeer. The results in Paper III indicate that lactating reindeer are more susceptible to environmental heat stress.

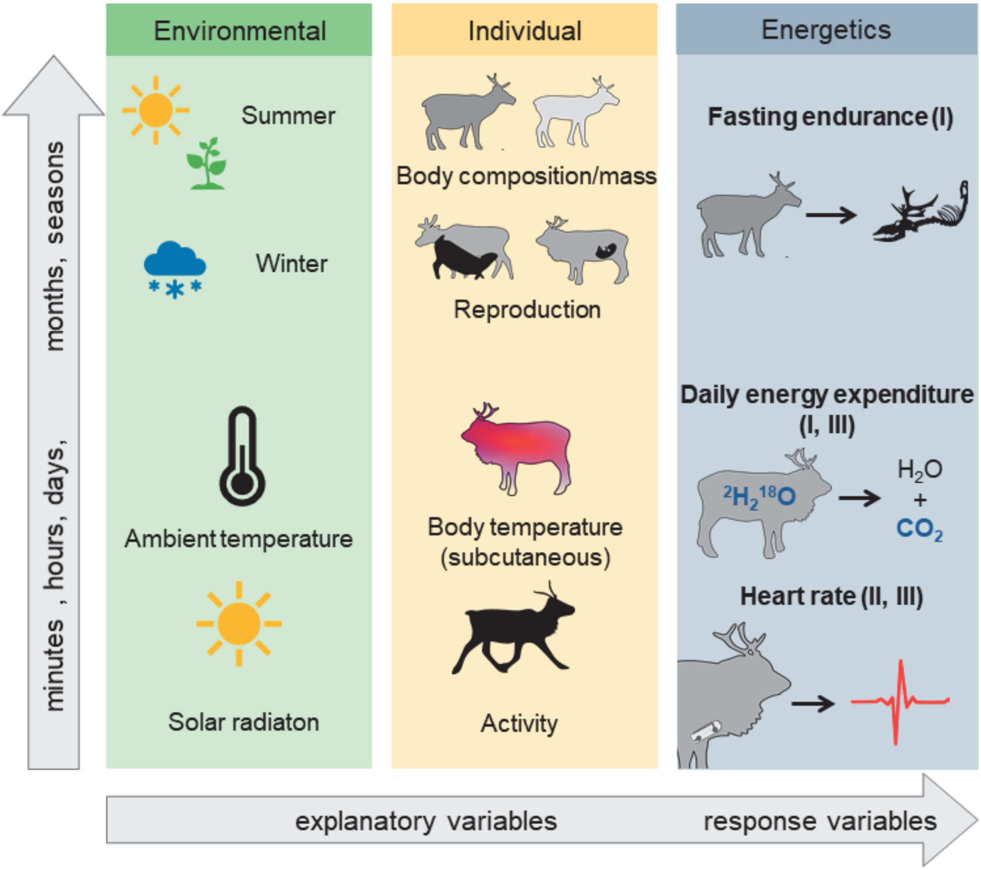

Figure 4: Overview of parameters used in this thesis. The vertical arrow on the left indicates the time scale at which different variables are measured. Parameters are categorized into environmental (green, left column) and individual (yellow, central column) explanatory variables. The grey column on the right contains the three main response variables evaluated in this thesis and in which papers they are used (in parentheses).

Discussion

The findings in my thesis demonstrates that the determinants of energy expenditure can vary under different seasonal, reproductive and individual contexts (Figure 5). The role of body mass was equivocal in the two study systems. In Svalbard reindeer, body mass was an important determinant of daily energy expenditure (DEE), and body mass interacted with both activity levels and T_{sc} in its effects on heart rate. In domestic reindeer, body mass did not explain any variation in DEE, reproductive output or responses to high ambient temperatures. Temperature influenced energy expenditure differently between the systems and seasons. In Svalbard reindeer, T_{sc} was an important driver of DEE (Paper I), and the relationship between heart rate and T_{sc} varied depending on reproductive status, activity levels, and age (Paper II). These results suggest a state- or condition dependent benefit of reducing T_{sc} to save energy. The relationship between heart rate and T_{sc} also differed for lactating and non-lactating females in both systems, but in opposite directions. In Svalbard reindeer, only lactating females increased their heart rate when body temperature (via T_{sc}) increased. In contrast, lactating domestic reindeer lowered their heart rates in response to high T_{sc}. This difference suggests that domestic reindeer were closer to, or more susceptible to heat stress, than Svalbard reindeer. Indeed, ambient temperature had a negligible effect on heart rate in Svalbard reindeer (Paper II), while in domestic reindeer, resting heart rates declined while T_{sc} increased with increasing ambient temperatures (Paper III). Lactation contributed to explain much less variation in energy expenditure in either system (Papers II and III) than expected, although lactating reindeer had greater total energy budgets (Paper III). Activity levels (resting/active), and intensity of activity, were the most consistent determinants of both DEE (Papers I and III) and heart rate (Papers II and III). Here, I discuss the relevance of the key findings from each paper and how they expand our knowledge of eco-physiological processes in seasonally adapted mammals.

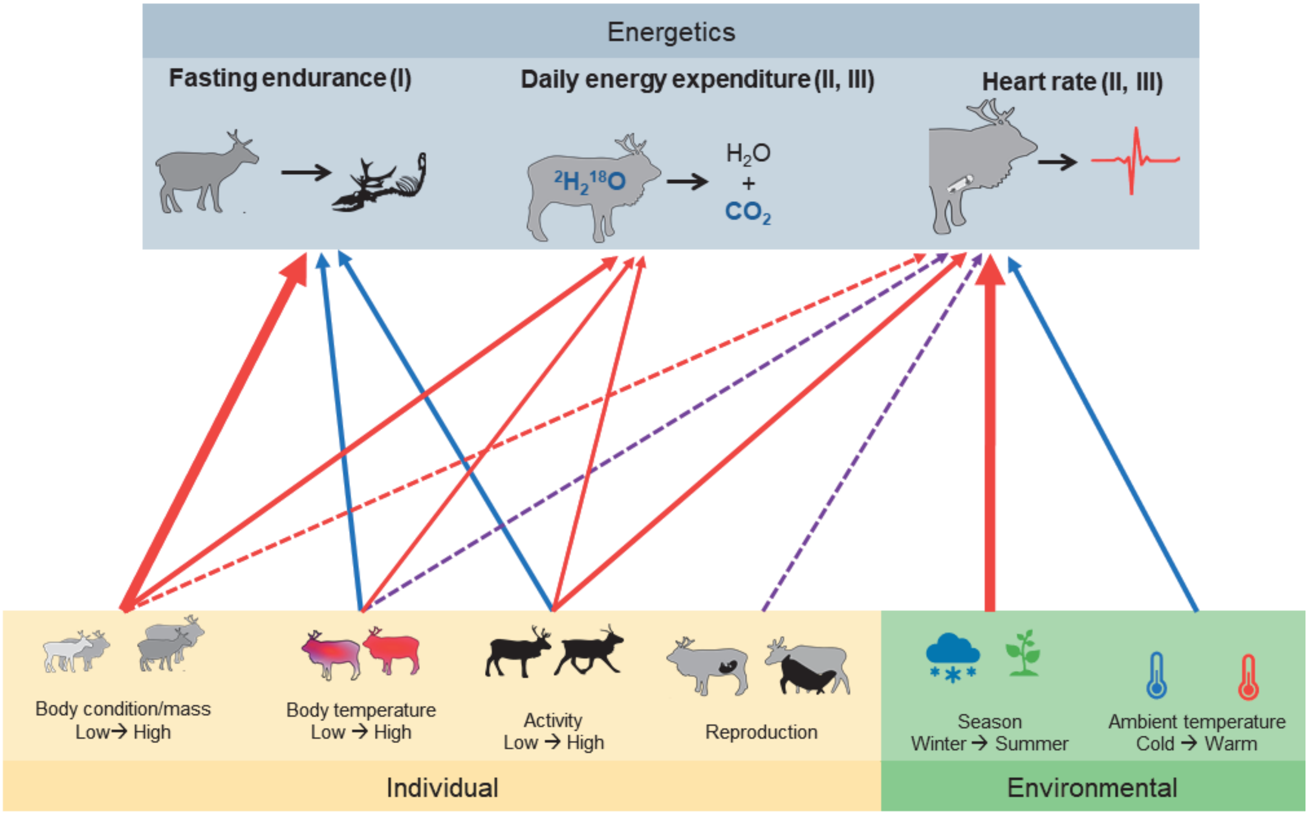

Figure 5: Schematic diagram of the main results from the papers in my thesis. Solid lines show direct effects while dotted lines indicate effects via interactions with other explanatory variable (the interactions are not shown). Blue colour represents negative effects, red – positive, and purple – either positive or negative (interaction-dependent). Arrow thickness represents the relative size of each effect.

Body mass and daily energy expenditure

For Svalbard reindeer, both body mass and body composition were important variables as a driver of energy expenditure. In Paper I, I found that lean (fat-free) body mass explained as much as 39% of the variation in winter DEE. It is expected that the relationship between body mass and DEE is weaker at the intraspecific than at the interspecific level (Anderson and Jetz 2005; Nagy 2005; Speakman and Król 2010), because of the smaller range of body mass and hence less intraspecific variation in mass. Surprisingly, despite a wider absolute range of body mass in domestic reindeer (66–90 kg vs. 46–58 kg in Svalbard reindeer), DEE did not correlate with body mass at all (Paper III). However, seasonal differences could potentially explain this difference since the measurements in Svalbard reindeer were taken in winter and the measurements in domestic reindeer in the summer. For example, in North American red squirrels, DEE only correlates with body mass in winter but not during lactation nor hoarding (summer and autumn, respectively; Fletcher et al. 2012). The authors suggested that other sources of variation that increase energy expenditure could be more prevalent in periods of high energy expenditure, which reduces the influence of body mass, *per se*. In winter, when red squirrel DEE was much closer to resting metabolic rates (Fletcher et al. 2012), body mass could be a greater determinant of DEE as resting metabolic rate typically correlates with body mass (McNab 2002). In fact, winter DEE measured in Svalbard reindeer (range 5.5-8.1 MJ day^{-1}; Paper I) overlapped with previously measured resting metabolic rates in captive-raised (non-pregnant) Svalbard reindeer females of similar body mass (ca. 6.5 MJ day^{-1}, recalculated from Nilssen et al. 1984b). DEE of domestic reindeer in (Paper III) did not overlap with resting metabolic rates reported for Norwegian reindeer (17.4 MJ day^{-1} for a body mass of 67kg Nilssen et al. 1984b), or North American reindeer (13–22 MJ day^{-1} for reindeer weighing 90–99kg, recalculated from White and Yousef 1978). Some caution should be made when using these estimates comparatively, since derivatives from mass-specific metabolic rates do not necessarily correlate with whole-body metabolism (Hayes 2001). Still, this difference indicates that domestic reindeer in summer have DEEs considerably above resting metabolic rates, while Svalbard reindeer in winter do not. This corresponds with the suggestion by Fletcher et al. (2012), that when DEE is closer to resting metabolic rates, body mass can explain more variation in DEE.

Allometric scaling and seasonal discrepancies

From allometric equations of field metabolic rates, DEE of a 50 kg Svalbard reindeer should be around 14 MJ day^{-1} (Nagy 2005; Riek et al. 2019). The daily energy expenditure measured in Paper I was only 44% of the predicted value (mean of 6.2 MJ day^{-1}). This discrepancy is likely a consequence of seasonal differences in daily energy expenditures within a species being unaccounted for in allometric equations (Nagy 2005). Indeed, most allometric scaling equations are based on species averages without considering seasonal context (e.g. Nagy 2005; Anderson and Jetz 2005), although some account for individual variations within species (Hudson et al. 2013) or effects of ambient temperature (Speakman and Król 2010). In llamas for instance, DEE measured in winter was only 42% of that predicted from the allometric equation, whereas summer, DEE was 89% of the predicted values (Riek et al. 2019). For other species with known seasonal variation in energy expenditure, the greatest differences between observed and predicted DEE (from allometric scaling) occur in winter in temperate regions (Fletcher et al. 2012) or during the dry season in hot, arid regions (e.g. Nagy and Knight 1994; Williams et al. 2001). Adaptations to specific diets containing low digestibility and high turnover times, such as the bamboo-eating giant panda (*Ailuropoda melanoleuca*) can also lead to exceptionally low DEE (Nie et al. 2015). In contrast, DEE of domestic reindeer measured in Paper III (mean of 26.2 MJ day^{-1}) was on average 30% greater than that predicted (using a mean body mass of 75 kg) for ungulates (20.3 MJ day^{-1}; Riek et al. 2019). The strong seasonal adaptations observed in reindeer and other species, demonstrate that interspecific scaling relationships are not always useful for predicting intraspecific patterns (Dunbrack and Ramsay 1993; Nagy 2005; Riek et al. 2019).

Body condition as a moderator of heterothermy?

Winter DEE in Svalbard reindeer was positively correlated with subcutaneous temperature (T_{sc}), suggesting that reductions in T_{sc} can facilitate reductions in energy expenditure (Paper I). In Paper II, individuals of different body mass displayed contrasting responses in heart rate to elevated activity and T_{sc}. For example, in winter, small individuals reduced heart rates when subcutaneous temperatures were lower, but the largest individuals maintained constant heart rates over the same range of T_{sc}. Although data on body composition was not available for the study subjects in Paper

II, body mass and body condition are positively correlated in Svalbard reindeer. In general, adult females of low body mass are in poorer condition that those of high body mass (Reimers and Ringberg 1982), albeit with substantial variation (Paper I). Body condition has been shown to be relevant for temperature-relationships in a variety of species. For instance, in musk oxen (*Ovibos moschatus*), the energy saving benefits of heterothermy appears to be greater in individuals of poor body condition (Desforges et al. 2021b). In moose, a decline in ingesta-free body fat led to a greater amplitude of core body temperature via lowered minimums and daily means in core temperature (Thompson et al. 2019). Similarly, low T_{sc} coincide with the period (March) when body condition is lowest in Przewalski horses (Arnold et al. 2006; Kuntz et al. 2006). It would be reasonable to assume that individuals which are not able to meet their energetic demands may be more inclined to employ peripheral thermoregulation than those who do (Hetem et al. 2016). Indeed, food-restricted Shetland ponies display lower T_{sc} (Brinkmann et al. 2012), and exhibit a greater amplitude in mean daily core temperature (Brinkmann et al. 2014) compared to individuals with free access to food. These studies, together with the findings of Papers I and II, support the notion that thermoregulation may be linked to body condition and that peripheral heterothermy can be a plastic trait (Hetem et al. 2016).

The (un)importance of lactation as a seasonal driver of energy expenditure

Seasonality in reproduction has been proposed as one of the major causes of seasonal variation in energy expenditure among mammals (Fournier et al. 1999) because of the elevated energetic demands of lactating females (Oftedal 1985). I found that lactation status did not contribute to a large difference in heart rates in Svalbard reindeer, with lactating females having heart rates only 6 bpm (6%) higher than non-lactating females (Paper II). In domestic reindeer this difference was comparatively greater (10 bpm, 16%) for resting heart rates, but not for active heart rates (no significant difference), and DEE between reproductive groups were similar (Paper III). There are few studies available that compare energy expenditure in lactating and non-lactating females in ungulates. While Arnold (2020) showed that the voluntary food intake is greater in lactating red deer compared to non-lactating hinds, both follow the same seasonal fluctuation in food intake. Further, previous estimates of reindeer DEE in non-reproductive females (Gotaas et al. 2000) is similar to the DEE measured in lactating domestic reindeer (Paper III). It is more likely that the seasonal change in food availability has led to an adaptation to maximise energy turnover in summer and minimise energy expenditure in winter irrespective of reproductive status (Kuntz et al. 2006; Arnold et al. 2018). Costs of reproduction could also manifest itself as a differences in the ability to replenish body reserves prior to winter. For example, wild caribou and Svalbard reindeer females who successfully reproduce have poorer body condition (Chan-McLeod et al. 1999) or lower body mass (Albon et al. 2017) compared to non-reproductive females. In contrast, North American domestic reindeer have been shown to regain body mass later in the season, when the energy demands of lactation are lower (Luick et al. 1974). Similarly, the lactating domestic reindeer in Paper III did not lose more mass than non-lactating reindeer, and autumn body masses did not differ from those of non-lactating reindeer. These differences in the ability to 'recover' from the energetic costs of lactation may be caused by differences in food availability, as the domestic reindeer were provided with supplementary food (Luick et al. 1974, Paper III). The trade-off between allocation of energy to reproduction and to body reserves could thus be stronger in wild subspecies where nutrition and energy availability fluctuate with environmental conditions (Parker et al. 2009).

Activity as a proxy of energy expenditure

Activity is an important variable in all papers of this thesis. First, activity contributed to explain up to 10% of the variation in winter DEE (Paper I). Second, active heart rates were on average 10 bpm greater than resting heart rates in both winter and summer, although the relative difference was greater in winter (29% vs 10%, respectively) (Paper II). Third, both activity levels and vectorial overall dynamic body acceleration (VeODBA) contributed to explain up to 50% of variation in DEE across reproductive groups in domestic reindeer (Paper III). Finally, the intensity of activity caused a linear increase in heart rates in both Svalbard and domestic reindeer (Papers II and III). These results are unsurprising given that any form of movement is more energetically expensive than resting, and that both oxygen consumption and heart rate increase linearly with running speeds in reindeer on treadmills (Nilssen et al. 1984a). Indeed, estimates of dynamic body acceleration has been shown to correlate well with oxygen consumption in chickens (Green et al. 2009) and with measurements of energy expenditure via doubly labelled water in other species (Elliott et al. 2013; Plasqui et al. 2013; Hicks et al. 2017; Pagano and Williams 2019).

A caveat of using acceleration as a proxy of energy expenditure is that only activity-related energy expenditure is quantified, and not energy expenditure related to maintenance, reproduction, growth or digestion (Gleiss et al. 2011). Combining acceleration estimates with heart rate, for instance, could provide additional information on processes unrelated to activity levels (Green et al. 2009). However, I failed to find a correlation between mean heart rates and DEE in reindeer (Paper III). This could be due to the temporal resolution over which heart rates were measured (4 sec every 15 minutes). In effect, heart rate was measured over a total of 6.4 minutes per day, provided that no values were filtered out (Paper II). In contrast, the DLW method estimates the total energy expended over the measurement period and divides this by the time over which it has been measured. The heart rate records provided only a glimpse of what happened in the past few minutes, and the average of these records may not reflect whole-organism metabolism over a long period (Denny 2017). Although some studies successfully calibrate heart rate measurements to oxygen consumption (and hence energy expenditure) (Boyd et al. 1995; Davidson et al. 1997; Currie et al. 2014) it is clear that thorough calibrations, including different physiological states (Currie et al. 2018), activity types (Fancy and White 1986; Green et al. 2001), seasonal stages (Dalton et al. 2014) and individual variation (Aharoni et al. 2003), all are necessary for the heart rate method to successfully replace quantitative (but not necessarily qualitative) estimates of energy expenditure with the DLW method (Halsey and Bryce 2021).

Modelling energetics

Models of animal energetics can be a powerful tool for research and conservation purposes (Tomlinson et al. 2014). For instance, mechanistic models can help us understand population dynamics (Desforges et al. 2021a) and species responses to climate change (Kearney et al. 2010). Fasting endurance has been predicted in other species by use of mechanistic models (e.g. Mathewson and Porter 2013) and combinations of empirical data and mechanistic modelling (Desforges et al. 2019). Many mechanistic models are based on the physical principles of heat exchange (Scholander et al. 1950). Levesque et al. (2016) argued that these models are often too simplistic, because endotherms use heterothermy to regulate energy expenditure, which is not necessarily accounted for. Indeed, Desforges et al. (2021b) incorporated measurements of body temperature in their mechanistic model and demonstrated that the benefit of heterothermy for winter energetics and fasting endurance was greater in individuals of poor body condition. In Paper I, I constructed a model that predicted the fasting endurance of Svalbard reindeer using a mathematical model of weight loss under total starvation in humans (Speakman and Westerterp 2013), combined with empirical data of energy expenditure (measured by DLW method), T_{sc} and activity patterns. While this modelling approach includes many assumptions (described in Paper I), combining empirical data such as energy expenditure measured by DLW or heart rates, body temperature and activity levels can improve model predictions (Tomlinson et al. 2014; Levesque et al. 2016). The fasting endurance model in Paper I uses a framework to estimate depletion of body reserves in relation to body composition, but does not take into account variations in foraging patterns or availability (e.g.: state-dependent foraging; Denryter et al. 2020) or impacts of environmental conditions (e.g.: snow; Loe et al. 2021; Desforges et al. 2021a). Future improvements could potentially include high-resolution biologger data to estimate energetics in relation to environmental and nutritional states (Kearney et al. 2012; Denryter et al. 2021).

Ambient temperature and seasonal thresholds

Heat stress and heat load is likely to be an increasingly common challenge for large mammals in a warming climate (Fuller et al. 2010). Temperate and arctic ungulates, which are adapted to cold environments, have been shown to display altered behavioural when summer temperatures rise. For example, Alpine ibex reduce mid-day activity but increase foraging in the colder hours of the mornings and evenings on hot summer days (Semenzato et al. 2021). Similar responses have been shown in moose (Ditmer et al. 2018; Borowik et al. 2020). The use of thermal refugia such as cool ground, snow patches and forest canopies providing shade, also increases with increasing air temperature in mountain goats (*Oreamnos americanus;* Sarmento et al. 2019), alpine ibex (Brivio et al. 2019) and moose (Beest et al. 2012; McCann et al. 2016). In Paper III, both activity levels and heart rate declined with increasing ambient temperature, while at the same time subcutaneous body temperature increased. However, there was no clear threshold in the response of heart rate and T_{sc} (only a continuous decline), while reindeer reduced activity levels when ambient temperatures exceeded 18°C. On the very hottest occasions, T_{sc} was close to previously recorded core body temperature in reindeer (37°C vs 38–39°C; Johnsen et al. 1985) In reindeer, foraging efforts have been found to decline at ambient temperatures above 14°C (Thompson and Barboza 2014). Reduced activity levels and lower resting heart rates most likely reflect a decreased foraging effort, which also reduces the internal heat load (via heat increment of feeding) (Beatty et al. 2006; Andrade Ferrazza et al. 2017). The implications for reduced foraging efforts in response to hot environmental conditions could manifest itself as reduced mass gain during the summer period (Beest and Milner 2013), or reduced reproductive output (Igono et al. 1992; Kadzere et al. 2002; Dash et al. 2016), but data was not available to investigate this in Paper III. In Svalbard reindeer, the effect of ambient temperature on heart rates was almost negligible in both summer and winter, and there were no clear signs of heat stress in summer or hypothermia in winter over the observed range of ambient temperatures (5°C to 25°C in summer; −22° to −2°C in winter, Paper II). This is surprising given that Williamsen et al. (2019) showed that Svalbard reindeer in the same study population seek cool snow patches to rest on when ambient temperatures exceed 10°C in summer. The lack of a clear effect of ambient temperature on heart rates could be due to the relatively low temporal resolution in measurements of ambient temperature (4-hr intervals), and few observations of temperatures above 15°C. Also, it is possible that behavioural thermoregulation was sufficient to reduce heat load which could explain the lack of a clear response in heart rates.

Reindeer in a warming Arctic

The Arctic is one of the regions undergoing the most extreme changes in climate (Meredith et al. 2019). For arctic herbivores, the major impacts of climate change involve more frequent extreme weather events, warmer summers and milder winters. Mild spells in winter with rain falling on snow, can lead to ground icing which restricts access to forage (Putkonen and Roe 2003). In the most extreme events ground icing can lead to population crashes (Hansen et al. 2013), but can also stabilise populations as only the robust individuals survive (Hansen et al. 2019). Climate change has already shown contrasting effects on different seasons and subspecies of reindeer. For instance, changes in vegetation growth in spring leads to phenological mismatch for migrating reindeer (Post et al. 2009), while warmer summers and delayed onset of snow in autumn have been found to increase seasonal body mass growth in Svalbard reindeer (Albon et al. 2017; Loe et al. 2021). In fact, Loe et al. (2021) found that late snow onset in the autumn (and thereby prolonged access to vegetation) compensated for ground icing in winter in all but the most extreme years. Since animals with greater fat reserves are better equipped to withstand long periods of food shortage (Paper I), increased fat deposition is probably an important mechanism to increase winter survival. This may benefit reindeer in future winters, which may be icier but also shorter.

Concluding remarks

The findings from my thesis demonstrate that the relative contribution of body mass and body composition, temperature (ambient and body), reproduction and activity as drivers of energy expenditure depend on seasonal, individual and reproductive contexts. From these results it appears that the strong adaptations to seasonality result in different summer and winter phenotypes in which there is apparently less scope for additional variation in energy expenditure (Paper II). However, this variation seems to be greater in summer, under conditions where food is abundant (Papers II and III). Biologger data and estimates of energy expenditure can be important parameters in predictive models for species responses to climate change (Chmura et al. 2018). For instance, biologger data in Paper III captures the co-current behavioural and physiological response to hot environmental conditions, which may become increasingly challenging in a warming climate. The consistently significant relationship between activity and energy expenditure, both in terms of DEE and heart rate, demonstrate that acceleration data can provide important parameters for predicting energy expenditure (Wilson et al. 2020), although calibrations are necessary for quantitative predictions. I also show that both T_{sc} and body mass can be important factors influencing energy expenditure (Paper I). Future research should consider how activity patterns, thermoregulation and body mass can be incorporated into models of mammalian energetics to predict and understand responses to fluctuating environmental conditions.

Perspectives – future work

Do seasonal heart rate variations correspond with changes in plant phenology?

The seasonal pattern in heart rate in Svalbard reindeer shows a rapid increase from April to July before beginning to decline already in August, coinciding with the seasonal peak in vegetation (Arnold et al. 2018). Loe et al. (2021) recently demonstrated that late winter body mass is positively correlated with snow onset in autumn (later onset, greater winter mass). This indicates that the availability of vegetation in autumn positively influences somatic growth (via increased foraging). Plasticity in calving dates in response to climatic conditions have been demonstrated in domestic reindeer (Paoli et al. 2018), although in Svalbard reindeer the major determinant for calf production is maternal body mass, not spring phenology (Veiberg et al. 2017). Whether between-year variations in the seasonal adjustments of heart rate coincide with the annual variations in plant phenology (Van Der Wal and Stien 2014) or other climatic variables, has not been investigated. This could allow us to determine the degree of plasticity in the timing of seasonal adjustments in energy expenditure.

Do Arctic ungulates employ adaptive heterothermic strategies?

Desert ungulates like the Arabian Oryx (*Oryx leucoryx)*, have adapted to high heat loads by storing body heat during the hot desert days and releasing heat during night ('adaptive heterothermy'; Hetem et al. 2016). Whether temperate and arctic ungulates are able to employ similar strategies is unknown, but some ungulates display nocturnal hypometabolism during winter which includes reductions in T_{sc} (Arnold et al. 2004) or rumen temperature (Signer et al. 2011). A relatively large range of T_{sc} (20–38 °C) was observed in the papers of this thesis, while previously observed range of rumen temperatures are 37–39°C (Arnold et al. 2018). Reindeer might alter the size of their body core to save heat when it is cold - shrinking the core by reducing peripheral temperature, and release heat when it is warm - expanding the core by increasing peripheral temperature (Speakman and Król 2010). Indeed, the increase in T_{sc} in response to ambient temperatures in domestic reindeer suggest that this is the case (Paper III), but whether these responses are adaptive to the current environmental conditions is not known. Confirming this would require simultaneous measurements of peripheral and core body temperatures across a range of ambient temperatures.

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RESEARCH ARTICLE

Functional Ecology BRITISH ECOLOGICAL SOCIETY

Fat storage influences fasting endurance more than body size in an ungulate

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Abstract

1. The fasting endurance hypothesis (FEH) predicts strong selection for large body size in mammals living in environments where food supply is interrupted over prolonged periods of time. The Arctic is a highly seasonal and food-restricted environment, but contrary to predictions from the FEH, empirical evidence shows that Arctic mammals are often smaller than their temperate conspecifics. Intraspecific studies integrating physiology and behaviour of different-sized individuals may shed light on this paradox.
2. We tested the FEH in free-living Svalbard reindeer *Rangifer tarandus platyrhynchus*. We measured daily energy expenditure (DEE), subcutaneous body temperature (T_{sc}) and activity levels during the late winter in 14 adult females with body masses ranging from 46.3 to 57.8 kg. Winter energy expenditure (WEE) and fasting endurance (FE) were modelled dynamically by combining these data with body composition measurements of culled individuals at the onset of winter (14 years, $n = 140$) and variation in activity level throughout winter (10 years, $n = 70$).
3. Mean DEE was 6.3 ± 0.7 MJ/day. Lean mass, T_{sc} and activity had significantly positive effects on DEE. Across all 140 individuals, mean FE was 85 ± 17 days (range 48–137 days). In contrast to the predictions of the FEH, the dominant factor affecting FE was initial fat mass, while body mass and FE were not correlated. Furthermore, lean mass and fat mass were not correlated. FE was on average 80%

(45 days) longer in fat than lean individuals of the same size. Reducing activity levels by ~16% or T_{sc} by ~5% increased FE by 7% and 4% respectively.

4. Our results fail to support the FEH. Rather, we demonstrate that (a) the size of fat reserves can be independent of lean mass and body size within a species, (b) ecological and environmental variation influence FE via their effects on body composition and (c) physiological and behavioural adjustments can improve FE within individuals. Altogether, our results suggest that there is a selection in Svalbard reindeer to accumulate body fat, rather than to grow structurally large.

KEYWORDS

activity, biologging, daily energy expenditure, doubly labelled water, intraspecific scaling, subcutaneous body temperature, Svalbard reindeer, the Arctic

1 | INTRODUCTION

The effects of the environment on body size and shape of animals (Bergmann, 1847), as well as the relationship between body size and metabolism (Kleiber, 1961), are fundamental principles in ecological and physiological theory. In particular, environmental temperature and resource availability are central drivers of animal energetics, and have subsequently shaped the evolution of body size in animals (Brown et al., 2004; McNab, 2010). The fasting endurance hypothesis (FEH) predicts that animals should be larger in environments where food supply is interrupted over prolonged periods (Lindstedt & Boyce, 1985; Millar & Hickling, 1990), because fat stores increase with body size at a steeper rate than energy expenditure (Calder, 1984). A caveat of this hypothesis is that selection is an intraspecific process, while the scalars used to explain this pattern are derived interspecifically. Although often proposed as a mechanism for observed body size patterns, few studies have tested the FEH at the intraspecific level (Boos et al., 2005; Lundblad & Conway, 2020; Swenson et al., 2007).

There are at least three non-trivial factors that may contribute to the lack of studies properly addressing the role of fasting endurance (FE) as a determinant of body size patterns within a species. First, variation in body size and mass observed within species is typically much smaller than between species (Dunbrack & Ramsay, 1993). Furthermore, intraspecific variation in structural body size may be small, while body mass can vary greatly due to differences in body condition (Dunbrack & Ramsay, 1993; Sheriff et al., 2011). Thus, to evaluate the importance of FE within species, it is necessary to quantify FE across the relevant ranges of both body size and body mass. Second, ecological factors may be more important in influencing the size of internal body stores than structural size. In seasonal environments where food availability varies throughout the year, the amount of resources available largely determines the degree of fattening prior to the food-depleted season (Loe et al., 2021; Pagano et al., 2018). In periods of low resource availability body mass declines, largely driven by the depletion of fat stores (Reimers & Ringberg, 1982). Therefore, body composition may be more relevant for determining FE than body mass itself. Third, the rate at which internal body stores are depleted depends on the total energy expenditure (Speakman & Westerterp, 2013). Many species inhabiting seasonal environments decrease energy expenditure during the food-depleted seasons (Fletcher et al., 2012; Riek et al., 2019). Reductions in locomotor activity (Arnold et al., 2018), decreased endogenous heat production and an increased tolerance towards low peripheral body temperatures may further facilitate the seasonal drop in energy budgets (Arnold, 2020; Trondrud, Pigeon, Albon, et al., 2021). Individual and temporal variations in energy expenditure may therefore play an additional explanatory role in determining FE within a species (Desforges et al., 2021). Understanding the relative influence of environmental and individual variation on metabolic phenotypes and the capacity of free-living animals to withstand food scarcity can provide potentially novel insights into the role of FE as a driver of natural selection.

The Arctic is one of the most seasonal and temporally food-restricted environments. While the prediction from the FEH would be a selection for large body size in Arctic animals, empirical evidence shows that Bergmann's rule, that is, increasing body size with increasing latitude, does not hold above the Arctic circle (66°N; Huston & Wolverton, 2011). This suggests that Arctic species might have alternative strategies to increase FE rather than having a large body size. To explore this phenomenon, we tested the FEH in a species inhabiting the High Arctic, the Svalbard reindeer *Rangifer tarandus platyrhynchus*. They are among the structurally smallest subspecies of *Rangifer*, yet the relative size of their autumn fat reserves is unprecedentedly large (Pedersen et al., 2019). Variation in body size and mass between cohorts can be up to 20%, as a result of early life conditions (Douhard et al., 2016). Furthermore, individual body mass varies greatly throughout the year with rapid growth and fattening during the short Arctic summer, and a depletion of up to 50% of body mass by the end of winter (Reimers & Ringberg, 1982). Still, Svalbard reindeers' fat reserves are insufficient to cover the energy demands over of the entire winter and must be subsidised by food intake (Tyler, 1987) in a season when snow and ice conditions restrict access to forage. Late winter body mass explains most of the annual variation in reproduction and survival, and is negatively related to, both early onset of snow in the autumn (Loe et al., 2021) and the severity of rain-on-snow in the subsequent winter (Albon et al., 2017). From the predictions of the FEH, long periods of low food availability, coupled with abrupt food shortage, should have led to a selection for a large body size. Yet, Svalbard reindeer are smaller than contemporary conspecifics in all candidate areas of origin (Yannic et al., 2014), indicating a selection for smaller size and a case of insular dwarfism (Foster, 1964).

In this paper, we first quantify the drivers of energy expenditure in free-living Svalbard reindeer. Specifically, we measured daily energy expenditure (DEE) in females during the late Arctic winter (March/April) using the doubly labelled water (DLW) method (Butler et al., 2004), in relation to body composition, activity and subcutaneous body temperature (T_{sc}). Second, we constructed and parameterised a dynamic model of energy use (Speakman & Westerterp, 2013) using the measurements of DEE and body composition data collected from autumn culls of 140 Svalbard reindeer females over 14 years (Albon et al., 2017) to quantify winter energy expenditures (WEE) and FE. This enabled us to test the central prediction of the FEH that larger individuals, both skeletally and by mass, have greater FE than smaller individuals. Furthermore, we evaluated the relative importance of adjustments in activity levels and T_{sc} for FE. Investigating individual variation in energetics provides a potential advance in understanding environment–body size relationships and their implications for survival.

2 | MATERIALS AND METHODS

2.1 | Animals and study system

The study was conducted in Nordenskiöld Land, Svalbard (77°50′–78°20′N, 15°00′–15°60′E) during the late winter (March–April) 2017 and 2018. We used a total of 21 adult Svalbard reindeer females in their prime age (3–7 years old, body mass [M_b] range 46.3–57.8 kg), which had been marked as calves as part of a long-term capture–mark–recapture study (Albon et al., 2017). Males have not been followed in detail. In the study area, the plant growing season typically lasts from snow melt (early June) until late August, when temperatures and day length decline (Van Der Wal & Stien, 2014). Reindeer mate in October and gestation lasts for about 7.5 months, with calving occurring in early June (Albon et al., 2017).

2.2 | Capture and handling

Animals were caught using snowmobiles (Omsjø et al., 2009), and blood samples were collected from the jugular vein. Animals were weighed (±0.5 kg), length of hind leg (±1 mm, hereafter 'leg length') was measured as an indicator of body size and pregnancy status was checked using ultrasound (Kaixin Electronic Instrument Co.; Table S2). All animals were fitted with GPS collars containing activity sensors (Vectronic Aerospace GmbH), and in 2018, also fitted with a biologger to record T_{sc} (details in 2.4).

2.3 | Daily energy expenditure by doubly labelled water technique

All 21 animals were caught and dosed with DLW to measure DEE. The estimates of DEE were obtained in 2017 ($n = 6$) and 2018 ($n = 17$), with two animals studied in both years (Table S2). This technique has been validated by indirect calorimetry in a wide range of mammals, providing an accurate measure of DEE over periods of several days (Butler et al., 2004). Animals were first blood sampled for background levels of ^{18}O and ^{2}H (Method A: Speakman & Racey, 1987) and then dosed with ~0.3 ml of DLW (65 atom% ^{18}O, 35 atom% ^{2}H) per kg of M_b. The DLW was injected into the jugular vein, on the opposite side from the blood sampling. The animals were recaptured 2 days later to obtain initial (equilibrated) blood samples, and then 7–10 (2017) or 14–20 (2018) days later to collect final blood samples. The differences in timing between the 2 years reflected a lack of knowledge of reindeer metabolism in the first year, resulting in large excess of isotopes in the final 2017 samples. Therefore, the time span between initial and final sampling was extended in 2018 to improve the protocol. Blood samples were stored in flame-sealed glass capillaries at room temperature for further processing. Details on the isotope analyses and calculations are provided in Appendix A1; the results are presented in Table S3. Total body water (TBW) was calculated from the deuterium dilution space (N_d) assuming that N_d corresponds to 104% of TBW (Schoeller et al., 1986). The conversion of TBW (N_d/1.04) to lean mass assumed that lean tissue contains on average 73% water (Marken Lichtenbelt et al., 1994). This calculation does not account for water content in the digestive tract, which may lead to a bias in estimating lean mass (Andrew et al., 1995). We therefore assumed that the gut fill accounts for 22% of the reindeer's body mass, and that the water content of the gut is 85%, as reported for Svalbard reindeer, previously (Sørmo et al., 1999; Staaland et al., 1979). The estimated gut water content was subtracted from the lean mass. Hereafter, these values are referred to as isotope-derived lean mass corrected for gut water ($M_{lean(I)}$).

2.4 | Biologging of subcutaneous body temperature (T_{sc})

In 2018, the measurements of DEE were coupled with the recordings of T_{sc} using a small biologger (Star-Oddi DST-CentiHRT, Star-Oddi). Loggers were implanted subcutaneously on the chest, left sternum or caudal to the left elbow, under anaesthesia with intranasal medetomidine (details in Appendix A2). T_{sc} was recorded with an accuracy of 0.1°C every 15 min by a calibrated thermistor in the biologger (details in Appendix A3). Out of the 17 biologgers deployed in 2018, only 14 were successfully retrieved the following year and only seven had successfully stored data for the period October–April

(Table S2). For the model of T_{sc} over winter, we also included data from three individuals with biologger but without DEE measurements. To investigate the effect of T_{sc} on DEE, we used the mean T_{sc} recorded in the DLW measurement period.

2.5 | Locomotor activity and environmental data

The GPS collars contain an activity sensor that recorded gravitational acceleration along two axes, *X* and *Y*, which represented back–forward and right–left movements respectively. Activity was recorded as the difference in acceleration between two consecutive measurements, characterising the mean acceleration in each axis over a 5-min period within a relative range between 0 and 255 (Krop-Benesch et al., 2011). When exploring the effect of daily activity on DEE, we used the sum of *X* and *Y* over the DLW measurement period, divided by the duration of this period (in days). Daily activity values ranged from 12,809 to 23,651 across individuals. To parameterise our model of FE, we used activity data derived from 70 individuals between 2009 and 2019 (267 individual-year combinations) for the 7-month winter period (October 20–May 20, 212 days, defined by Tyler, 1987). Here, activity was calculated as the daily sum of acceleration of *X* and *Y* (range 0–64428, $n = 38{,}646$). Activity data were downloaded directly from the collar during the capture event in 2019. The GPS collars also recorded temperature (hereafter T_{ac}), which is a reliable source of fine-scale temperature for wildlife ecological studies (Ericsson et al., 2015). Because the temperature logger was situated on top of the collar, it absorbed solar radiation and to some extent reflected the microclimate the animal was experiencing.

2.6 | Autumn body composition

To estimate the body composition of reindeer in autumn, we used data from adult female Svalbard reindeer (3–9 years old, $n = 140$) from the same population as the current study, which were culled in late October (19–27) from 1994 to 2007 (Albon et al., 2017). To derive representative body size (leg length, mm) and body composition phenotypes, we selected the mean, the 10th and 90th quantiles of lean mass derived from regressions of body composition ($M_{lean(R)}$). On these, we added the fat stores, hereafter 'fat mass' (M_{fat}), derived from the mean, the 10th and 90th quantiles of M_{fat} as % of $M_{lean(R)}$ (20, 13 and 27% respectively). This resulted in five representative body size and composition phenotypes: (a) 'average', (b) 'small and lean', (c) 'small and fat', (d) 'large and lean' and (e) 'large and fat' (Table 1; Figure S1). We set 40% loss of muscle and organ mass as the threshold before an animal dies from starvation (Tyler, 1987). Body composition details are provided in Table 1. The calculations of body composition for selection of phenotypes are described in detail in Appendix A4.

2.7 | Statistical analyses

All statistical analyses were conducted using R version 4.0.0 (The R Core Team, 2020). DEE was modelled using linear regression based on 14 individuals with complete data, with the following candidate variables: mean M_b (kg) between initial and final blood sample points, pregnancy status, age (as a continuous variable), mean daily T_{sc}, mean daily activity and $M_{lean(I)}$. Because $M_{lean(I)}$ and M_b were correlated, we compared models containing either $M_{lean(I)}$ or M_b using Akaike's information criteria for small sample size (AICc; Burnham & Anderson, 2002). The inclusion of $M_{lean(I)}$ yielded a more parsimonious model (ΔAICc = −1.9) and therefore $M_{lean(I)}$ was selected as the candidate variable to represent mass. The full model was simplified using a backward stepwise deletion approach, presented in Appendix B (Crawley, 2015).

Daily activity between October 20 and May 20 was modelled in relation to T_{ac} and body size (leg length). We used a generalised additive mixed-effects model (gamm), using the *bam* function for very large datasets (MGCV package; Wood, 2017). We fitted time (days) as a thin plate regression spline, with year and individual as random effects for both slope and intercept (separately). An autoregressive

TABLE 1 Characterisation of five body size and composition phenotypes of Svalbard reindeer females based on the data from the adult females culled in October 1994–2007 ($n = 140$, Albon et al., 2017). Selection of phenotypes is described in Appendix A4. WEE was based on average activity levels, and fasting endurance (FE) assumes an activity level at zero to infer metabolism at rest. Details on these calculations are described in Appendix C1

Phenotype	M_b	$M_{lean(R)}$	M_{fat} %	LL	M_{fat}	E_{tot}	% E_{fat}	WEE	% E_{food}	FE	% M_b loss
Average	68	52.8	20	281	10.6	492	84	1728	74	79 ± 1	38
Small–lean	57	46.1	13	276	6.0	315	78	1936	66	101 ± 2	42
Small–fat	63	46.1	27	276	12.4	547	87	1874	80	56 ± 1	33
Large–lean	73	60.3	13	284	7.9	408	79	1572	68	101 ± 2	41
Large–fat	80	60.3	27	284	16.3	711	88	1548	83	56 ± 1	33

Abbreviations and units: M_b, body mass (kg); $M_{lean(R)}$, regression-derived lean mass (kg); M_{fat} %, fat mass as percent of $M_{lean(R)}$; LL, length of hind leg (mm); M_{fat} fat mass (kg); E_{tot}, total energy reserve (MJ); % E_{fat}, percentage of E_{tot} derived from fat (lipids), remaining percent is derived from proteins; WEE, winter energy expenditure (MJ); % E_{food}, percentage of WEE contributed by food intake; FE, mean ± SD of fasting endurance (days); % M_b loss, percent loss of M_b at end of fasting period (death).

function was used to account for within-individual temporal autocorrelation. In addition, T_{sc} data were available for nine individuals. We fitted a gamm modelling daily mean T_{sc} (3,228 records) against activity and T_{ac} (fixed effects), with the same structure as the activity model, except that year was excluded as random effect because the data were from a single winter.

To estimate FE, we used predictions based on the best fitting model of DEE, which included $M_{lean(I)}$, mean daily T_{sc} and mean daily activity as explanatory variables (Table S4). For each winter day, DEE was predicted using $M_{lean(R)}$, predicted activity and T_{sc} from the models described above, for all 140 individuals and the five body size and composition phenotypes. At each time step *t* (one day), the mass of each body compartment was recalculated based on the depletion of energy reserves from fat and protein (Speakman & Westerterp, 2013; Figure S4), and DEE was predicted from the recalculated $M_{lean(I)}$. A graphical presentation of the model input and steps is presented in Figure 1 and further details are provided in Appendix C. In the simulations of fasting, metabolisable energy from food was set to zero, and thus all energy expended was derived from internal stores. FE was modelled for all individuals assuming resting conditions (zero activity) or with predicted activity levels (see below). The model was iterated 100 times per individual and 1000 times per phenotype. Details on how uncertainty was included, and sensitivity analyses are provided in Appendix C.

To estimate the influence of adjustments in activity and/or T_{sc} levels, we extracted the theoretical 10th and 90th quantiles based on random effect variance of the individual random intercepts in activity (−3,982, +3,982) and T_{sc} (−1.9, +1.9) models. These values were then added to the mean predictions to generate high and low levels of activity and/or T_{sc}, resulting in a mean difference of ±16% in activity and ±5% in T_{sc}. We modelled FE at all nine combinations (low, mean and high level of both activity and T_{sc}) for the five body size and composition phenotypes, resulting in 45 combinations of body phenotype, activity level and T_{sc} level. Using the FE model, we calculated the minimum amount of metabolisable energy obtained from food required to survive the winter to derive an estimate of WEE. For simplicity, this was fixed at a constant value throughout the winter. We selected the lowest value that would allow survival for 212 days, while sustaining normal activity and T_{sc}, for each of the 140 individuals. Model uncertainties were not included in these analyses.

3 | RESULTS

3.1 | Drivers of daily energy expenditure

Daily energy expenditure in late winter ranged between 5.5 and 8.1 MJ/day, with a mean ± standard deviation (*SD*) of 6.3 ± 0.7 MJ/day. DEE was higher in animals with higher lean mass, higher mean T_{sc} and higher activity levels (Figure 2). Together, $M_{lean(I)}$, T_{sc} and activity explained 52% of the individual variation in DEE ($p = 0.02$). Singularly, $M_{lean(I)}$, T_{sc} and activity explained 39%, 20% and 10% of the variance in (they sum to >52% due to colinearity between T_{sc} and activity; more details in Figure S2).

3.2 | Estimates of fasting endurance

Fasting endurance was mostly determined by fat reserves rather than body size or mass, in contrast to the prediction from the FEH. Across all 140 individuals, mean ± *SD* of FE was 85 ± 17 days (range 48–137 days) under the theoretical assumption that individuals are continuously resting (Figure 3). The most important determinant of FE was initial fat mass (M_{fat}, Adj. $R^2 = 0.74$, $p < 0.001$), followed by lean mass ($M_{lean(R)}$; Adj. $R^2 = 0.18$, $p < 0.001$). M_{fat} and $M_{lean(R)}$ were not correlated ($r = 0.07$, $p = 0.4$) and had opposite effects on FE: greater M_{fat} increased FE (Figure 3a), while greater $M_{lean(R)}$ caused a decrease in FE (Figure 3b). In contrast, M_{fat} and M_b were positively correlated ($r = 0.5$, $p < 0.001$), but M_b was not a significant determinant of FE (Adj. $R^2 = -0.01$, $p = 0.8$, Figure 3c). Finally, while FE decreased with increasing body size (leg length: Figure 3d), leg length explained relatively little of the variation in FE (Adj. $R^2 = 0.04$, $p < 0.01$).

Variation between the five body size and composition phenotypes reinforces this pattern. The 'average' individual would survive 79 ± 1 days without food (with assumed continuous rest). The two fat phenotypes (both small and large size) would survive 101 ± 2 days, and the two lean phenotypes (both small and large size) were predicted to survive 56 ± 1 days. Thus, FE was 45 days (~80%) longer in fat than in lean individuals of the same size (Table 1). FE did not differ between small and large size, within fat and lean phenotypes (Table 1).

3.3 | Effects of activity and subcutaneous body temperature (T_{sc}) on fasting endurance

Reductions in both activity levels and T_{sc} increased FE (Figure 4). Daily activity decreased with time over winter, reaching a minimum around April 27, before increasing again in May (Figure S3). Furthermore, daily activity decreased with ambient temperature (T_{ac}) and was higher in individuals with larger skeletal size, with the fixed and random effects explaining 49% of the variation in activity (Table S5). T_{sc} increased with daily activity and T_{ac}, which together with the random effects explained 89% of the variation in T_{sc} (Table S6). Sustaining lower activity levels (~16% reduction) could prolong FE by 7% on average, whereas increasing activity levels (~16% increase) would shorten FE by 6%. While lowering T_{sc} (~5% reduction) would only improve FE by on average 4%, sustaining increased T_{sc} (~5% increase) would shorten FE by as much as 22%, on average. In the simulations, the greatest FE (81 days) was predicted for the large and fat phenotype sustaining low activity and low T_{sc}, whereas the shortest FE (28 days) was predicted for the small and lean phenotype sustaining high activity and high T_{sc}.

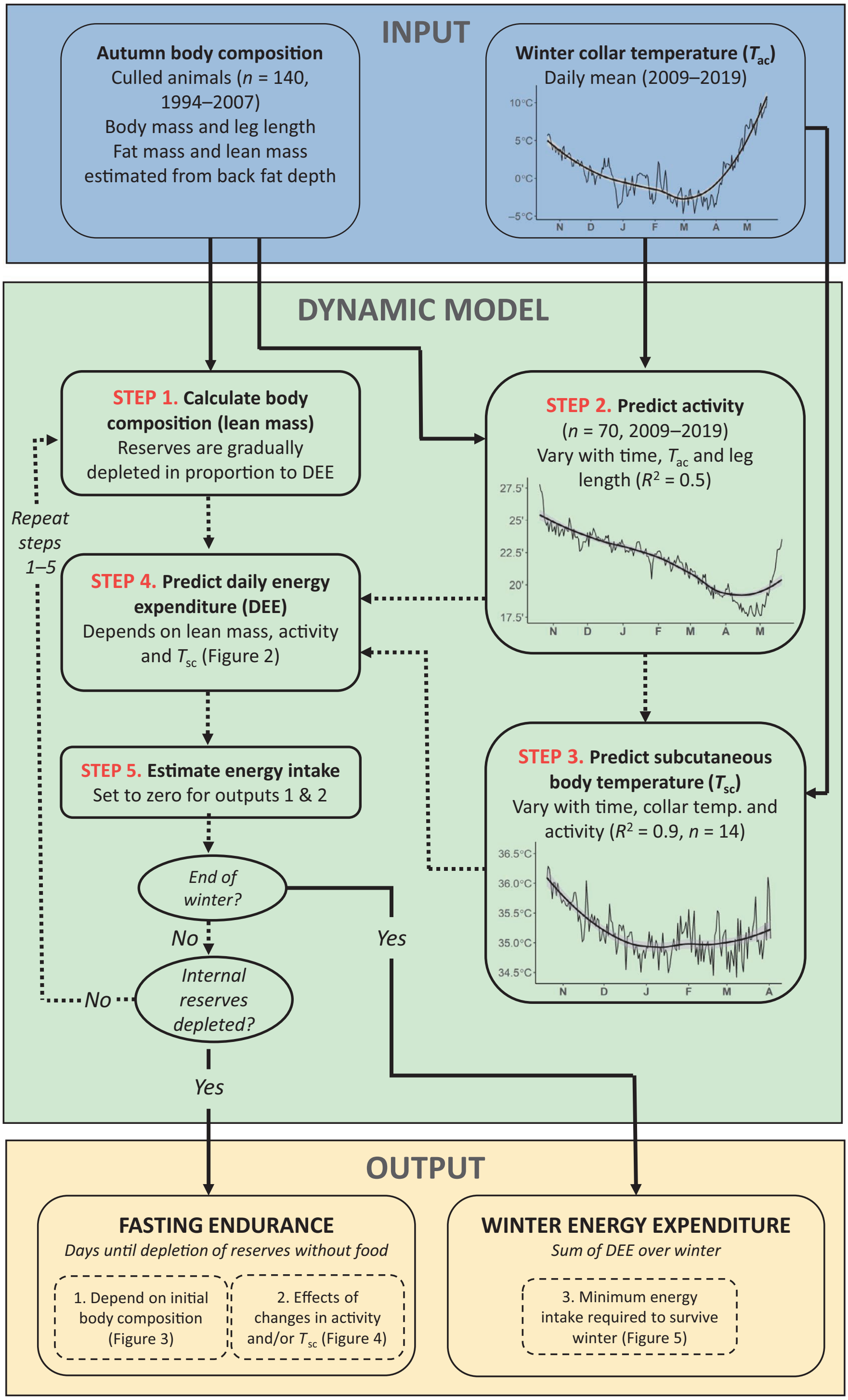
INPUT
Autumn body composition
Culled animals (n = 140, 1994–2007)
Body mass and leg length
Fat mass and lean mass estimated from back fat depth
Winter collar temperature (Tac)
Daily mean (2009–2019)
10°C
5°C
0°C
−5°C
N D J F M A M
DYNAMIC MODEL
STEP 1. Calculate body composition (lean mass)
Reserves are gradually depleted in proportion to DEE
STEP 2. Predict activity
(n = 70, 2009–2019)
Vary with time, Tac and leg length (R2 = 0.5)
27.5
25
22.5
20
17.5
N D J F M A M
Repeat steps 1–5
STEP 4. Predict daily energy expenditure (DEE)
Depends on lean mass, activity and Tsc (Figure 2)
STEP 3. Predict subcutaneous body temperature (Tsc)
Vary with time, collar temp. and activity (R2 = 0.9, n = 14)
36.5°C
36.0°C
35.5°C
35.0°C
34.5°C
N D J F M A
STEP 5. Estimate energy intake
Set to zero for outputs 1 & 2
End of winter?
No
Yes
Internal reserves depleted?
No
Yes
OUTPUT
FASTING ENDURANCE
Days until depletion of reserves without food
1. Depend on initial body composition (Figure 3)
2. Effects of changes in activity and/or Tsc (Figure 4)
WINTER ENERGY EXPENDITURE
Sum of DEE over winter
3. Minimum energy intake required to survive winter (Figure 5)

FIGURE 1 Schematic diagram of calculations steps to derive fasting endurance (FE) and winter energy expenditure (WEE) in Svalbard reindeer. The model calculates each step for 1 day at a time, making a loop that is broken when energy reserves are depleted, or when reaching end of winter (day 212). Solid arrows represent data fed into the model prior to the loop, while dashed lines represent steps that are repeated each day consecutively. For the WEE calculation, a fixed value of energy from food (in MJ) is fed into the loop (step 5) and is increased by increments of 0.5 MJ until the loop reaches the end of winter before energy reserves are depleted. To estimate FE, energy from food is set to zero

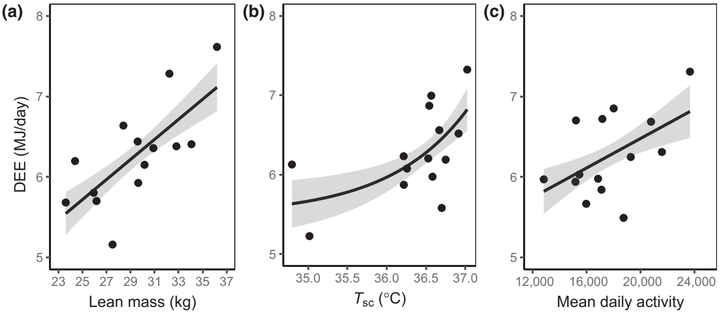

FIGURE 2 Daily energy expenditure (DEE) of Svalbard reindeer females ($n = 14$) as a function of (a) isotope-derived lean mass corrected for gut water ($M_{lean(I)}$, kg), (b) subcutaneous body temperature (T_{sc}; °C) and (c) mean daily activity level. In each regression, the points are adjusted for the other variables. The straight lines represent the predicted relationship, and the shaded areas are the 95% confidence intervals of the fitted model

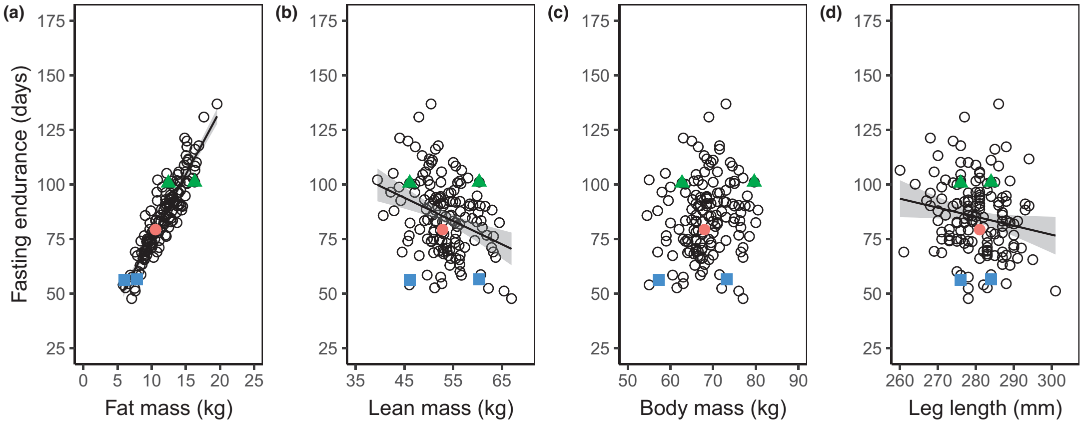

FIGURE 3 Fasting endurance (FE) estimated for Svalbard reindeer females ($n = 140$, open circles) and five body size and composition phenotypes ($n = 5$, filled shapes) under the assumption of continuous rest and zero food intake, against (a) initial body mass ($p = 0.8$), (b) initial fat mass (Adj. $R^2 = 0.74$, $p < 0.001$), (c) initial lean mass (Adj. $R^2 = 0.18$, $p < 0.001$) and (d) leg length (Adj. $R^2 = 0.02$, $p = 0.04$). Pink circles, green triangles and blue squares represent average, fat and lean individuals respectively (see Table 1 for details)

3.4 | Contribution of food intake to winter energy expenditure

Individuals with fat mass greater than that predicted for their body mass would survive with a lower food intake compared with individuals that fall below the fitted regression line of fat mass to body mass (Figure 5). The mean estimated WEE was 1,742 ± 129 MJ (range 1,404–2,055 MJ) over 212 winter days. The average phenotype would have to obtain a minimum of 1,248 ± 146 MJ (range 958–1,704 MJ) as metabolisable energy from food to survive the winter. Thus, internal fat stores contributed on average to 28 ± 6% (range 16%–44%) of WEE at mean activity and T_{sc} levels (Figure 5; Table 1). The difference in WEE between lean and fat individuals of similar size was small (Table 1).

4 | DISCUSSION

The FEH has been criticised for lacking ecological context (McNab, 2010), but many of the tools to investigate this idea in large mammals have only recently become available. The FEH, as originally proposed by Millar and Hickling (1990) was based on the interspecific scaling exponents of fat and body mass (Calder, 1984). As a result, the FEH assumes that the only way to store additional

FIGURE 4 Fasting endurance (FE) estimated for five body size and composition phenotypes of Svalbard reindeer females, sustaining low (circles), mean (triangles) and high (squares) activity levels, and maintaining low (blue), mean (green) and high (red) subcutaneous body temperature (T_{sc}) during winter. The result is a constantly lowered or increased activity (±16%) and/or T_{sc} (±5%) sustained until energy reserves are depleted (death occurs). The vertical lines represent the 95% confidence interval for each prediction, drawn from 100 simulations

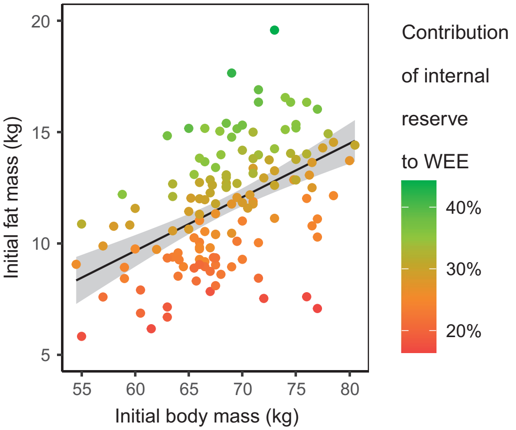

FIGURE 5 Relationship between initial fat mass and initial body mass in Svalbard reindeer females ($n = 140$). Data were collected at the beginning of winter (late October). The colour gradient represents the contribution of internal body reserves to the winter energy expenditure (WEE) over 212 days. The straight line shows the regression of initial fat mass to initial body mass, and the shaded area represents the 95% confidence intervals of the regression

fat is to become larger. We found no correlation between FE and body size and only a small correlation with mass, suggesting that animals of any size can increase the size of their fat stores to increase FE. Consistent with our findings, there is likely a strong selection in Svalbard reindeer to accumulate body fat ahead of the long winter rather than investing in structural growth from an early age. This may explain the paradox of their relatively small size in an environment strongly favouring enhanced FE.

Our estimated mean DEE (6.3 MJ/day) was similar to previously measured resting metabolic rate of captive Svalbard reindeer (6.5 MJ/day; Nilssen et al., 1984). Since DEE includes energy expended both during activity and rest, this result suggests that Svalbard reindeer in the wild have a greater ability to reduce energy expenditure during winter than previously assumed. More than half of the variation in DEE was explained by $M_{lean(I)}$, T_{sc} and activity. The fact that lean mass explains more variation in metabolic rate than body mass is comparable to results in humans and apes (Pontzer et al., 2016). Lean mass comprises the 'metabolically active' tissue, while fat is relatively 'metabolically inert' (Elia, 1992), although fat tissue may play an important role in metabolic regulation (Kaiyala et al., 2010). Positive relationships between DEE by the DLW technique and activity or displacement (movement) have been shown in other larger mammals (Pagano et al., 2018; Riek et al., 2019; Scantlebury et al., 2014). Although T_{sc} increases with activity, we also show a positive relationship between T_{sc} and DEE, independent of activity. T_{sc} varies more than core body temperature, particularly in large mammals (Irving & Krog, 1955). In our study, mean T_{sc} ranged from 34.8 to 37.0°C, whereas the rumen temperature of Svalbard reindeer in winter is typically between 38 and 38.5°C (Arnold et al., 2018). The positive relationship between T_{sc} and DEE suggests that peripheral cooling (heterothermy) is a potential mechanism to conserve energy during winter (Arnold et al., 2004; Trondrud, Pigeon, Albon, et al., 2021).

In contrast to the predictions of the FEH, we found that FE did not increase with body size or mass, but rather decreased with body size (measured as both lean mass and leg length) and increased with fat mass. Because larger bodies also have greater lean mass, which is expensive to maintain, a larger body alone does not appear to be advantageous when food is depleted in the winter. The FEH predicts that it is better to be larger because the relative scaling exponents across species suggest that the only way to store more fat is to have a bigger body with a larger lean mass. In fact, animals within a species can add fat without the need to be structurally larger. As adipose tissue is the main energy reserve in Svalbard reindeer, high fat mass is crucial for enhanced FE. The disparity between expected scaling of body reserves to body size and FE is also evident for polar bears (Dunbrack & Ramsay, 1993); survival through prolonged seasonal fasting in polar bears is mainly determined by body condition (fatness) and not size (Mathewson & Porter, 2013). Although FE has been found to increase with body mass in muskoxen *Ovibos moschatus*, Desforges et al. (2019) did not investigate variation in body reserves within a given body mass. In many species, the ratio of fat to body mass is constrained by physiological and/or ecological factors (Dunbrack & Ramsay, 1993; Jacobs et al., 2011). Body fatness is not constrained by predation risk in Svalbard reindeer because polar bears rarely attempt to kill them (Derocher et al., 2000). However, there is a substantial variation in fatness relative to body mass, indicating that some individuals are not able to maximise energy storage before the onset of winter. For example, non-lactating females in October were ~5% heavier than lactating females, suggesting that lactation hinders greater accumulation of body fat (Albon et al., 2017). Furthermore, later onset of snow in autumn has been shown to positively influence late winter body mass (Loe et al., 2021). Consequently, the high seasonality in energy supply and stochasticity during winter likely contributes to shaping a selection for fat and small phenotypes in Svalbard reindeer.

Many ungulates display pronounced seasonal adjustments in locomotor activity, body temperature and energy expenditure (reviewed in Arnold, 2020), which is also evident for Svalbard reindeer (Trondrud, Pigeon, Albon, et al., 2021). We found that adjustments in T_{sc} and activity levels can alter the FE of Svalbard reindeer by up to 20% if both parameters are reduced at the same time. A caveat of the effect of activity is the assumption made by our model that animals are fasting even during high activity, although increased activity could reflect greater foraging efforts. The greatest change to FE was induced by having a high T_{sc}, which is likely an effect of the exponential relationship between T_{sc} and DEE. Similar results have been demonstrated for musk oxen, using a process-based modelling approach, in that lower body temperatures led to reductions in the rate of body reserve depletion and to increased fitness (Desforges et al., 2021). Furthermore, Desforges et al. (2021) found that initial body condition was important for determining the benefit of reducing body temperature—the greater initial body condition, the lower the benefit of reduced body temperature for overwinter survival. Although we did not find any major differences in the effect of adjusting activity or T_{sc} between individuals of different body conditions, our results do demonstrate a general benefit of combined behavioural and physiological adjustments to conserve energy in response to food scarcity.

5 | CONCLUSIONS

The FEH is one of several central hypotheses that aim to explain the evolution of mammalian body size, in which the main premise is that the only way to get fatter is to get bigger. We demonstrate that this reasoning does not necessarily hold true within a species. Instead, we show that FE was primarily dependent on the amount of fat that individuals were able to accrue before winter. Consequently, individuals have comparatively limited scope to reduce their energy expenditure by lowering their T_{sc} and/or activity levels. Nonetheless, we emphasise that extending FE even by a few days through behavioural and physiological adjustments could prevent death, as most mortalities occur at the very end of the winter (Reimers, 1983). Our study is a demonstration that the theoretical premises of the FEH may not hold empirically, due to the large impact of environmental factors on intraspecific variation in body composition.

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AUTHORS' CONTRIBUTIONS

L.E.L., S.A., J.R.S., E.K. and L.M.T. conceived the ideas and planned the study; R.J.I. and V.V. collected the body composition harvest data; S.A., R.J.I., L.E.L., E.R., A.S. and V.V. collected activity data; A.L.E. and E.R. performed surgery; E.K., E.R., L.E.L., S.A., R.J.I., A.S., W.A., A.L.E., V.V. and L.M.T. performed the DLW field study; C.H. analysed DLW results; L.M.T. and G.P. did the statistical analyses, in interactions with E.K., J.R.S. and W.A.; L.M.T. led the writing of the manuscript. All the authors contributed to editing and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.83bk3j9qv (Trondrud, Pigeon, Król, et al., 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Supporting Information for

Fat storage influences fasting endurance more than body size in an ungulate

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Contents

Table S1. Abbreviations and units used in the Supporting Information.

Abbreviation	**Explanation and units**
BFD	Back fat depth, mm
DEE	Daily energy expenditure, MJ day^{-1}
DLW	Doubly labelled water
DW	Dressed carcass weight, kg
ED_{lipids}	Energy density of fat, MJ kg^{-1}
ED_{prot}	Energy density of protein, MJ kg^{-1}
EE_{food}	Energy expenditure contributed by food intake, MJ day^{-1}
$EE_{reserve}$	Energy expenditure contributed by body reserves, MJ day^{-1}
$ELIP_{fat}$	Energy from lipids contributed by adipose mass, MJ
$ELIP_{muscle}$	Energy from lipids contributed by muscle and organ mass, MJ
$EPROT_{fat}$	Energy from protein contributed by adipose mass, MJ
$EPROT_{muscle}$	Energy from protein contributed by muscle and organ mass, MJ
$ETOT_{lipids}$	Total energy from lipids, MJ
$ETOT_{prot}$	Total energy from proteins, MJ
$EUSE_{lipids}$	Energy used from lipid stores, MJ day^{-1}
$EUSE_{prot}$	Energy used from protein stores, MJ day^{-1}
FE	Fasting endurance, days
GISP	Greenland Ice Sheet Precipitation
k_d	Deuterium elimination rate
k_o	^{18}O elimination rate
M_b	Total body mass, kg
M_{bone}	Mass of skeleton, kg
M_{fat}	Fat mass available as energy reserve, kg
$M_{Tfat(I)}$	Total body fat, isotope derived, kg
$M_{Tfat(R)}$	Total body fat, regression-derived, kg
$M_{lean(I)}$	Lean (fat-free) mass, isotope-derived from DLW, kg
$M_{lean(R)}$	Lean (fat-free) mass regression-derived, kg
M_{muscle}	Muscle and vital organ mass, kg
M_{skin}	Mass of skin and hooves, kg

N_d	Deuterium dilution space, kg
N_o	Oxygen dilution space, kg
PP_{fat}	Proportion of protein contributed by fat tissue
PP_{muscle}	Proportion of protein contributed by muscle and organ mass
PL_{fat}	Proportion of lipids contributed by fat mass
PF_{muscle}	Proportion of lipids contributed by muscle and organ mass
R_{fat}	Ratio of contribution by fat and protein to DEE
SLAP	Standard Light Antarctic Precipitation
SMOW	Standard Mean Ocean Water
T_{ac}	Ambient temperature by collar, °C
T_{sc}	Subcutaneous body temperature, °C
WEE	Winter energy expenditure, MJ

Appendix A. Additional methods

A1 Isotope analyses of doubly labelled water (DLW) samples and calculation of daily energy expenditure (DEE)

Samples of blood were vacuum distilled into glass Pasteur pipettes (Nagy 1983). The resultant water was used for analysis of ^{18}O:^{16}O and ^{2}H:^{1}H performed on a laser-based Isotopic Water Analyzer IWA-*35*-EP (Los Gatos Research, San Jose, CA, USA), as described in Berman et al. (2012). Each batch of samples was run alongside five lab and three international standards (SMOW, GISP and SLAP) to correct for day-to-day differences in machine performance. All isotope enrichments were expressed in delta (δ) per mil (‰) relative to the working standards and converted to parts per million (ppm) using the established isotope ratios for the reference materials. Inter-sample memory was routinely addressed by injecting water samples multiple times and ignoring the results from the first few injections (Lis, Wassenaar, & Hendry, 2008). The measures of isotope enrichment were based on a minimum of five readings; all subsequent calculations were performed on the mean values. For the two animals with no initial blood samples (Table S2), the initial isotope enrichment was extrapolated from the relationship between the mass of DLW injectate relative to M_b versus the enrichment of ^{18}O and ^{2}H in ppm, established for the animals with complete set of the DLW data. Initial dilution spaces for ^{18}O (N_o) and ^{2}H (N_d for deuterium) were calculated by the intercept method (Coward & Prentice, 1985), converted from moles to grams assuming a molecular mass of body water as 18.0153 g mol^{-1}, and then expressed as a percentage of M_b before initial blood sample. We used the intercept rather than the plateau method because estimates generated by the intercept method are robust to the timing of the initial blood sample, as long as sufficient time elapsed for complete mixing of DLW with body water pools and if the processes that occurred during the equilibration period were the same as those that generated the isotope elimination curves (Speakman & Król, 2005). Final dilution spaces were inferred from the final M_b, assuming the same percentage of M_b as measured for the initial dilution spaces. The isotope elimination rates for k_o and k_d were calculated following Nagy (1983). The mean rate of CO_2 production (rCO_2 mol day^{-1}) was calculated using a two-pool model, equation A6 from (Schoeller et al., 1986), as recommended for large animals and humans (Speakman, 1993). Because of the reduced food intake in winter, the loss of ^2H through methane and its impact on the rCO_2 estimates were considered negligible. Energy equivalents of the rate of CO_2 production were established using a conversion factor of 22.949 J mL^{-1} CO_2, derived from the Weir equation (Weir, 1949) for a respiratory quotient of 0.9 (Cuyler & Øritsland, 1993). The differences in timing protocol in 2017 and 2018 had no significant effect on the results ($P > 0.05$). Neither DEE nor M_b differed significantly between the two years (Table S3, Welch's two sample t-test for unequal variances); data from 2017 and 2018 were consequently pooled in analyses.

Table S2. Characteristics of 21 Svalbard reindeer females dosed with doubly labelled water (DLW) to measure daily energy expenditure (DEE) in late winter 2017 and 2018. The data from 2017 were used only in the expanded model of DEE excluding subcutaneous body temperature (T_{sc}). Two individuals represented in both years were treated as separate data points.

Animal	Born	Age (years)	Injected with DLW	M_b before injection (kg)	Pregnant[a]	T_{sc}[b]	Activity[c]
1	2009	7	24.03.2017	56.0	+	−	+
2	2010	6	24.03.2017	50.0	+	−	−
3[d]	2010	6	25.03.2017	46.0	+	−	+
4	2013	3	23.03.2017	56.0	+	−	+
5[d]	2013	3	23.03.2017	49.5	+	−	+
6	2013	3	25.03.2017	49.5	+	−	+
7	2011	6	04.04.2018	57.8	+	−	+
8	2011	6	04.04.2018	56.3	+	+/−	+
9	2011	6	03.04.2018	57.8	+	+/+	+
10	2011	6	06.04.2018	51.3	+	+/−	+
11	2011	6	03.04.2018	50.8	+	+/+	+
12	2011	6	03.04.2018	48.8	+	+/+	+
13	2012	5	04.04.2018	49.8	−	+/+	+
14	2012	5	06.04.2018	52.8	−	−	−
15	2012	5	03.04.2018	54.8	+	−	+
16[e]	2012	5	04.04.2018	46.3	+	−	+
17	2012	5	04.04.2018	47.8	+	+/+	+
18	2010	7	03.04.2018	51.8	+	+/−	+
3[d, e]	2010	7	03.04.2018	46.8	+	+/−	+
19	2013	4	03.04.2018	47.3	+	+/+	+
5[d]	2013	4	07.04.2018	48.8	−	+/+	+
20	2013	4	03.04.2018	51.3	+	+/−	+
21	2013	4	06.04.2018	53.8	+	+/−	+

Superscripts: **a** pregnant (+) or non-pregnant (−); **b** with (+) or without (−) **T_{sc}** measurements during DLW period/with (+) or without (−) **T_{sc}** in Oct-April, **c** with (+) or without (−) activity measurements; **d** studied in 2017 and 2018; **e** no initial sample taken.

Table S3. Results of the doubly labelled water (DLW) experiment performed on 21 Svalbard reindeer females in years 2017 and 2018.

Parameter	Mean ± standard deviation	
	2017	2018
Number of animals	6[a]	17[a]
M_b at DLW injection (kg)	51.2 ± 4.0	51.4 ± 3.7
Dose of DLW (g kg^{-1} M_b)	0.330 ± 0.045	0.293 ± 0.077
Time between injection and initial blood sample (h)	48.6 ± 7.9	46.1 ± 3.3
M_b at initial blood sample (kg)	50.3 ± 3.8	50.2 ± 3.3
Time between initial and final blood samples (days)	8.4 ± 1.1	14.8 ± 1.7
M_b at final blood sample (kg)	46.6 ± 3.8	48.2 ± 3.5
k_o (h^{-1})[b]	0.0032 ± 0.0005	0.0034 ± 0.0004
k_d (h^{-1})[b]	0.0025 ± 0.0005	0.0026 ± 0.0004
k_o/k_d	1.307 ± 0.084	1.280 ± 0.040
N_o (% of M_b)[c]	62.8 ± 1.2	62.5 ± 4.5
N_d (% of M_b)[c]	63.4 ± 1.7	63.6 ± 4.2
N_d/N_o	1.009 ± 0.010	1.016 ± 0.009
DEE (MJ day^{-1})	6.0 ± 0.5	6.2 ± 0.8
TBW (kg)	30.6 ± 1.7	30.7 ± 3.1
Gut content (22% of M_b)[d]	11.1 ± 0.8	11.2 ± 0.6
Water in gut (85% of gut content)[d]	9.4 ± 0.7	9.5 ± 0.5
$M_{lean(I)}$ (kg) corrected for gut water	29.1 ± 1.5	29.5 ± 3.7
$M_{fat(I)}$ (kg)	9.2 ± 2.0	9.3 ± 2.9
M_{fat} (% of M_b)[d]	17.8 ± 2.7	18.2 ± 5.4

Superscripts: [a] two of these animals were studied in both years; [b] elimination rates of ^{18}O (k_o) and ^2H (k_d for deuterium); [c] oxygen (N_o) and deuterium (N_d) dilution spaces expressed as % of M_b at injection, [d] based on previous literature: Staaland et al. 1979, Sørmo et al. 1999.

A2 Surgical protocol for subcutaneous implantation of biologger device

All implants were sterilized with ethylene oxide gas (Anaprolene AN74i 60 L, Andersen Europe, Kortrijk, Belgium). We programed temperature loggers (DST Centi-HRT, Star Oddi, Gardabaer, Iceland, 46 x 15 mm; 19 g) to record subcutaneous body temperature (T_{sc}) at intervals of 15 min. With a memory capacity of 175,000 temperatures, the data loggers could record T_{sc} every 15 min for up to 5 years. Before implantation, animals were sedated with intranasal medetomidine (Domitor vet, Orion Pharma Animal Health, Turku, Finland; dose ~0.14 mg kg^{-1} M_b) or dexmedetomidine (Dexdomitor vet, Orion Pharma Animal Health, Turku, Finland, dose ~0.07 mg kg^{-1} M_b). During the procedure, surgical anaesthesia was assessed by checking the eyelid reflexes, limb movements, pulse and breathing rate. The surgical area was shaved and cleaned with chlorhexidine spirit (Fresenius Kabi Norge AS). Bupivacaine (Marcaine 5 mg/mL, AstraZeneca, Cambridge, United Kingdom), 2.5–5 mg was used for local anaesthesia. For post-operative analgesia, 0.5 mg kg^{-1} M_b meloxicam (Metacam®, Boehringer Ingelheim Vetmedica GmBH, Ingelheim am Rhein, Germany) was administered subcutaneously before surgery. We made a 1 cm incision either on the left side of the chest or behind the left axilla and inserted the logger into the subcutaneous space. The incision was closed with 2-0 monofilament absorbable suture PDS® II (polydioxanone) (Ethicon, Johnson & Johnson, New Brunswick, NJ, United States). After the surgery, anaesthesia was reversed with intramuscular atipamezole (Antisedan® 5 mg mL^{-1}, Orion Pharma Animal Health, Turku, Finland; 10 mg per mg dexmedetomidine or 5 mg per mg medetomidine). Once animals regained consciousness and coordination, they were released and monitored until running normally (~5 min post-atipamezole injection). Animals were allowed to recover from surgery for 12–21 (mean 17±3) days before recapture and injection of DLW. At logger retrieval, reindeer were captured and given local anaesthesia at the location of the logger (Bupivacaine, Marcain 2.5–5 mg) before making a small incision through where the logger was removed. The incision site was closed with 2-0 PDS suture.

A3 Temperature logger calibration

The loggers' temperature was individually calibrated by the manufacturer for 41 set points over the range 5°C to 45°C, with a guaranteed accuracy of ±0.2°C for the full temperature range one-year post calibration. The equipment used for the calibration of the loggers, as stated on the calibration certificate from the manufacturer, is a Hart 7012 temperature bath and the reference measurements are conducted with a Hart 1504 thermometer and a Hart 5610–9 thermistor probe with combined absolute accuracy better than ±0.010°C. Each set point measurement was taken when the temperature was stable within 0.001°C.

A4 Body composition calculations and selection of body phenotypes

We used the relationship between back fat depth (BFD; mm), dressed carcass weight (DW, kg) and body fat described for Svalbard reindeer in Figure 3 in Reimers and Ringberg (1983) to estimate both dissectible fat (hereafter fat mass, M_{fat}, eq. 1) and total fat mass ($M_{Tfat(R)}$, eq. 2), the latter being chemically determined fat from the ingesta-free body:

$$M_{fat} = -3.5371 + 0.0990\,BFD + 0.0014\,BFD^2 + 0.2093\,DW \tag{1}$$

$$M_{Tfat(R)} = 2.8835 + 0.2440\,BFD + 0.0004\,BFD^2 \tag{2}$$

Both $M_{Tfat(R)}$ and previously described $M_{Tfat(I)}$ represent total body fat, but $M_{Tfat(R)}$ is estimated from the above-mentioned regression whereas $M_{Tfat(I)}$ is isotope derived. Total body fat incorporates all lipids in the body, both structural (phospholipids) and non-structural (triglycerides). Structural fat is generally not considered as a source of energy (McCue, 2012). As most triglycerides are found in the white adipose tissue, we used $M_{Tfat(R)}$ to calculate $M_{lean(R)}$ ($M_b - M_{Tfat(R)} = M_{lean(R)}$) for model predictions, while M_{fat} was used to indicate utilisable body fat reserves. The mass of structural fat for each individual was considered to remain constant and calculated as the difference between initial $M_{Tfat(R)}$ and M_{fat}. We used the distributions of body composition to select the mean, lower and upper 10% quantiles of $M_{lean(R)}$ (58, 46 and 60 kg, respectively) and M_{fat} as % of $M_{lean(R)}$ (20, 13 and 27%, respectively). We estimated the mass of the skeleton (M_{bone}) as well as skin and hooves (M_{skin}) as 11% of initial $M_{lean(R)}$ (Reimers, Ringberg, & Sørumgård, 1982) and we assumed that these masses did not change over time:

$$M_{bone} = 0.11 \times \mathrm{M_{lean(R)}} \tag{3}$$

$$M_{skin} = 0.11 \times \mathrm{M_{lean(R)}} \tag{4}$$

Subtracting M_{fat}, M_{bone}, M_{skin} from M_b yielded the remaining weight, assumed to be the vital organs, skeletal muscles and ligaments in the body (hereafter muscle and organ mass, M_{muscle}):

$$M_{muscle} = M_b - M_{fat} - M_{bone} - M_{skin} \tag{5}$$

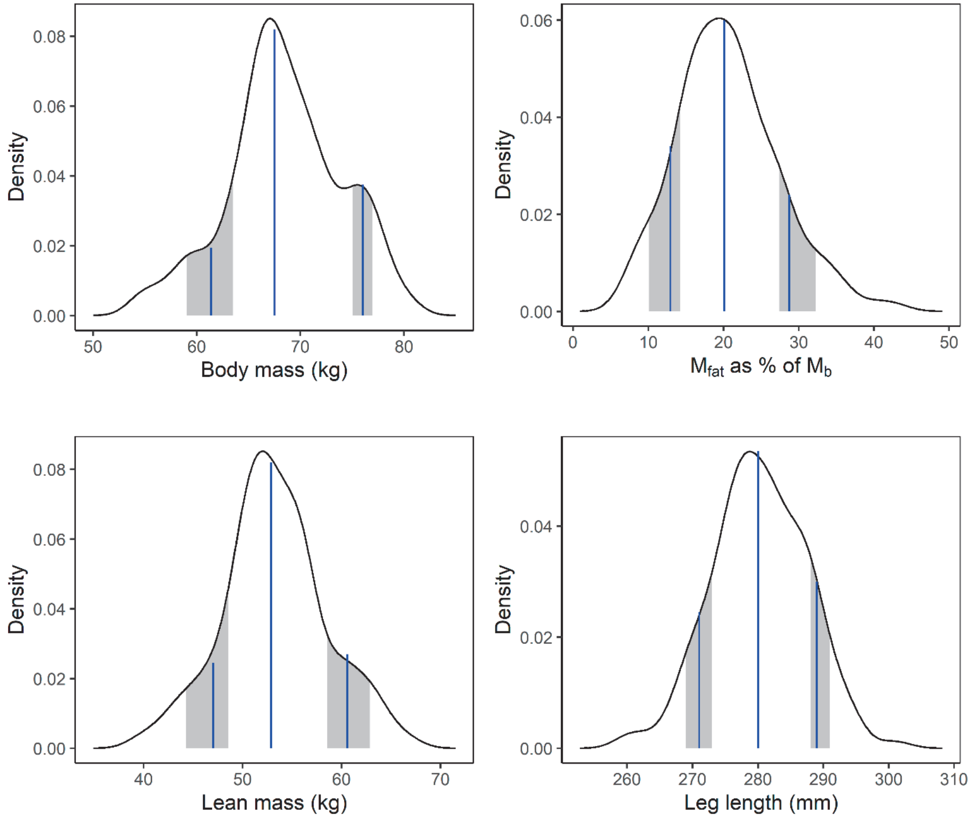

Figure S1. Density distributions of body size and composition in adult Svalbard reindeer females (ages 3–9 years) culled in October in years 1994–2007 (n = 140; Albon et al. 2017). The shaded areas represent the 5–15% and 85–95% quantiles, while the blue lines represent the 10, 50 and 90% quantiles for each distribution.

Appendix B. Additional results

Table S4. Selection and parameter estimates of the model used to identify drivers of individual variation in daily energy expenditure (DEE, MJ day^{-1}) in Svalbard reindeer females. The final model contained isotope-derived fat-free mass corrected for gut water ($M_{lean(I)}$, kg), mean daily subcutaneous temperature (T_{sc}, °C) and mean daily activity as explanatory variables of variation in DEE (Adj. $R^2 = 0.52$, $n = 14$, $P = 0.02$). Activity was borderline not significant for the subset of individuals with data on T_{sc} ($n = 14$, $P = 0.09$), but significant when including individuals with missing data on T_{sc} ($n = 21$, $P = 0.01$). Activity was therefore retained in the model. Parameter estimates are reported along with standard error (SE), t-value, P-value and confidence intervals. Model selection was done in a stepwise manner, based on likelihood ratio testing. The parameter estimates of each removed variable together with SE, F-value (degrees freedom, df, and sample size, n), P-value, and 95% confidence intervals (CI) are presented with the first variable removed at the bottom.

Variable included	**Estimate**	**SE**	**t**	**P**	**95% CI**	
					lower	**upper**
Intercept	6.27	0.14	46.38	< 0.001	5.97	6.57
Activity	0.27	0.15	1.83	0.10	−0.06	0.59
T_{sc} (exponential)	0.33	0.14	2.38	0.04	0.02	0.64
$M_{lean(I)}$	0.46	0.15	3.16	0.01	0.14	0.78
Variable removed	**Estimate**	**SE**	**F (df, n)**	**P**	**95% CI**	
					lower	**upper**
Age	−0.17	0.16	1.24 (9, 14)	0.31	−0.53	0.19
Pregnancy	0.41	0.38	1.19 (8, 14)	0.31	−0.46	1.29

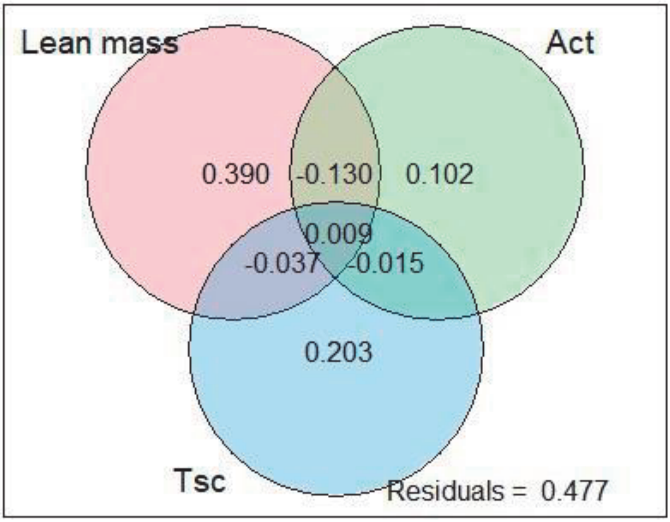

Figure S2. Variance partitioning of the variables explaining DEE (Table S4.1). Testable individual fractions were available for [a] $M_{lean(I)}$ | Activity (Act) + T_{sc} (0.39), [b] = Act | $M_{lean(I)}$ + T_{sc} (0.10) and [c] = T_{sc} | Activity + $M_{lean(I)}$ (0.20). The remaining fractions (values in overlapping sections) were not testable but included to show that the sum of values returns the total variance explained by the model (Adj. $R^2 = 0.52$). Residual variance is extracted from $(1 - R^2)$ of the model. To partition the variance explained by each variable, we used redundancy analysis ordination (Legendre & Legendre, 2012) with the *varpart* function from the 'vegan' package in R (Oksanen et al., 2019). Because Act and T_{sc} were co-linear, the sum of the individual variances is greater than the variance explained by the full regression.

Table S5. Model parameter estimates of activity in 70 adult female Svalbard reindeer from October 20 to May 20 as a function of ambient temperature recorded by the collar (T_{ac}) and leg length (mm) and fitted with thin-plate regression splines for time (days). Year and individual were fitted as random effects for both slope and intercept. Estimated degrees of freedom (EDF), which represents curvature of the smoothing parameter (the higher the number, the more variation), F-statistic and P-value are shown for the smoothing terms. Adj. $R^2 = 0.49$, n = 30696.

Variable included	**Estimate**	**SE**	**t**	**P**
Intercept	22055.6	760.7	29.0	<0.001
T_{ac} (scaled)	604.2	75.7	8.0	<0.001
Leg length (scaled)	−343.4	36.6	−9.4	<0.001
Smooth terms	EDF		F	P
Time (days)	8.9		158	<0.001
Year (random intercept)	9.0		81600	<0.001
Year (random slope)	8.9		8563	<0.001
ID (random intercept)	71.3		7511	<0.001
ID (random slope)	67.8		878	<0.001

Table S6. Model parameter estimates of subcutaneous body temperature (T_{sc}) in 9 adult female Svalbard reindeer from October 20 to May 20 as a function of activity and ambient temperature recorded by the collar (T_{ac}), fitted with thin-plate regression splines for time (days) and individual slopes and intercepts as random effects. Estimated degrees of freedom (EDF), which represents curvature of the smoothing parameter (the higher the number, the more variation), F-statistic and P-value are shown for the smoothing terms. Adj. $R^2 = 0.89$, n = 1463.

Variable included	**Estimate**	**SE**	**T**	**P**
Intercept	35.04	0.44	78.7	< 0.001
Activity (scaled)	0.10	0.02	5.2	< 0.001
T_{ac} (scaled)	0.21	0.02	11.7	< 0.001
Smooth terms	EDF	F		P
Time (days)	5.3	20		< 0.001
ID (random intercept)	11.3	60544		< 0.001
ID (random slope)	10.4	38882		< 0.001

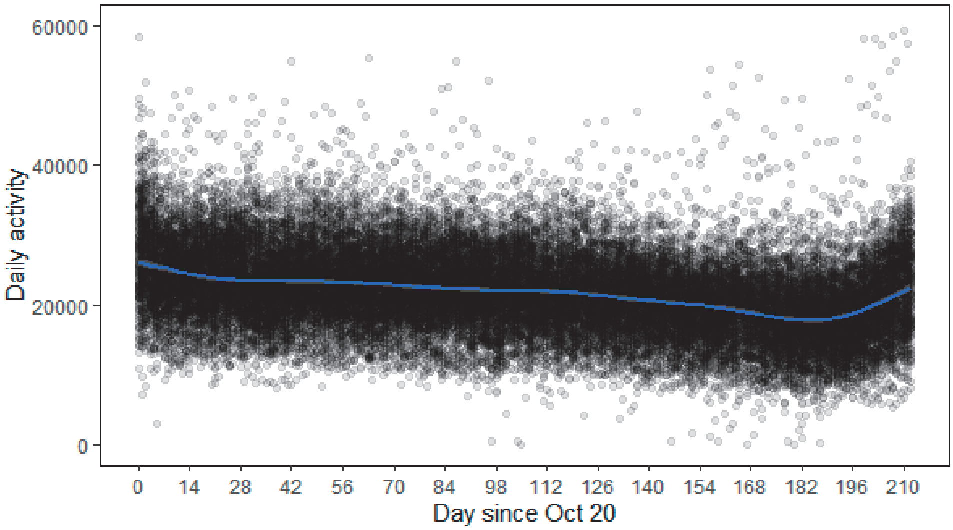

Figure S3. Daily activity levels (sum of every 5 min measurement, where one measurement represents the average 8 Hz acceleration ranging from 0 to 255) across all years (2009-2019) and for all individuals (n = 70, 267 id-year combinations) shown for the period October 20 (day 0) to May 20 (day 212). The date with lowest activity in each year ranged between January 26 to May 5 (day 98 to day 197 since October 20) with a mean date of April 4. However, when averaging activity levels across all years, the lowest activity level occurred around April 27 (day 189 since October 20) before increasing, because this across-year mean was pulled down by two years with particularly low activity levels in the end of the winter. The blue line shows a generalized additive model prediction with time (days) fitted with a spline function.

Appendix C. Fasting endurance model

C1 Fasting endurance (FE) model steps

For all 140 individuals as well as the five specific body size and composition phenotypes, we predicted DEE for each day of winter using the linear regression of DEE against $M_{lean(R)}$, activity and T_{sc} from models described in Tables S4.1–3. This approach assumes equivalent gradients for the within- and between-individual effects on DEE, and that $M_{lean(I)}$ and $M_{lean(R)}$ provide the same information on body composition. On the first winter day (t = 0), all body compartments were derived from the cull data set as described in Appendix A4. One increment in time t represents one day. Because M_{fat} and M_{muscle} reflects the utilizable energy reserves, but DEE is predicted from $M_{lean(R)}$, $M_{Tfat(R)}$ was calculated each day as the sum of structural fat (constant value) and M_{fat}, and then subtracted from M_b to get $M_{lean(R)}$. Tissue composition for M_{fat} was taken from Reimers et al. (1982): 92% lipids, 2.5% protein and 5.5% water. For the composition of M_{muscle}, we used values for lean mass: 5% lipids, 22% protein and 73% water from Lichtenbelt et al. (1994). The following calculation steps are derived from the mathematical model of starvation in humans by Speakman and Westerterp (2013). Stored energy from body reserves at time t was calculated from the mass of each tissue, the composition and the energy density of lipids (ED_{lipids}, 39 MJ kg^{-1}) and protein (ED_{prot}, 18 MJ kg^{-1}) (Hall, 2008), respectively:

$$ELIP_{fat(t)} = M_{fat(t)} \times 0.92 \times ED_{lipids} \tag{6}$$

$$ELIP_{muscle(t)} = M_{muscle(t)} \times 0.05 \times ED_{lipids} \tag{7}$$

Where ELIP is the energy stored as lipids (MJ) and ED_{lipids} the energy density (MJ kg^{-1}) of lipids, and

$$EPROT_{fat(t)} = M_{fat(t)} \times 0.025 \times ED_{prot} \tag{8}$$

$$EPROT_{muscle(t)} = M_{muscle(t)} \times 0.22 \times ED_{prot} \tag{9}$$

Where EPROT is the energy stored as protein and ED_{prot} the energy density (MJ kg^{-1}) of protein.

The total stored energy was summed for fat and protein separately:

$$ETOT_{lipids(t)} = ELIP_{fat(t)} + ELIP_{muscle(t)} \tag{10}$$

and

$$ETOT_{prot(t)} = EPROT_{fat(t)} + EPROT_{muscle(t)} \quad . \tag{11}$$

Where ETOT is the sum of available energy (MJ) in the form of lipids (eq. 10) or protein (eq. 11) contributed by both fat stores and by muscle stores. For simplicity, we considered that glycogen stores were depleted within the first day of fast and were not considered as a source of energy during prolonged fasting (Elia, Stubbs, & Henry, 1999; Øritsland, 1990). The relative contribution of each body compartment was derived by estimating the P-ratio, which explains how the depletion of body stores differs with initial fat; the higher the fat content in the body, the higher the P-ratio (Forbes, 1987; Hall, 2007). The relative contribution of fat and protein to DEE was estimated with the P-ratio (R_{fat}) (Forbes, 1987; Hall, 2007) using equation 23 from Speakman and Westerterp (2013):

$$R_{FAT} = \Lambda + \Gamma \times \left[\frac{ETOT_{fat}}{ETOT_{fat} + ETOT_{prot}}\right] \tag{12}$$

with the empirically derived values for humans being $\Lambda = 0.489$ and $\Gamma = 0.547$ (Speakman and Westerterp 2013, equation 27). Energy used from fat ($\mathrm{EUSE_{fat}}$) was calculated as $\mathrm{DEE} \times \mathrm{R_{fat}}$ and from protein ($\mathrm{EUSE_{prot}}$) as $\mathrm{DEE} \times (1 - \mathrm{R_{fat}})$. Because both M_{muscle} and M_{fat} contain both lipids and protein, we needed to calculate the relative depletion of each store. We used the proportion of lipids and protein contributed by each compartment to $\mathrm{ETOT_{fat}}$ and $\mathrm{ETOT_{prot}}$ to estimate their relative contributions to $\mathrm{EUSE_{fat}}$ and $\mathrm{EUSE_{prot}}$, respectively, at each time step:

$$PP_{muscle(t)} = \frac{[M_{muscle(t)} \times 0.22 \times ED_{\mathrm{prot}}]}{ETOT_{\mathrm{prot}(t)}} \tag{13}$$

thus

$$PP_{fat(t)} = 1 - PP_{muscle(t)} \tag{14}$$

Similarly, for lipids:

$$PL_{muscle(t)} = \frac{[M_{muscle(t)} \times 0.05 \times ED_{lipids}]}{ETOT_{lipids(t)}} \tag{15}$$

thus

$$PL_{fat(t)} = 1 - PL_{muscle(t)} \tag{16}$$

where PP and PL are proportions of protein and lipids contributed by each tissue (M_{fat} and M_{muscle}) to the total energy stores of protein and lipids, respectively. The energy reserves from each tissue compartment the next day (time t+1) are therefore:

$$ELIP_{fat(t+1)} = ELIP_{fat(t)} - [EUSE_{fat} \times PL_{fat(t)}] \tag{17}$$

and

$$ELIP_{muscle(t+1)} = ELIP_{muscle(t)} - [EUSE_{fat} \times PL_{muscle(t)}] \tag{18}$$

$$EPROT_{fat(t+1)} = EPROT_{fat(t)} - [EUSE_{prot(t)} \times PP_{fat(t)}] \tag{19}$$

and

$$EPROT_{muscle(t+1)} = EPROT_{muscle(t)} - [EUSE_{prot(t)} \times PP_{muscle(t)}] \tag{20}$$

Knowing the composition of each tissue, we calculated the mass of adipose and lean tissue at time t+1:

$$M_{muscle(t+1)} = \frac{ELIP_{M_{muscle}(t+1)}}{0.05 \times ED_{lipids}} + \frac{EPROT_{M_{muscle}(t+1)}}{0.22 \times ED_{prot}} \tag{21}$$

and

$$M_{fat(t+1)} = \frac{ELIP_{M_{fat}(t+1)}}{0.92 \times ED_{lipids}} + \frac{EPROT_{M_{fat}(t+1)}}{0.025 \times ED_{prot}} \tag{22}$$

The body mass at time t+1 was calculated by subtracting the utilized M_{fat} and M_{muscle} from the body mass at time t:

$$M_{b(t+1)} = M_{b(t)} - \left[M_{muscle(t)} - M_{muscle(t+1)}\right] - \left[M_{fat(t)} - M_{fat(t+1)}\right] \quad (23)$$

and

$$M_{lean(t+1)} = M_{b(t)} - \left[M_{fat(t+1)} + \text{structural fat (constant)}\right] \quad (24)$$

Uncertainty was accounted for by adding an error term which was randomly drawn from a normal distribution with mean of zero and SD equal to the standard error of the model predictions of DEE, activity and T_{sc}. We ran 100 simulations and extracted the mean, SD, and upper and lower 95% quantiles per daily prediction per individual. Sensitivity of the model was assessed using one-parameter-at-a-time analyses and varying each default parameter value by 5, 10 and 20% in both directions, described further in Appendix C2.

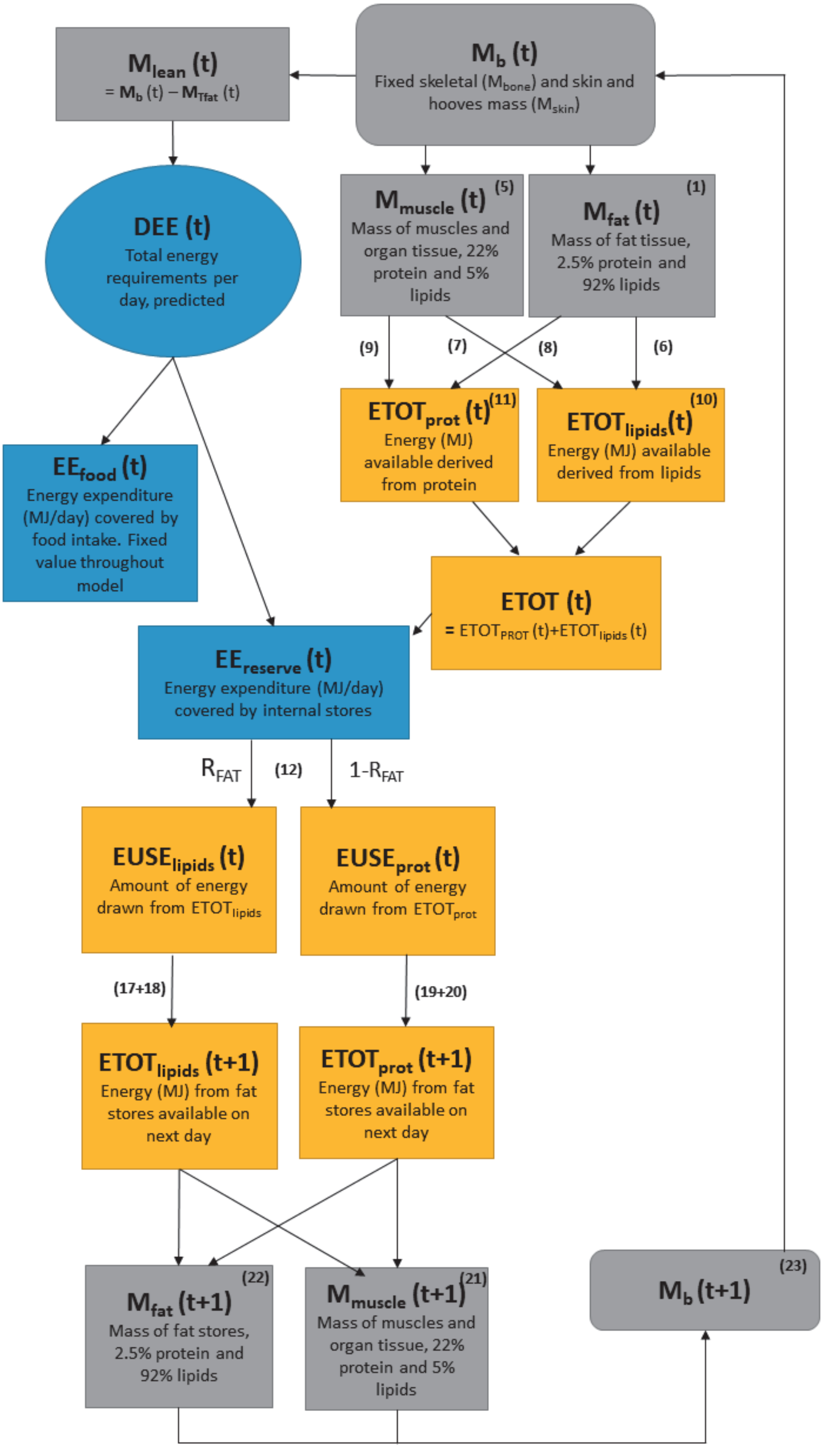
M_{lean} (t)
= M_b (t) – M_{Tfat} (t)
M_b (t)
Fixed skeletal (M_{bone}) and skin and hooves mass (M_{skin})
DEE (t)
Total energy requirements per day, predicted
M_{muscle} (t) (5)
Mass of muscles and organ tissue, 22% protein and 5% lipids
M_{fat} (t) (1)
Mass of fat tissue, 2.5% protein and 92% lipids
(9)
(7)
(8)
(6)
$ETOT_{prot}$ (t) (11)
Energy (MJ) available derived from protein
$ETOT_{lipids}$ (t) (10)
Energy (MJ) available derived from lipids
EE_{food} (t)
Energy expenditure (MJ/day) covered by food intake. Fixed value throughout model
ETOT (t)
= $ETOT_{PROT}$ (t)+$ETOT_{lipids}$ (t)
$EE_{reserve}$ (t)
Energy expenditure (MJ/day) covered by internal stores
R_{FAT}
(12)
1-R_{FAT}
$EUSE_{lipids}$ (t)
Amount of energy drawn from $ETOT_{lipids}$
$EUSE_{prot}$ (t)
Amount of energy drawn from $ETOT_{prot}$
(17+18)
(19+20)
$ETOT_{lipids}$ (t+1)
Energy (MJ) from fat stores available on next day
$ETOT_{prot}$ (t+1)
Energy (MJ) from fat stores available on next day
M_{fat} (t+1) (22)
Mass of fat stores, 2.5% protein and 92% lipids
M_{muscle} (t+1) (21)
Mass of muscles and organ tissue, 22% protein and 5% lipids
M_b (t+1) (23)

Figure S4. Conceptual framework of the calculation of fasting endurance with respect to energy stores. Each shape represents a type of variable: rectangles are calculated values, semi-round rectangles are empirical data, and ovals reflect values predicted from models and thus includes error estimates. Numbers in bold refer to equations in section 1.4 and 3.1. The arrows represent the direction of effects and/or steps in the calculation. Each variable is color-coded based on grey = mass, yellow = energy stores, blue= energy expenditure. Values followed by "(t)" change for each time step t, whereas the other values are fixed throughout the model. One increment in time t represents one day, so the calculation of M_b (t + 1) is derived from the depletion of reserves at time t. Abbreviations are explained in Table S1.

C2 Sensitivity analyses of fasting endurance model

We investigated the sensitivity of the FE model using one-parameter-at-a-time analyses and varying each default parameter value by 5, 10 and 20% in both directions. We followed the FE (days) as the model output for the average body size and composition phenotype, under the assumption of zero food intake and for both normal and zero activity. The model uncertainties were not included in these analyses. The following parameters were tested (default value): bone mass (11% of $M_{lean(R)}$), skin and hooves mass (11% of $M_{lean(R)}$), energy contribution from M_{muscle} (40%), energy content of protein (18 MJ kg^{-1}) and values for Λ (0.489) and Γ (0.547) for calculating R_{FAT}. The outputs of the sensitivity analyses were compared to the default values using a sensitivity index (SI) as described in Pethybridge et al. (2013), providing the percentage error in between baseline and the new values for each output. The model output (FE) did not vary with changing proportions of M_{bone} or M_{skin} (Table S5.1). The changes were only slightly different in the model output based on normal activity in the case of ED_{PROT} and available M_{muscle}. When changing Λ and Γ, the effect was almost halved in all parameter deviations (± 5, ± 10 and ± 20%) during activity as compared to resting (Table S5.1). Changing the ED_{PROT} had relatively small effects on the output, changing by less than 1% for ± 5% and ± 10% deviations, and only 2–3% change when ED_{PROT} deviated by ± 20%. The most influential parameters where the proportion of muscle and organ mass, M_{muscle}, that are available for energy utilization, Λ and Γ. Deviations of ± 5% and ± 10% for these parameters did not influence the model output by more than 5% and model output varied by maximum 9% at the highest deviation (± 20%).

Table S7. Results of classical single parameter sensitivity analyses (changing one parameter at a time) for the fasting endurance of an average adult female Svalbard reindeer during rest and during activity. The parameters were tested for ± 5, ± 10 and ± 20% deviations from the default value. Sensitivity index (SI) was calculated as the percentage difference (error rate) between the model output with default parameter values and the output with the deviated parameter value. Presented are the values for each parameter in each deviation, and the SI (% deviation) for the model output during rest and activity.

Parameter		**−20%**	**−10%**	**−5%**	**0**	**5%**	**10%**	**20%**
Available M_{muscle}	Value	0.320	0.360	0.380	0.400	0.420	0.440	0.480
(% M_{muscle})	SI (resting)	8.0	4.5	1.8	0.0	2.7	4.5	9.8
	SI (active)	7.0	2.8	1.4	0.0	1.4	4.2	8.5
Skeletal mass	Value	0.088	0.099	0.105	0.110	0.116	0.121	0.132
(% M_{lean})	SI (resting)	0	0	0	0	0	0	0
	SI (active)	0	0	0	0	0	0	0
Skin and hooves	Value	0.088	0.099	0.105	0.110	0.116	0.121	0.132
Mass (% M_{lean})	SI (resting)	0	0	0	0	0	0	0
	SI (active)	0	0	0	0	0	0	0
Energy in protein	Value	14.4	16.2	17.1	18.0	18.9	19.8	21.6
(MJ)	SI (resting)	1.8	0.9	0.0	0.0	0.9	0.9	1.8
	SI (active)	1.4	1.4	0.0	0.0	0.0	1.4	2.8
Lambda	Value	0.391	0.440	0.465	0.489	0.513	0.538	0.587
(constant)	SI (resting)	8.0	4.5	1.8	0.0	1.8	3.6	8.0
	SI (active)	4.2	2.8	1.4	0.0	1.4	2.8	5.6
Gamma	Value	0.438	0.492	0.520	0.547	0.574	0.602	0.656
(constant)	SI (resting)	7.1	3.6	1.8	0.0	1.8	3.6	6.3
	SI (active)	4.2	2.8	1.4	0.0	1.4	1.4	4.2

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Paper II

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Determinants of heart rate in Svalbard reindeer reveal mechanisms of seasonal energy management

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Seasonal energetic challenges may constrain an animal's ability to respond to changing individual and environmental conditions. Here, we investigated variation in heart rate, a well-established proxy for metabolic rate, in Svalbard reindeer (*Rangifer tarandus platyrhynchus*), a species with strong seasonal changes in foraging and metabolic activity. In 19 adult females, we recorded heart rate, subcutaneous temperature and activity using biologgers. Mean heart rate more than doubled from winter to summer. Typical drivers of energy expenditure, such as reproduction and activity, explained a relatively limited amount of variation (2–6% in winter and 16–24% in summer) compared to seasonality, which explained 75% of annual variation in heart rate. The relationship between heart rate and subcutaneous temperature depended on individual state via body mass, age and reproductive status, and the results suggested that peripheral heterothermy is an important pathway of energy management in both winter and summer. While the seasonal plasticity in energetics makes Svalbard reindeer well-adapted to their highly seasonal environment, intraseasonal constraints on modulation of their heart rate may limit their ability to respond to severe environmental change. This study emphasizes the importance of encompassing individual state and seasonal context when studying energetics in free-living animals.

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1. Introduction

The interplay between energy requirements and availability is fundamental to all living organisms [1]. Because both energy requirements and availability depend on environmental conditions, the balance between them contributes to shaping ecological interactions at the level of individuals [2], populations [3], species [4,5] and whole ecosystems [6]. Seasonal changes in energy supply have led to the evolution of life-history strategies and a wide range of physiological, morphological and behavioural adaptations that enable animals to adjust their metabolic phenotype to the prevailing season of the year [7]. While seasonal plasticity is relatively well studied, little is known about the capacity of seasonally adapted animals to respond to short-term environmental fluctuations within the season [8–10]. It has been suggested that strong phenotypic plasticity may limit the potential for evolutionary responses to climate change [11]. Thus, understanding the relationship between interseasonal and intraseasonal plasticity of metabolic adaptations is of fundamental importance for predicting animal resilience to climate change [12].

For mammals and birds, the cost of maintaining high core body temperature increases as ambient temperatures decline [13]. However, in many seasonal environments, the availability and quality of food plants decline simultaneously with decreasing ambient temperature, particularly in temperate and arctic environments [14]. Hence when herbivorous endotherms have potentially the greatest thermoregulatory demands due to low ambient temperatures, they also have the lowest supply of food to meet such demands [15]. By contrast, when food is more plentiful, animals must both reproduce and replenish energy reserves. In the most extreme seasonal environments, this period of high energy supply can be as short as two months [7]. Trade-offs between energy investment in offspring and energy acquisition to replenish reserves are often shaped by physiological [16,17] or nutritional [18] constraints.

The most pronounced temporal reductions in energy expenditure are observed in species exhibiting daily torpor and hibernation, characterized by substantially lowered metabolic rate, body temperature and reduced movement [19]. However, accumulating evidence shows that many non-hibernating temperate animals also display seasonal adjustments in metabolic rate [20–23] through reduced body temperature and activity levels [24–26]. Similar responses have been observed in desert ungulates during the hot, dry season when food is limited [27–29]. Adjustments in behavioural and physiological traits are clearly important to reduce the energetic costs in periods of low resource availability, and simultaneously maximize replenishment of body reserves and reproduction in periods of high resource availability [30].

Heart rate is a key physiological parameter of animal performance because it correlates with oxygen consumption and hence is often used as a proxy for metabolic rate [31,32]. Also, heart rate is a key biologging parameter because the heart beat generates an electrical signal readily monitored by small implantable devices, providing information on energetics of wild animals in their natural habitat over long time periods [4], at fine temporal scales [33], and in remote or harsh environments ([34,35], reviewed in [36]). Furthermore, heart rate can be influenced by locomotor activity [37,38], ambient temperatures [5,39] and reproduction [40,41]. Although substantial knowledge about the relationship between energy expenditure (indexed by heart rate) and specific physiological or environmental processes exists, few studies have explored how these potentially interact to influence variation within periods of energy surplus and deficit [5,41,42].

We quantified the effects of environmental, behavioural and physiological factors on variation in heart rate of Svalbard reindeer (*Rangifer tarandus platyrhynchus*)—a keystone species in the High Arctic with strong seasonal changes in nutrition and metabolic activity. Inhabiting a predator-free environment, they forage for up to 70% of their time in summer to meet the energetic demands of reproduction [43] and accumulate large fat reserves critical for survival during the long, cold winter [44]. Svalbard reindeer exhibit the largest seasonal amplitude in daily resting heart rate recorded in any ungulate [34] and downregulate metabolic rate during winter even when fed *ad libitum* in captivity [45]. Despite such strong adaptations to seasonal energetic constraints, there is considerable uncertainty in how flexible they are in their response to short-term environmental and physiological challenges.

We deployed internal biologging devices to measure heart rate and subcutaneous body temperature (T_{sc}) in 19 adult female Svalbard reindeer of known body mass, age and reproductive status. In addition, animals were fitted with a global positioning system (GPS) collars containing activity sensors. Using these data, we quantified variation in heart rate in relation to animal age, reproductive status, body mass, T_{sc}, activity level and environmental temperature within the seasonal peak (summer) and trough (winter) of heart rate. Identifying the correlates of intraseasonal variation in metabolic rate, as indexed by heart rate, is important for understanding the challenges faced by Svalbard reindeer in a rapidly warming Arctic [46].

2. Methods

(a) Study area and animals

The study was conducted in Nordenskiöld Land, Svalbard (77°50′–78°20′ N, 15°00′–15°60′ E). At this latitude, there is continuous daylight from 19 April to 23 August and continuous darkness from 14 November to 29 January. The plant growing season typically lasts from June until late August [47]. Monthly mean air temperatures in July 2018 and January 2019 were 6.8 ± 1.5°C and −10.1 ± 5.5°C, respectively (Svalbard airport, SN99840; http://eklima.met.no). Further information on the study system is provided in the electronic supplementary material, §1.a. Gestation in Svalbard reindeer lasts for approximately 7.5 months from October until calving in early June [8]. Peak lactation is expected 3–5 weeks postpartum based on domestic reindeer *Rangifer t. tarandus* [48]. We selected July as the representative month for mid-summer due to the seasonal peak in heart rate, and January to represent mid-winter. During these periods, circadian rhythmicity is weak [34].

(b) Animal capture and data collection

Adult females (ages 5–8 years, marked as calves) were captured in March–April 2018 for biologger deployment and in April 2019 for biologger retrieval. On both occasions, animals were caught by net using snowmobiles [49]: we recorded their body mass (±0.5 kg) and checked for pregnancy using an ultrasound scanner (Kaixin Electronic Instrument Co., Xuzhou, China). Body mass and age were not correlated ($r = -0.07$, $p = 0.9$). In August 2018, surveys were conducted on foot to relocate marked animals and assess calf status. Eight out of 19 individuals were not observed, and their calf status was inferred from pregnancy status in April and activity pattern in early June as described in [50] (electronic supplementary material, table S1). Omitting individuals with inferred calf status resulted in similar parameter estimates and *p*-values in the analyses described below. All females with a calf at heel in August were classified as lactating.

(c) Biologger programming, deployment and retrieval

We fitted each animal with a combined heart rate and temperature logger (DST centi-HRT, Star-Oddi, Gardabaer, Iceland; approximately 19 g), which was implanted subcutaneously on the left side of the sternum or behind the left axilla, while animals were under anaesthesia. Surgical procedures are described in the electronic supplementary material, §1.b. Heart rate was automatically calculated from a 4 s electrocardiogram (ECG) at 150 Hz measurement frequency and stored alongside a quality index of signal clarity. We programmed the loggers to record heart rate and subcutaneous body temperature (T_{sc}) every 15 min, and to store a raw ECG signal every 6 h for manual validation. Validation and filtering steps are described in the electronic supplementary material, §1.c. Briefly, values were filtered based on minimum and maximum values that could be validated (20 and 175 beats per minute (bpm), respectively) and the loggers' internal quality assessment (keeping only high quality—level 0). On average, 63% of recordings per animal per day were retained for analysis. T_{sc} was recorded with an accuracy of 0.2°C and calibrations were conducted by the manufacturer prior to implantations and validated again 12–13 months later, after removal. After retrieval, data were downloaded with the Mercury software program and a communication box [51]. Of the animals recaptured in April 2019, nine had uninterrupted recordings of heart rate and T_{sc} available for the whole year, while ten stopped recording before January due to battery failure. Consequently, summer analyses (July 2018) were based on the data available for all 19 females, while winter analyses (January 2019) relied on data for nine animals. Subsetting the summer data using just the nine individuals from the winter dataset resulted in qualitatively similar results.

The animals were also fitted with a collar (Vertex Plus, Vectronic Aerospace GmbH, Berlin, Germany, approximately 750 g) containing a GPS receiver, an activity sensor and an Iridium Communication (satellite) system. The GPS receiver had a fix rate of 8 h and was used to locate animals prior to capture. The activity sensor measured acceleration along two orthogonal axes representing back–forward and right–left movements at 4 Hz intervals. An internal algorithm calculates activity as the difference in acceleration between two consecutive measurements and is given within a relative range between 0 and 255, providing a mean value of acceleration in each axis every 5 min [52]. Because heart rate was recorded every 15 min, we used the sum of all activity recorded in both axes between two heart rate timestamps. For example, for a heart rate recorded at 16 : 15, we used the sum of activity recorded at 16 : 05, 16 : 10 and 16 : 15. Activity values therefore ranged between 0 and 1530, where 0 represented no activity and 1530 maximum activity. We categorized behaviour into resting/stationary (less than 50; hereafter 'resting') and moving (≥50; hereafter 'active') based on the bimodal distribution of activity data (details provided in the electronic supplementary material, §1.d).

In the main valley of our study area, we had a black bulb thermistor (15 cm in diameter) containing an iButton temperature logger (iButton Link) situated 1.5 m above the ground. These black spheres are designed to measure effective environmental temperatures, as the temperature inside the black bulb is influenced by solar radiation, wind chill and precipitation in addition to air temperature [53]. Temperatures were recorded every 4 h throughout the study period. Therefore, we matched recordings by 2 h in each direction of the time stamp to match with heart rate and T_{sc} records. For example, black bulb temperature recorded at 1600 h was matched to all heart rate and T_{sc} records between 1400 and 1800 h. Information about the construction of the black bulb is provided in electronic supplementary material, §1.e. Hereafter, black bulb temperatures are referred to as effective environmental temperature (T_e).

(d) Statistical analyses

All statistical analyses were conducted in R v. 4.0.0 [54]. First, we modelled heart rate over the whole year ($n = 393\,708$ recordings in total) with a generalized additive mixed-effects model using the '*bam*' function for large datasets [55] to analyse the seasonal trend. We fitted heart rate as the response variable and time (days) as a thin plate regression spline with smoothing parameter $k = 20$ and a penalization value (λ) of 1.4 [55]. k was selected and assessed using the '*gam.check*' function from the 'mgcv' package. An individual term was fitted as both random slope and intercept. We used an autoregressive structure (AR1) to account for intraindividual temporal autocorrelation. Since the filtering of recordings left missing values in the dataset, we added a weighting parameter that gave missing values a weight of 0 and non-missing values a weight of 1.

Second, we investigated the drivers of variation in resting and active (defined above) heart rate during July and January with separate models for each activity level and season. We used a linear mixed-effects (lme) model using the 'nlme' package with the individual as a random intercept and fitted an AR1 structure as described above. All models were initially fitted with the same explanatory variables using maximum likelihood and simplified through a stepwise backward model selection approach [56], with a likelihood ratio test performed at each removal step (electronic supplementary material, tables S2–S5). The explanatory variables fitted were time (calendar day), T_e, body mass recorded during capture (April 2018 for summer models, March 2019 for winter models), reproductive status (lactation status in summer, pregnancy in winter; categorical 'yes/no') and age, T_{sc}, as well as several biologically relevant interactions between the variables. For the models of active heart rate, an additional term for activity (continuous values from 50 to 1530) was fitted, together with additional interactions between activity and other variables (all parameters are listed in the electronic supplementary material, tables S2–S5). The final models were fitted with restricted maximum likelihood to account for random effects [56]. All continuous variables (activity, body mass, T_e, T_{sc} and time) except age were scaled within seasons to a mean of zero and standard deviation relative to the variance. Time was fitted as a quadratic term in the summer models to account for the peak in heart rate in mid-July and as a linear term in the winter models. We used the function '*rsquared.GLMM*' from the 'MuMIn' package to derive the coefficients of determination (R^2) for fixed effects (marginal R^2) and fixed and random effects combined (conditional R^2) to assess the amount of variation explained by each model [57]. The generation of figures from model predictions is described in the electronic supplementary material, §2.c.

3. Results

Predicted heart rates from the generalized additive mixed-effects model peaked in mid-July at 103 bpm, declined to 40 bpm in December and then remained relatively stable until April, when the loggers were removed (figure 1). Day

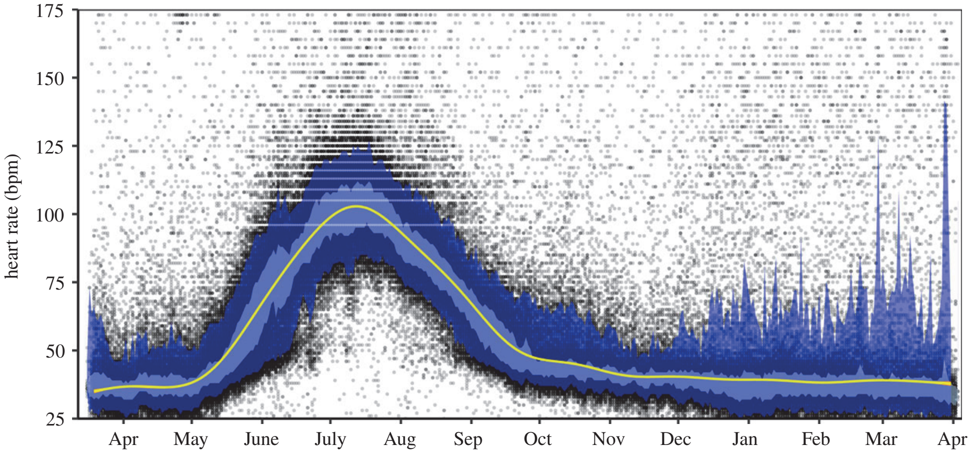

Figure 1. Annual variation in heart rate across activity and reproductive states in Svalbard reindeer females, based on the data for 19 individuals (sample size declines throughout the year; see §2 for details). The *x*-axis spans from March 2018 to April 2019, and each tick mark indicates the first day of the month. The solid yellow line represents predictions of heart rate across all individuals, fitted with a generalized additive mixed model as a function of time with the individual as a random term ($R^2 = 0.75$). The black points are raw data values; the dark blue area represents values within the lower 5% and upper 95% quantiles, and the light blue area represents values within the lower 25% and upper 75% quantiles of the data.

of the year and individual variation explained 75% of the variation in heart rate. In both winter (January) and summer (July), arithmetic means of heart rate were 10 bpm lower when resting compared to active heart rate (electronic supplementary material, table S2, and figure S9). In winter, animals were active 44% of the time compared to 66% in summer (electronic supplementary material, table S2).

The lme models of winter heart rate explained relatively little variation. Together, fixed and random effects explained only 5% (fixed effects 2%) and 7% (fixed effects 6%) for resting and active heart rate, respectively. The summer lme models explained considerably more variation in heart rate, accounting for a total of 42% (fixed effects 16%) of variation in the resting state and 38% (fixed effects 24%) of variation in the active state. Despite the marked difference in the fit of the final models in each season, there were many similarities in the model parameters included. Therefore, we continue by describing the explanatory variables from lme models in turn, presenting results of model predictions with 95% confidence intervals (CI) in parentheses.

Body mass did not influence heart rate in resting animals in either summer or winter (table 1). In the active state, however, the increase in heart rate with increasing activity was greater in heavier animals (table 1). In winter, predicted heart rate rose from 34 bpm (CI: 31–39) to 54 bpm (CI: 51–58) at the highest activity levels in heavy (approx. 56 kg) individuals compared with a rise from 35 bpm (CI: 32–39) to 45 bpm (CI: 42–49) in light individuals (approx. 43 kg, figure 2*a*). This interaction was also significant in summer (table 1), but less pronounced with only a 4 bpm difference between heavy individuals at 113 bpm (CI: 110–115) and light individuals at 109 bpm (CI: 106–113) at the highest activity levels (figure 2*b*). In the active state, body mass also interacted with T_{sc}, but only in winter (table 1). Whereas lighter animals reduced heart rate markedly from 47 bpm (CI: 44–50) at T_{sc} 37°C to 28 bpm (CI: 21–34) at T_{sc} 31°C, heavy individuals maintained a constant heart rate at approximately 44 bpm (CI: 41–47) across the range of T_{sc} (electronic supplementary material, figure S10).

Reproductive status affected heart rate in summer and during activity in winter. The effect size in summer, when lactating, was greater (on average 4 bpm higher in reproductive females) than in winter when pregnant (2 bpm difference) (figure 3*a*). The reproductive status also interacted with T_{sc}, but differently for summer and winter (table 1). During summer, lactating females had higher heart rates at higher T_{sc}, both during activity and at rest (table 1, figure 3*b*). In winter, the interaction between reproductive status and T_{sc} was only significant when active (table 1), with the heart rate of pregnant females displaying both a positive relationship with, and a greater range of T_{sc} (electronic supplementary material, figure S11). Finally, in summer, lactating females spent on average 6% more time active (67% versus 61% in non-lactating females, $p < 0.001$), whereas in winter, there was no significant difference in time spent active between the two reproductive groups (electronic supplementary material, table S2).

Age had a pronounced effect on heart rate in summer, regardless of reproductive and activity states. Predicted heart rate declined by 10 (resting) and 12 (active) bpm in 8-year olds compared to 5-year olds (table 1). Furthermore, there was an interaction between age and T_{sc} during summer, with a greater effect in older animals. When resting, an 8-year old who lowered T_{sc} to 30°C had a predicted heart rate of 84 bpm (CI: 82–87) compared to 100 bpm (CI: 98–102) in a 5-year old (figure 4). When active, the magnitude of the age difference was again greater at lower T_{sc} (figure 4). The interaction between age and T_{sc} was also significant in winter; however, differences were small (differences of 1–2 bpm) and no 8-year olds were present in the winter dataset (electronic supplementary material, table S1 and figure S12).

Being active raised heart rate as expected, but relatively more so in winter than in summer (table 1). However, the effect of activity on heart rate interacted with body mass, as described above (figure 2), and furthermore with T_{sc}, especially in winter (table 1). The relation of heart rate with

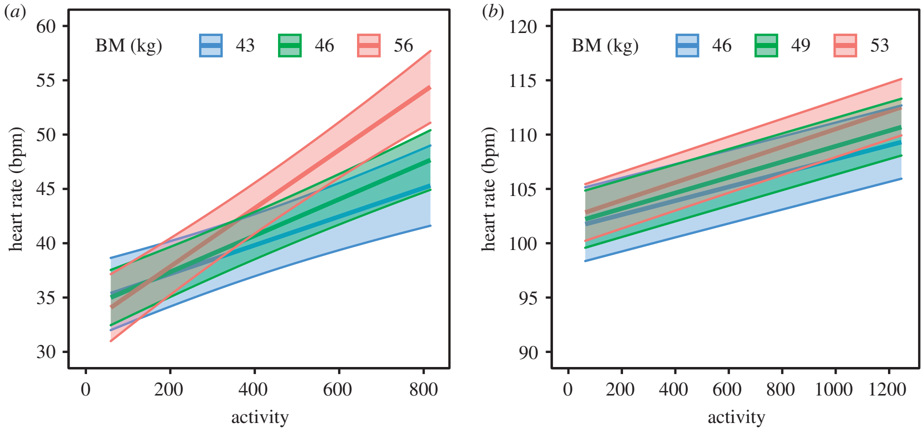

Figure 2. Predicted heart rate (±95% CI) of Svalbard reindeer females, plotted against activity levels in interaction with body mass grouped based on the 0.15, 0.5 and 0.85 quantiles of the distribution of body mass in (*a*) winter and (*b*) summer.

Table 1. Results of linear mixed-effects models on the heart rate of Svalbard reindeer females, during rest and while active in both summer (July) and winter (January). The values are parameter estimates of the fixed effects, with upper and lower 95% CIs given in brackets. The standard deviations of the random intercepts of each model were 1.2 and 2.1 for resting and active in winter, and 5.6 and 4.4 for resting and active in summer, respectively. All continuous variables except age were scaled with a mean of 0 within each season. The final models were fitted with restricted maximum likelihood. Sample size '*N*' represents the number of unique individuals, while '*n*' represents the number of unique observations. For each model, reference levels for the intercepts are based on non-reproductive females (0). BM, body mass (kg); '–', not applicable/tested in model; ns, not significant (removal based on maximum likelihood ratio test); RS, reproductive status (1 = lactating in summer or pregnant in winter, 0 = non-reproductive); T_e, environmental temperature; T_{sc}, subcutaneous body temperature.

	summer ($N = 19$)		**winter ($N = 9$)**	
model parameters (fixed effects)	**resting ($n = 11\,287$)**	**active ($n = 24\,436$)**	**resting ($n = 8936$)**	**active ($n = 4495$)**
intercept	113.8 (98.2, 129.5)	126.3 (113.9, 138.6)	33.2 (28.0, 38.5)	48.7 (37.8, 59.6)
time (days)	−0.5 (−0.7, −0.3)	−1.9 (−2.1, −1.7)	0.4 (0.2, 0.5)	0.2 (−0.3, 0.9)
time (days)^2	−1.9 (−2.1, −1.7)	−2.1 (−2.3, −2.0)	–	–
activity	–	3.2 (3.0, 3.4)	–	5.2 (4.3, 6.1)
age	−3.4 (−6.0, −0.8)	−3.9 (−6.0, −1.9)	−0.1 (−1.2, 0.9)	−0.8 (−2.9, 1.3)
BM	ns	1.4 (−1.1, 3.9)	ns	0.4 (−1.7, 2.6)
RS (1)	3.8 (−2.1, 9.7)	3.6 (−1.1, 8.3)	2.0 (−0.3, 4.4)	−5.9 (−12.8, 1.1)
T_e	−0.7 (−0.9, −0.6)	−0.2 (−0.4, −0.1)	−0.2 (−0.3, −0.1)	−0.8 (−1.3, −0.2)
T_{sc}	−2.3 (−3.2, −1.4)	−3.3 (−4.4, −2.4)	−0.8 (−1.7, 0.1)	1.1 (−7.8, 10.0)
activity × BM	–	0.5 (0.3, 0.8)	–	1.4 (0.5, 2.3)
activity × T_{sc}	–	0.3 (0.1, 0.5)	–	3.8 (2.4, 5.3)
age × T_{sc}	0.4 (0.2, 0.5)	0.6 (0.4, 0.7)	0.2 (0.0, 0.4)	−1.5 (−2.7, −0.3)
BM × T_{sc}	ns	ns	ns	−2.5 (−3.8, −1.3)
RS (1) × T_{sc}	0.6 (0.3, 1.0)	1.4 (1.0, 1.8)	ns	8.2 (2.2, 14.1)

T_{sc} was greatest at high activity levels and virtually absent at low activity levels (electronic supplementary material, figure S13a). Conversely, in summer, the relation of heart rate with T_{sc} was more similar at various activity levels, albeit still significantly different (table 1; electronic supplementary material, figure S13b).

Declining environmental temperature (T_e) raised heart rate in both winter and summer. The effect was slightly more pronounced when active compared to resting in winter, with predicted differences of 2.4 bpm and 0.7 bpm, respectively, when T_e declined from −2°C to −22°C (electronic supplementary material, figure S14). In summer, the effect was greater when resting compared to active: predicted difference of 4.2 bpm and 1.2 bpm, respectively, when T_e declined from 25°C to 4°C (electronic supplementary material, figure S14).

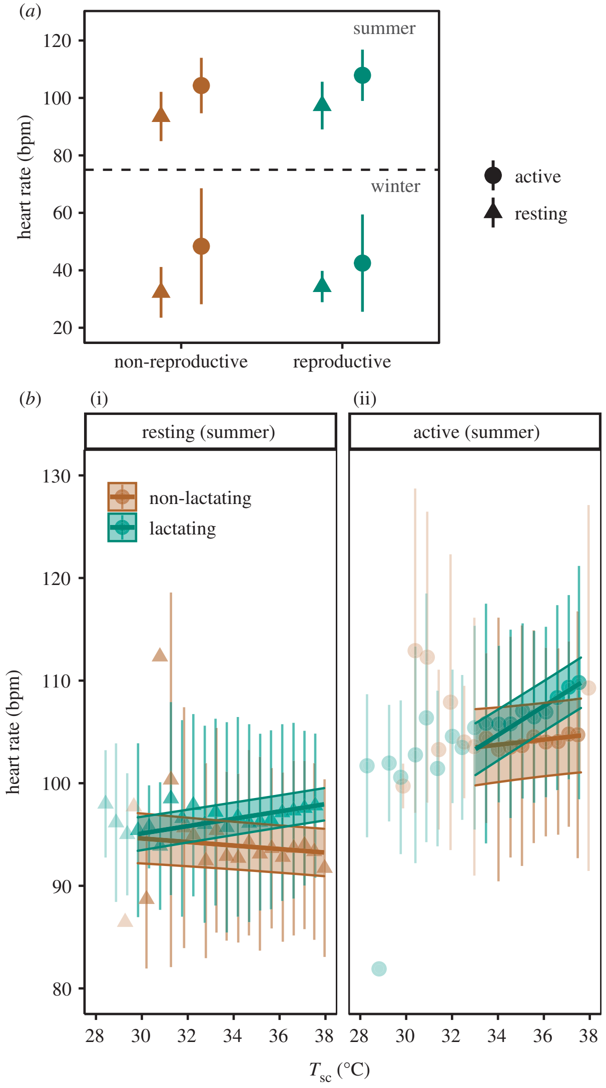

Figure 3. Predicted heart rate for reproductive (green) and non-reproductive (brown) females while resting (triangles) and while active (circles). (*a*) Predicted heart rate (±s.e.) grouped by reproductive state (summer: lactating $N = 13$, non-lactating $N = 6$; winter: pregnant $N = 7$, non-pregnant $N = 2$), activity state and season. (*b*) Predicted heart rates (±95% CI) for lactating (green) and non-lactating (brown) Svalbard reindeer females in summer, while resting (i) and while active (ii) in response to subcutaneous body temperature (T_{sc},°C). Points and their error bars represent mean ± s.d. of heart rate adjusted for the other model predictors (table 1). Points that fall outside the predicted range are values below the lower 0.01 and above the upper 0.99 quantiles of the T_{sc} distribution.

4. Discussion

Our results demonstrate that the impact of physiological and environmental factors on heart rate depends on both individual state and seasonal context. In particular, the relationship between heart rate and subcutaneous body temperature (T_{sc}) suggests that peripheral heterothermy, i.e. variability in the body 'shell' temperature [58], is an important mechanism for energy management, not only in winter, but also in summer. Furthermore, several interactions indicate that the benefit of heterothermy is dependent on activity, body mass, reproductive status and age, especially in summer. The most common drivers of energy expenditure such as reproduction [59] and activity [60] had only small effects on heart rate within the seasons (2–6% in winter and 16–24% in summer), whereas seasonality itself explained a large amount of the variation (75%) in annual heart rate. This seasonality is consistent with a strong selective pressure on energy conservation in winter and maximizing energy intake in summer for reproduction and replenishment of body reserves. Here, we discuss the observed energy management mechanisms that contribute to meeting these seasonal energetic constraints.

Endogenous heat production is an inevitable energetic cost for endotherms, and mechanisms to reduce this cost can be of great importance, particularly during periods of energy deficits and low ambient temperatures [15].

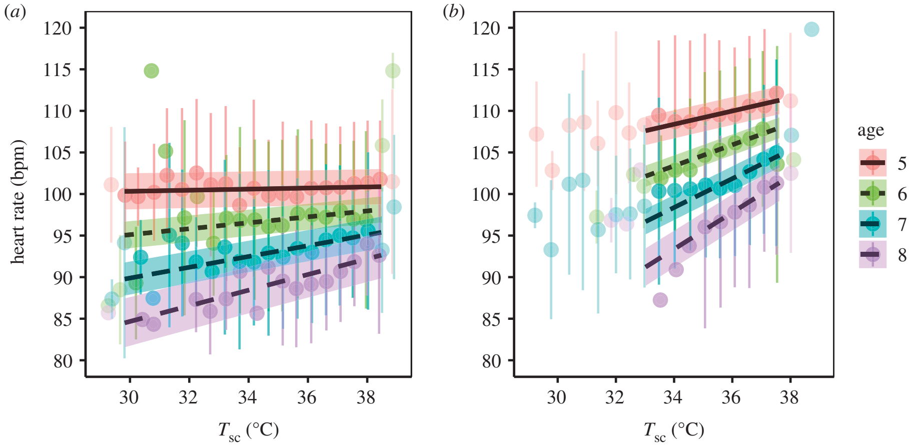

Figure 4. Predicted heart rate (±95% CI) plotted against subcutaneous body temperature (T_{sc},°C) in interaction with age (5-, 6-, 7- and 8-year olds) in summer (*a*) during rest and (*b*) while active. Points and their error bars represent mean ± s.d. adjusted the other model predictors (table 1). Points that fall outside the predicted range are values below the lower 0.01 and above the upper 0.99 quantiles of the T_{sc} distribution.

Reductions in heart rate have been strongly associated with a decrease in both core [25,34] and peripheral body temperatures [40,61,62]. We found that heart rate and T_{sc} were correlated in both seasons. Interestingly, this association was greater (more positive) in individuals with lower body mass in winter and in older females in summer. Peripheral heterothermy is likely to be an important mechanism to reduce heat loss and save energy by minimizing the temperature gradient between the body shell and the environment [58,63]. Winter body mass in Svalbard reindeer is strongly influenced by the size of the fat stores [8]. Body condition (indexed by fat stores) begins to decline around age seven *(Pigeon, unpublished data)*, at an age when teeth also start to wear down, which may cause a lower rate of energy assimilation [64] due to larger particles and longer retention time in the digestive system [65]. Our results indicate that peripheral heterothermy may be more pronounced in individuals of poorer body condition in winter, or with limitations on food intake in summer. Indeed, state-dependent heterothermy has been demonstrated in moose, where individuals of poor body condition displayed lower core body temperature [66]. Furthermore, the degree of hypothermia in both peripheral [67,68] and core body temperature [69] has been shown to increase in response to food restriction. Plasticity in the ability to employ heterothermy may therefore be a key factor that enables animals to respond to short-term energy deficits or limitations in energy uptake [12].

Reproduction, and lactation in particular, is considered the most energetically demanding part of the annual cycle for female mammals [70] and has been proposed as the main driver of seasonally elevated mammalian energy expenditure [71]. Although we found a significantly higher heart rate in lactating compared to non-lactating female Svalbard reindeer, the difference was surprisingly small (approx. 6%) and heart rates of non-lactating females were still more than twice that of winter rates (figure 2*a*). Our results demonstrate that elevated heart rate in summer is largely independent of reproduction, as has been observed in other seasonal animals [25,41]. The most likely explanation for the seasonal increase is the relatively narrow time window when forage is abundant, requiring a substantial upregulation of the metabolic machinery in order to recover body reserves and ensure survival during the coming winter, regardless of reproductive state [34]. The energetic cost of lactation is mainly determined by the amount of energy exported through the milk and is not necessarily reflected in an elevation of the total metabolic rate [72]. Further, the simultaneous increase of heart rate with T_{sc} found in lactating females only (figure 2*b*) may indicate that lactating females are constrained in dissipating surplus heat and thus are more susceptible to heat stress in summer [17]. Altogether, the relatively small increase of heart rate associated with lactation suggests that females may compensate for the additional cost of lactation by downregulating other metabolic processes such as ‘background’ metabolic rate [72,73] or replenishment of fat reserves [74]. In addition, lactating females were more active than non-lactating females in summer, suggesting higher foraging activity in response to elevated energy demands. However, this behavioural response is apparently not able to compensate fully for lactational costs as females that do not raise a calf have been found to be heavier, i.e. fatter, in autumn than those that reproduced successfully [8,74].

Although an increase in heart rate with increasing activity levels occurred in both seasons, in line with previous studies in other *Rangifer* subspecies [75], the relative increase in mean heart rate from resting to active was much greater in winter than in summer (29% versus 10%). Walking through snow and cratering in snow are both energetically costly activities and likely contribute to relatively greater increases in heart rate during activity in winter compared to summer [76,77]. This effect was even greater in heavier females, suggesting that the cost of locomotion increases disproportionally with body mass and the intensity of activity [78]. Also, the reduced time spent active in winter is indicative of behavioural compensation to reduce energy deficits during periods of low food supply [79]. In summer, a higher proportion of time spent foraging [42] is likely to lead to a greater degree of rumen filling and, in turn, precipitate increased energy uptake and necessary increase in blood supply to the rumen [80] contributing to increased heart rates, even when resting.

Overall, the strong seasonal pattern in heart rate contributes to the increasing evidence that seasonal animals upregulate energy expenditure in periods of high supply and downregulate it when food is scarce [22–24]. The relatively small elevations in heart rate in lactating females could indicate that breeding female reindeer are close to their upper limits of sustained metabolic rate in summer. Furthermore, the low proportion of variance explained in winter heart rate may indicate that Svalbard reindeer operate close to their lower limits of metabolic rate, a limit that may also be dictated by the cost of maintaining high core body temperature to maintain the rumen biota [80], and that is reflected in the high mortality observed in winters with severely restricted food access [81]. While enhanced insulation in winter counteracts thermoregulatory challenges in endotherms exposed to low ambient temperatures [13], a negative relationship between ambient temperature and heart rate within seasons suggests that thermoregulatory responses to low temperature are still present even in highly seasonal animals, albeit at a much smaller scale compared to the seasonal effect [5,39,41]. This could indicate that intraseasonal and interseasonal responses to environmental variation can differ within a species [42]. While the seasonal plasticity in energetics makes Svalbard reindeer well-adapted to their highly seasonal environment, intraseasonal constraints on yet further upregulation or downregulation of heart rate may limit their ability to respond to severe environmental change [12].

5. Conclusion

Here, we have highlighted the intraseasonal responses in heart rate to short-term environmental and physiological changes in a high-Arctic ungulate. We find that energy-saving mechanisms such as peripheral heterothermy depend on body condition, age and reproductive state. Overall, a strong seasonal pattern overshadowed relatively small intraseasonal responses in heart rate, emphasizing the importance of evaluating individual state and seasonal context when studying energetics in free-living animals [79].

Ethics. The animal handling procedure and surgery protocols were approved by the Norwegian Food Safety Authority (17/237024) and the Governor of Svalbard (16/01632-9).

Data accessibility. Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.mcvdnck0f [82].

Authors' contributions. L.E.L., A.E. and S.A. conceived the idea and planned the study. A.E., A.S., E.K., E.R., G.P., L.E.L., L.M.T., R.J.I., S.A., V.V. and W.A. participated in the field campaign and collected the data; A.E. and E.R. performed the surgery. L.M.T. and G.P. analysed the data. L.M.T. drafted the manuscript. All authors contributed to editing and approved the final version.

Competing interests. We declare we have no competing interests.

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Determinants of heart rate in Svalbard reindeer reveal mechanisms of seasonal energy management

Electronic Supplementary Material (ESM)

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Appendix 1: Additional methods

1.a Study system

The study area includes Colesdalen and Northern Reindalen with adjacent side valleys on the Nordenskiöld peninsula (Figure S1) on the western part of Spitsbergen, Svalbard. This part of Svalbard receives mild ocean currents from the south and sea ice forms relatively late in the year compared to the east coast of Svalbard [1]. The area is characterized by wide, U-shaped valleys which are mostly vegetated up to about 250 meters above sea level. Ridge communities on the upper part of the hillsides are characterized by the presence of the dwarf shrubs *Dryas octopetala* and *Salix polaris*, and on the shallow slopes graminoid vegetation can be found in patches [1]. Heathlands, together with moist moss vegetation and wetlands, are typical characteristics of the lower-lying parts of the valleys [1]. Although live vascular plant biomass in vegetated habitats averages 35 g m^{-2} (annual range 23–46 g m^{-2} [2]), the area supports a high density of reindeer compared to other parts of Svalbard [3]. In summer, reindeer forage mostly in lower-lying, wetter and more productive pastures. In winter, snow and ice normally limit the access to these areas, and reindeer tend to feed on wind-blown, vegetated ridges, where the quality and energy content of forage is poorer than in summer [1,4].

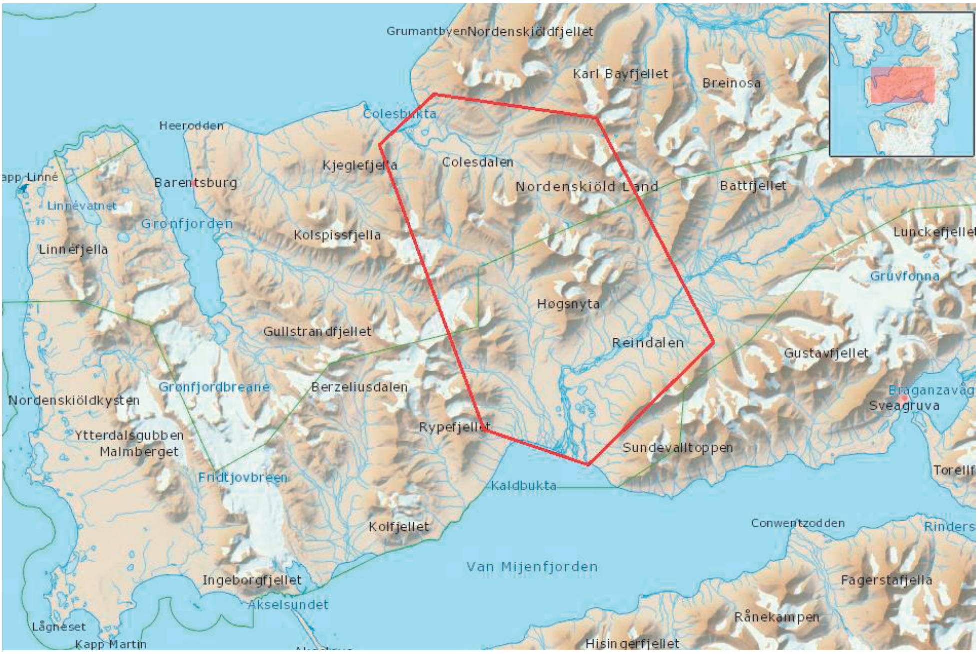

Figure S1. Map of the study area (red lines) on Nordenskiöld Land. The inset shows Nordenskiöld Lands position on Spitsbergen, the largest island on the Svalbard archipelago. ©Norwegian polar institute (https://toposvalbard.npolar.no/).

1.b Surgical procedure for biologger deployment and retrieval

All implants were sterilized with ethylene oxide gas (Anaprolene AN74i 60 L, Andersen Europe, Kortrijk, Belgium). Prior to biologger implantation, the animals were sedated with intranasal medetomidine (Domitor vet, Orion Pharma Animal Health, Finland; dose ~0.14 mg kg^{-1} body mass, BM) or dexmedetomidine (Dexdomitor vet, Orion Pharma Animal Health, Finland, dose ~0.07 mg kg^{-1} BM). For local anaesthesia, we used 2.5–5 mg of Bupivacaine (Marcaine 5 mg mL^{-1}, AstraZeneca, Cambridge, UK). Post-operative analgesia (0.5 mg kg^{-1} meloxicam; Metacam®, Boehringer Ingelheim Vetmedica GmBH, Germany) was administered subcutaneously prior to surgery. Surgical anaesthesia was assessed by checking the eyelid reflexes, limb movements, pulse and breathing rate. The surgical area was shaved and cleaned with chlorhexidine spirit. The logger was inserted into the subcutaneous space through a ~1 cm incision, which was then closed with 2-0 monofilament absorbable suture PDS® II (polydioxanone) (Ethicon, Johnson & Johnson, New Brunswick, NJ, United States). After surgery was completed, anaesthesia was reversed with intramuscular atipamezole (Antisedan® 5 mg mL^{-1}, Orion Pharma Animal Health, Turku, Finland; 5 mg mg^{-1} medetomidine or 10 mg mg^{-1} dexmedetomidine). Once animals regained consciousness and coordination, they were released and monitored until walking/running with normal balance (typically ~5 min post-injection of the antidote). Normal balance was considered as walking/running in straight lines and not stumbling when standing upright or walking.

When retrieving the loggers in 2019, animals were manually restrained and local anaesthesia was used (2.5–5 mg of Bupivacaine; Marcaine 5 mg mL^{-1}, AstraZeneca, Cambridge, UK) together with post-operative analgesia (0.5 mg kg^{-1} meloxicam; Metacam®, Boehringer Ingelheim Vetmedica GmBH, Germany). A small incision (~0.5 cm) was made at the top of the logger through which the logger was pushed out. The incision was left to heal without suture given its small size. The procedure took ~5 min.

1.c Validation and filtering of heart rate data

The DST centi-HRT (Star-Oddi, Gardabaer, Iceland; ~19 g) is a programmable heart rate- and temperature logger [5]. It is a leadless cylindrical device with a ceramic housing (Alumina) with the dimensions (diameter x length): 15 mm x 46 mm. Heart rate was automatically calculated from a 4-sec electrocardiogram (ECG) at 150 Hz measurement frequency and stored alongside a quality index of signal clarity in a non-volatile memory. Each logger can store up to 233,017 measurements per sensor, however several loggers experienced battery failure before maximum capacity was reached (noted in methods in main manuscript). We also programmed the loggers to save a raw ECG signal every 6 hrs alongside the calculated heart rate and quality index. This allowed us to manually validate the accuracy of the internal algorithms. To do so, we plotted the 4-sec ECG signal with a grid background divided into small squares (x), and counting the number of squares between two R waves (Figure S2). We calculated the number of beats per minute from this value (x) using the formula: $HR = \frac{60}{\left(\frac{x \times 4}{15}\right)}$ to derive the value in beats per minute. We calculated the percent deviation of the validated and the calculated heart rate, with the assumption that the validated heart rate was always correct. We considered the reading to be successful if the difference between the two values was less than 10%. From the validations, we asserted that only recordings with quality level 0 were reliable, with a success rate of 94% versus 67%, 31% and 9% for the quality levels 1, 2 and 3, respectively., we found that the reliability of the calculated heart rate decreased substantially at values above 175 bpm, even at quality level 0 (Figure S3), typically caused by “double counting”, where the R and T waves of the same ECG complex are counted as two separate complexes (Figure S4). In future studies, this can be avoided by increasing the minimum time gap between two counts (typically while programming the logger). We therefore filtered heart rate at 175 and 20 bpm. Removing these values in (and retaining only quality level 0) made little change to the results; while the range of heart rate records was reduced from 16–429 to 20–173 in the winter data and from 20–347 to 20–173 in the summer data, mean values changed only slightly: 39.2 ± 23.4 (pre-filtering) vs 37.2 ± 12.8 (post-filtering) in winter and 100.5 ± 21.3 (pre-filtering) vs 99.7 ± 12.1 (post-filtering) in summer. Furthermore, when comparing filtered validated heart rate and the recorded heart rate in the logger, 94% of the recordings deviated by less than ±5% from the validated heart rate and 98.5% less than ±10% from the validated heart rate. The percentage difference was normally distributed with a mean and standard deviation of 2.5 ± 6.1 %, and a median of 0.1% (figure S5). This potential error may contribute to a minor increase in unexplained variation in our results but is not likely to cause any bias in parameter estimates.

Figure S2. Example of a good quality (quality level 0) ECG signal with correct estimation by the internal algorithm (50 bpm) and the manual validation (4.5 red squares between two QRS complexes = 50 bpm).

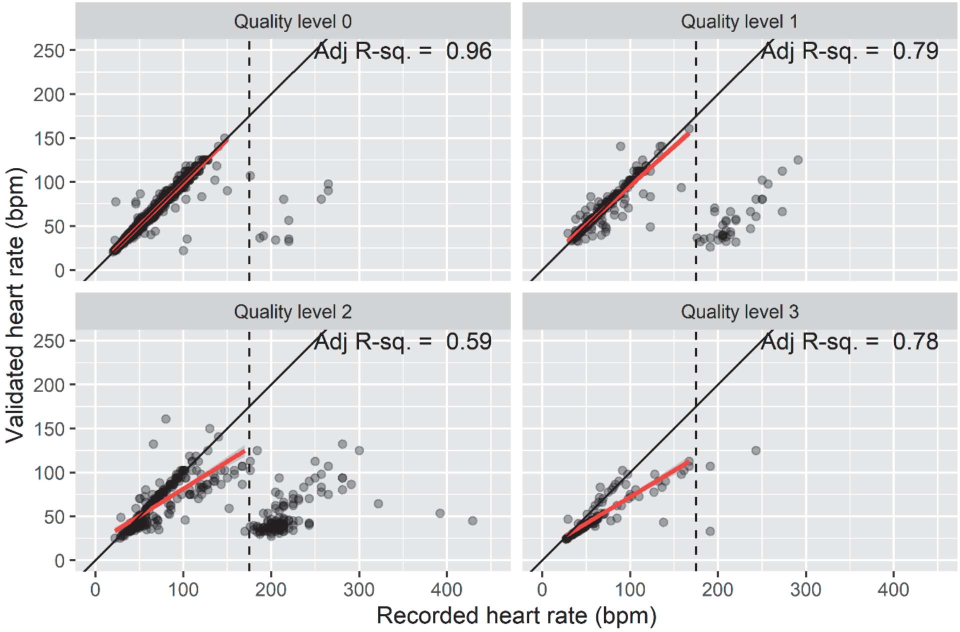

Figure S3. Correlation between recorded heart rate by the DST Centi-HRT loggers (x-axis) and manually validated heart rates from raw ECG signals (y-axis), grouped by quality level. In all quality levels, the difference between recorded and validated heart rate increased drastically for recorded values above 175 bpm (marked with a dashed line). The red lines represent the true correlation between the recorded and validated heart rates, with adjusted R-squared values presented in the panels for each quality level.

Figure S4. Example of an ECG signal (quality level 2) with incorrect estimation by the internal algorithm (225 bpm) and the manual validation (6.6 red squares between two R-waves = 34 bpm), typically resulting from double counts of one ECG cycle.

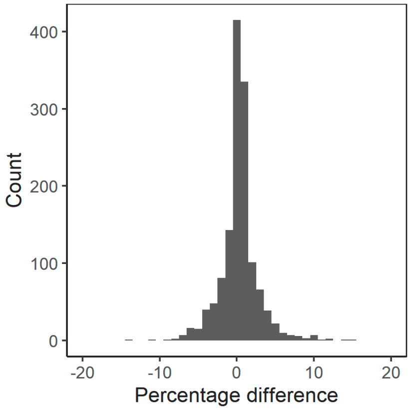

Figure S5. Distribution of the percentage difference between heart rates validated from an ECG signal and heart rates recorded by the logger, after filtering for quality level 0 and minimum and maximum values (>20 and <175 bpm).

1.d Activity data collection and processing

Most of the activity loggers were aligned with round 5-min intervals (16:05, 16:10, etc), but a few were not (e.g. recording at 16:03, 16:08). In the latter cases, the timestamp was rounded to the nearest 5-min timestamp. There was a strong correlation between activity recorded in each axis (X – forward/backward, Y – left/right; Figure S6). In both July and January, the total activity (X+Y) was bimodally distributed with a high frequency of low values (< 50) and a normal distribution of values between 50 and 510 (Figure S7). Acceleration data has not been validated with behavioural observations in Svalbard reindeer, so we could not distinguish activity types (walking, grazing, etc) except resting/stationary (hereafter "resting") and moving (hereafter "active"). We used the threshold of 50 (X + Y) to determine whether animals were resting/stationary based on observations of wild Norwegian reindeer (*R. t. tarandus*), where values below 25 in each axis (X and Y separately) were associated with "resting" and "standing still", whereas any form of non-stationary activity was characterized by higher values above 25 in either direction [7]. In Svalbard reindeer, the activity category "standing" only comprises 0.6% and 6.2% of Svalbard reindeer's time budget during summer and winter, respectively [8]

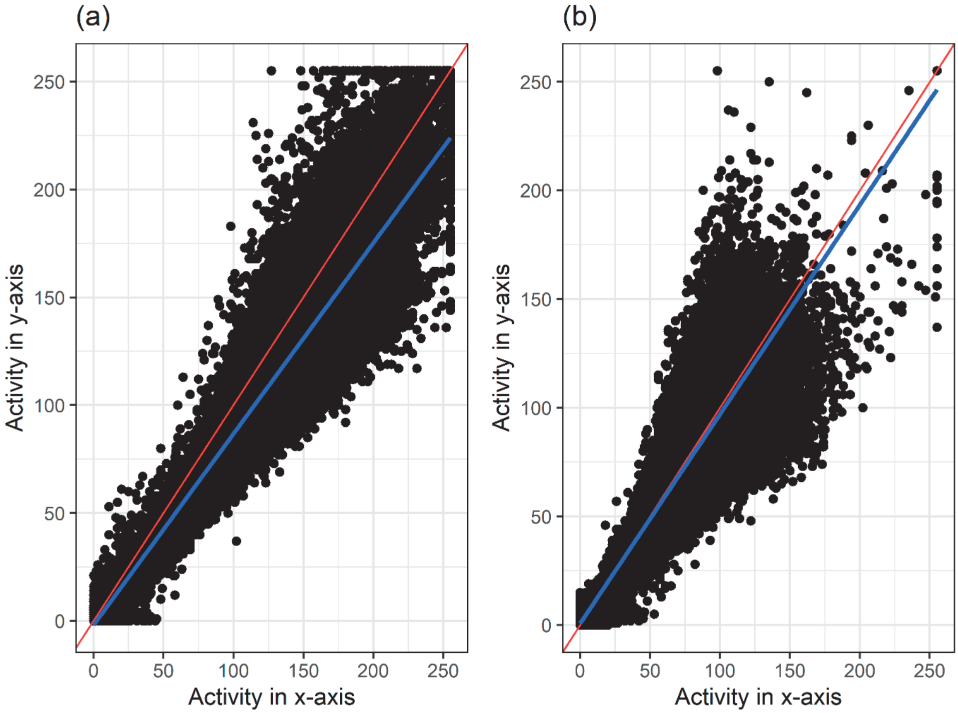

Figure S6. Relationship between activity recordings in the x-axis (back-forward movement) and the y-axis (right-left movement) (a) in summer and (b) winter. The red lines indicate a slope of 1 and intercept of 0, while the blue lines indicate the actual slopes for each season.

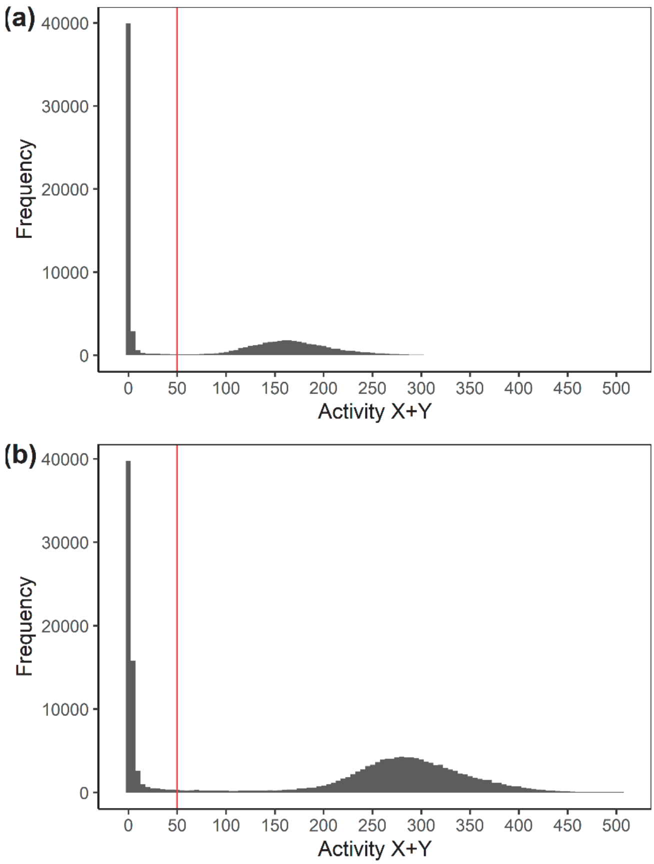

Figure S7. Distribution of all activity recordings (as the sum of activty in X- and Y-axes collected every 5 min) in (a) winter and (b) summer. The vertical red line indicates the treshold (value of 50) that was used to separate "resting" and "active" states.

1.e Temperature data collection and comparisons

The black bulb thermistor was constructed using a copper bulb of 15 cm in diameter painted black (figure S8a). Placed in the center of the bulb was a single Thermocron iButton temperature sensor (model no. DS1922L; iButtonLink, Whitewater, Wisconsin, US) which has a temperature range of –40°C to +85°C with a software corrected accuracy of ± 0.5°C in the range of –10°C to +65°C. The sensor was programmed to record temperature every 4 hrs and was situated centrally in Colesdalen (Figure S1, Figure S8b).

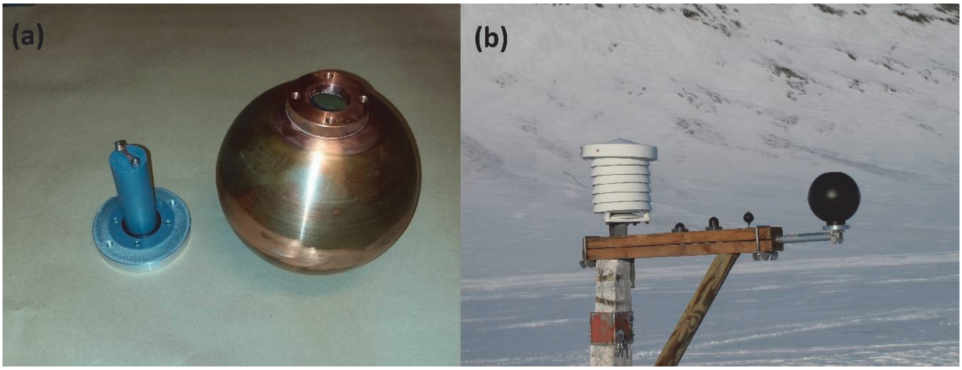

Figure S8. Construction and placement of black bulb thermistor in the study site. (a) Copper globe prior to painting. (b) Black bulb mounted on-site (right). The white structure (left) is a standard air temperature device, which together with the smaller globes (middle) are part of a separate study.

Appendix 2: Additional results

2.a Individual records

Table S1. Information about individuals used in the models of heart rate in summer (July 2018) and winter (January 2019), together with body mass (BM, kg) individual mean ± standard deviation of heart rate (in beats per minute) and subcutaneous body temperature (T_{sc}, °C). '–' means that the data was not available due to logger failure or mortality events, and the individual was not used in the analyses for winter heart rate. For both sets of data, body masses were recorded in March/April 2018 and April 2019. For calf and pregnancy statuses, '0' means not lactating or not pregnant, while '1' means lactating or pregnant, depending on season.

ID	**Year born**	**Summer data (2018)**				**Winter data (2019)**			
		BM	**Calf**	**Heart rate**	**T_{sc}**	**BM**	**Pregnancy**	**Heart rate**	**T_{sc}**
G118	2011	51.8	0	92.8±10.9	36.8±0.5	57.8	1	–	–
G140	2011	53.5	1[a]	94.6±10.3	36.2±1.1	61.3	1	36.1±12.5	33.8±2.2
G141	2011	49.5	0	89.3±9.5	36.6±0.9	52.3	1	–	–
G152	2011	47.3	1[a]	101.5±9.4	35.8±1.2	46.3	1	38.8±10.8	33.9±1.9
R289	2011	46.3	1	96.8±11.9	35.9±1.4	46.8	1	33.0±10.4	32.5±1.3
R290	2011	48.8	1	105.2±9.5	36.4±1.0	42.8	0	37.5±14.3	36.6±0.7
R297[b]	2012	47.3	0	103.8±10.0	36.0±0.9	–	–	–	–
R310	2012	50.3	0	101.7±10.3	36.3±1.1	48.3	1	–	–
R312	2012	56.3	0	99.6±13.1	36.7±0.9	55.8	1	–	–
R320	2012	46.3	1	103.7±14.7	36.8±0.8	44.8	1	40.7±20.8	35.3±1.3
W125	2010	60.0	1[a]	96.3±12.1	36.4±1.3	54.3	1	–	–
W127	2010	50.3	1	96.4±8.9	36.7±0.8	45.3	0	–	–
Y134	2013	58.8	1[a]	104.6±10.6	36.5±1.2	45.3	0	35.9±16.7	36.2±0.9
Y136	2013	45.3	1	105.0±8.4	35.5±1.9	54.3	1	37.7±7.9	31.6±4.1
Y137	2013	45.3	0	98.0±11.9	35.5±1.3	56.3	1	38.5±14.2	34.8±1.0
Y159	2013	49.3	1[a]	94.8±12.1	36.5±1.3	55.8	1	–	–
Y167	2013	52.8	1[a]	108.2±11.8	36.8±1.1	49.3	1	–	–
Y175	2013	57.0	1[a]	112.9±8.7	35.8±1.2	43.8	1	35.3±7.5	35.8±1.0
Y205	2013	53.1	1[a]	106.7±9.8	36.2±1.1	50.8	1	–	–

[a] Calf at heel inferred from pregnancy status in April and activity pattern in early July. [b] Died in March 2019, prior to scheduled body mass and pregnancy status assessment.

Table S2. Heart rate (beats per minute; bpm), subcutaneous body temperature (T_{sc}), percentage activity and environmental temperature (T_e) recorded in winter (January, N = 9) and in summer (July, N = 19). First presented are means ± standard deviation (SD) across all individuals, second for reproductive females only (lactating in summer/pregnant in winter), third for non-reproductive females only (not lactating in summer/not pregnant in winter) and finally the range (min, max) of individual means. Percent of time spent in activity was calculated as the proportion of all 5-min activity records classified as "resting" and those classified as "active". Since activity % is based on a binomial distribution (0, 1), SD was not calculated.

	Mean ± SD			**Range individual means**
	All individuals	**Reproductive**	**Non-reproductive**	
Heart rate (bpm)				
Winter				
Resting	33.7 ± 6.5	34.1 ± 5.6	32.6 ± 8.9	31.7, 35.3
Active	44.0 ± 18.4	43.8 ± 17.5	44.4 ± 20.8	36.7, 50.2
Summer				
Resting	93.0 ± 10.7	94.5 ± 10.8	90.6 ± 11.7	83.5, 108.3
Active	102.8 ± 11.3	104.0 ± 11.1	99.7 ± 11.7	91.8, 114.9
T_{sc} (°C)				
Winter				
Resting	34.1 ± 2.9	33.2 ± 3.0	36.4 ± 0.9	30.5, 36.7
Active	35.5 ± 1.2	35.1 ± 1.3	36.5 ± 0.4	34.3, 36.6
Summer				
Resting	36.2 ± 1.7	36.2 ± 1.9	36.4 ± 1.2	34.8, 37.5
Active	36.2 ± 1.0	36.2 ± 1.0	36.3 ± 1.0	35.1, 37.1
Activity %				
Winter	44	45	41	19, 49
Summer	65	67	61	42, 81
T_e (°C)				
Winter	−11.8 ± 6.5			
Summer	8.9 ± 3.7			

2.b Model selection results

Table S3. Model selection results using linear mixed-effects model with resting summer heart rate as the response variable, individual as random effect and an AR1 structure to account for within-individual temporal autocorrelation. Presented is the model structure, the likelihood ratio value (L. ratio), change in degrees of freedom (ΔDF) between original and reduced models, as well as the P-value indicating the significance level of the given change in model fit. The top model (in bold) is the one presented in the main results. Here, and in the following tables S4-S6, the abbreviated parameters refer to: t – time (in days), BM – body mass, ID- individuals, RS – reproductive status (lactation in summer, pregnancy in winter), T_e – environmental temperature and T_{sc} – subcutaneous body temperature.

#	Model parameters	L. ratio	ΔDF	P
4.	**$t + t^2 + Age + RS + T_e + T_{sc} + Age*T_{sc} + RS*T_{sc} + (1 \vert ID)$**	1.739	1	0.872
3.	$t + t^2 + Age + BM + RS + T_e + T_{sc} + Age*T_{sc} + RS*T_{sc} + (1 \vert ID)$	0.880	1	0.348
2.	$t + t^2 + Age + BM + RS + T_e + T_{sc} + Age*T_{sc} + RS*T_{sc} + T_e*T_{sc} + (1 \vert ID)$	0.117	1	0.732
1.	$t + t^2 + Age + BM + RS + T_e + T_{sc} + Age*T_{sc} + BM*T_{sc} + RS*T_{sc} + T_e*T_{sc} + (1 \vert ID)$			

Table S4. Model selection results using linear mixed-effects model with summer heart rate during activity as the response variable, individual as random effect and an AR1 structure to account for within-individual temporal autocorrelation. Presented is the model structure, the likelihood ratio value (L. ratio), change in degrees of freedom (ΔDF) between original and reduced models, as well as the P-value indicating the significance level of the given change in model fit. The top model (in bold) is the one presented in the main results.

#	Model parameters	L. ratio	ΔDF	P
4.	**$t + t^2 + Act + Age + BM + RS + T_e + T_{sc} + Act*T_{sc} + Act*BM + Age*T_{sc} + RS*T_{sc} + (1 \vert ID)$**	3.440	1	0.064
3.	$t + t^2 + Act + Age + BM + RS + T_e + T_{sc} + Act*T_a + Act*T_{sc} + Act*BM + Age*T_{sc} + RS*T_{sc} + (1 \vert ID)$	2.343	1	0.126
2.	$t + t^2 + Act + Age + BM + RS + T_e + T_{sc} + Act*T_e + Act*T_{sc} + Act*BM + Age*T_{sc} + BM*T_{sc} + RS*T_{sc} + (1 \vert ID)$	0.149	1	0.699
1.	$t + t^2 + Act + Age + BM + RS + T_e + T_{sc} + Act*T_a + Act*T_{sc} + Act*BM + Age*T_{sc} + BM*T_{sc} + RS*T_{sc} + T_e*T_{sc} + (1 \vert ID)$			

Table S5. Model selection results using linear mixed-effects models with resting winter heart rate as the response variable, individual as random effect and an AR1 structure to account for within-individual temporal autocorrelation. Presented is the model structure, the likelihood ratio value (L. ratio), change in degrees of freedom (ΔDF) between original and reduced models, as well as the P-value indicating the significance level of the given change in model fit. The top model (in bold) is the one presented in the main results.

#	Model parameters	L. ratio	ΔDF	P
5.	**$t + Age + RS + T_e + T_{sc} + Age*T_{sc} + (1\|ID)$**	0.031	1	0.860
4.	$t + Age + BM + RS + T_e + T_{sc} + Age*T_{sc} + (1\|ID)$	2.652	1	0.103
3.	$t + Age + BM + RS + T_e + T_{sc} + Age*T_{sc} + T_e*T_{sc} + (1\|ID)$	0.444	1	0.505
2.	$t + Age + BM + RS + T_e + T_{sc} + Age*T_{sc} + BM*T_{sc} + T_e*T_{sc} + (1\|ID)$	0.005	1	0.944
1.	$t + Age + BM + RS + T_e + T_{sc} + Age*T_{sc} + BM*T_{sc} + RS*T_{sc} + T_e*T_{sc} + (1\|ID)$			

Table S6. Model selection results using linear mixed-effects models with winter heart rate during activity as the response variable, individual as random effect and an AR1 structure to account for within-individual temporal autocorrelation. Presented is the model structure, the likelihood ratio value (L. ratio), change in degrees of freedom (ΔDF) between original and reduced models, as well as the P-value indicating the significance level of the given change in model fit. The top model (in bold) is the one presented in the main results.

#	Model parameters	L. ratio	ΔDF	P
3.	**$t + Act + Age + BM + RS + T_e + T_{sc} + Act*T_{sc} + Act*BM + Age*T_{sc} + BM*T_{sc} + RS*T_{sc} + (1\|ID)$**	1.439	1	0.230
2.	$t + Act + Age + BM + RS + T_e + T_{sc} + Act*T_{sc} + Act*BM + Age*T_{sc} + BM*T_{sc} + RS*T_{sc} + T_e*T_{sc} + (1\|ID)$	0.573	1	0.230
1.	$t + Act + Age + BM + RS + T_e + T_{sc} + Act*T_e + Act*T_{sc} + Act*BM + Age*T_{sc} + BM*T_{sc} + RS*T_{sc} + T_e*T_{sc} + (1\|ID)$			

2.c Additional results (figures)

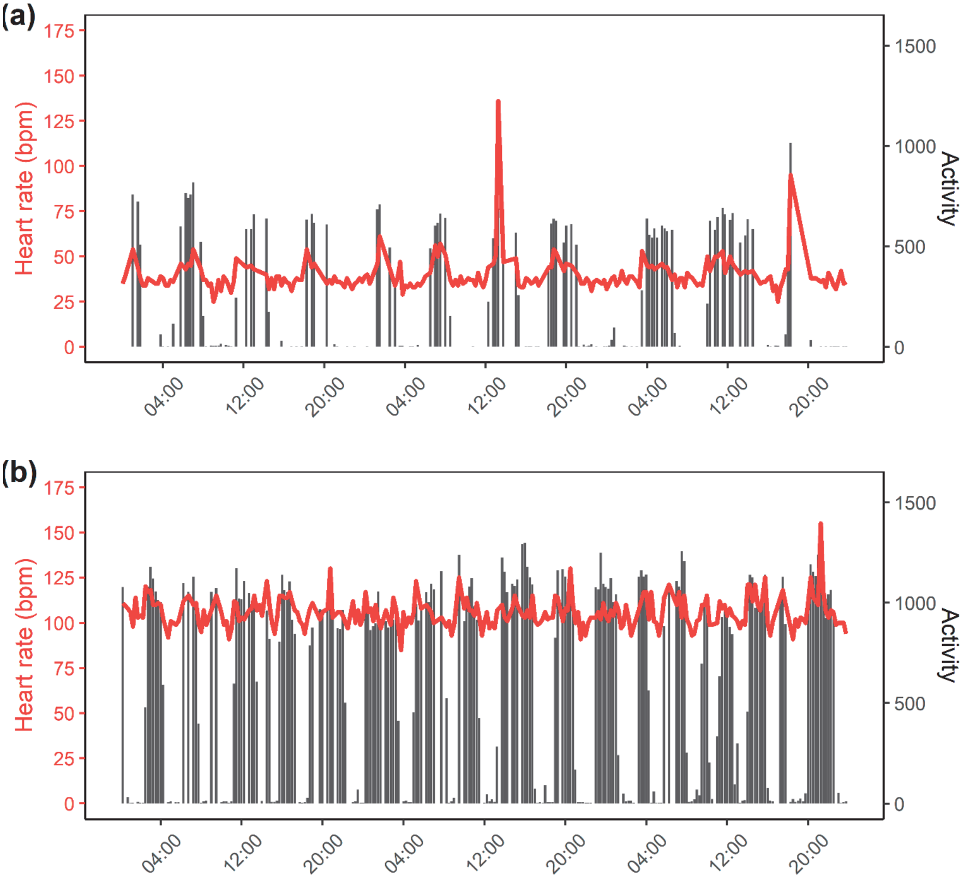

Figure S9. 15-min recordings of heart rate (left y-axis) and activity (right y-axis, ranging from 0 to 1530, where 0 represents no activity and 1530 maximum activity) spanning over 72 hours in a Svalbard reindeer female, in **(a)** January (11th – 13th) and **(b)** July (11th – 13th). The red lines show the heart rate (beats per minute, bpm) while the grey bars represent activity levels as a sum of acceleration in the X and Y directions every 15 minutes (details described in methods).

When generating predicted heart rates presented in figures and results, we expanded data sets starting at the lowest 1% to the upper 99% of the distribution of the predictor variable of interest, while other variables were fixed at mean values (this being 0 when scaled), except in interactions where the interacting variable was fixed at 3 categories, representing the 0.15, 0.5 and 0.85 quantiles of their distribution. Confidence intervals (95%) were generated by multiplying the standard error of the estimates by 1.96.

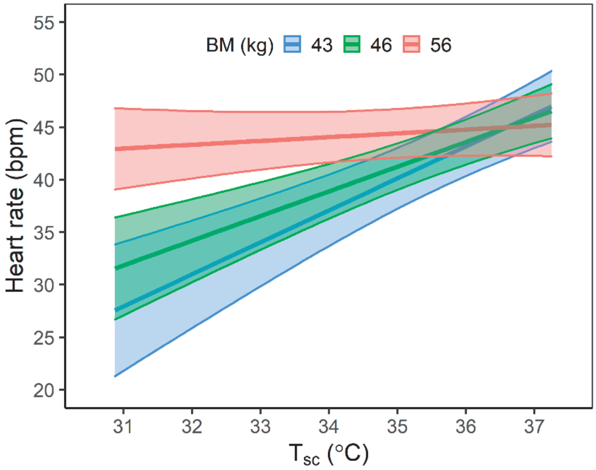

Figure S10. Predicted active heart rate (± 95% confidence intervals) of Svalbard reindeer females plotted against subcutaneous body temperature (T_{sc}, °C) during winter (N = 9, R^2 = 0.03; table 1) showing the interaction with April body mass (BM, kg). Body mass was fitted as a continuous variable, and the values here represent the 0.15, 0.5 and 0.85 quantiles from the distribution of body masses.

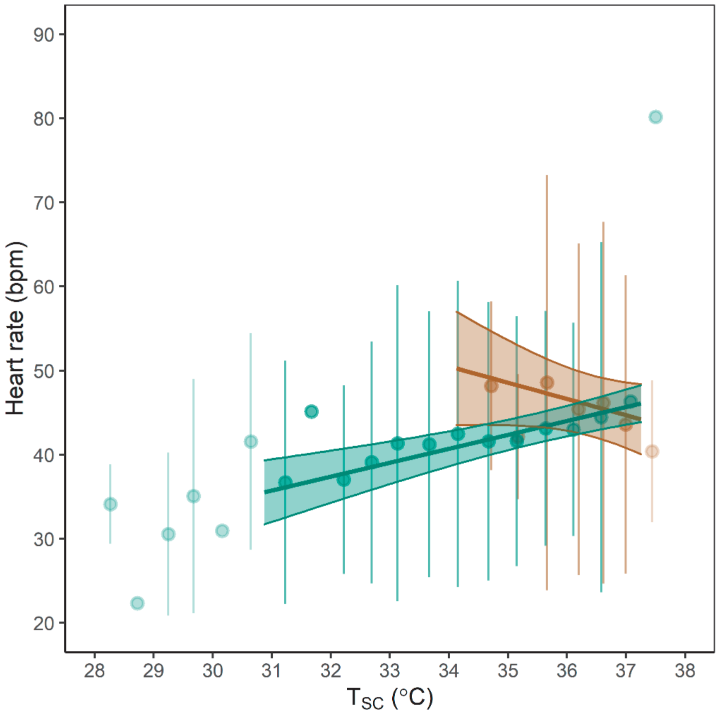

Figure S11. Predicted active heart rate (± 95% confidence intervals) of Svalbard reindeer females in winter, plotted against subcutaneous body temperature (T_{sc}, °C) in interaction with reproductive status (green = pregnant, brown = not pregnant). The solid lines represent predicted response in each activity state (see table 1 for details) and shaded areas represent 95% confidence intervals (CI) of the model predictions. Points and their error bars represent mean ± standard deviation of heart rate adjusted for the other model predictors (table 1). Points that fall outside the predicted range are values below the lower 0.01 or above the upper 0.99 quantiles of the T_{sc} distribution for each reproductive group.

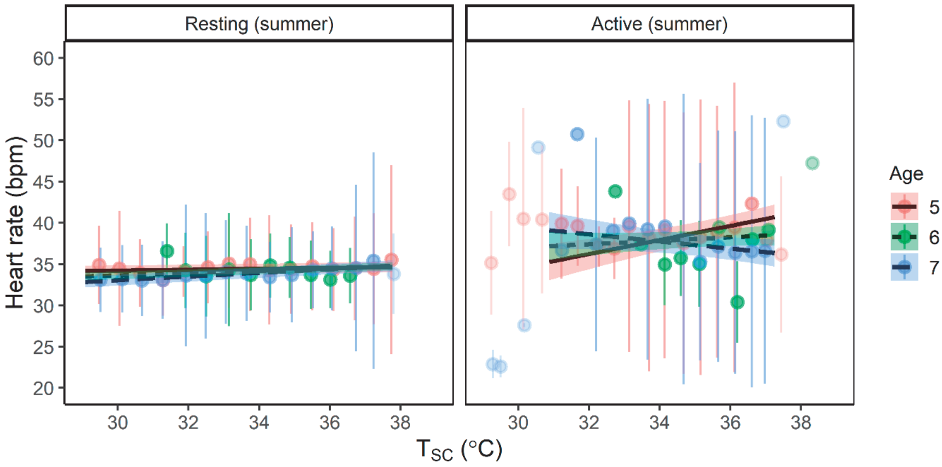

Figure S12. Predicted heart rate (± 95% confidence intervals) of Svalbard reindeer females winter, plotted against subcutaneous body temperature (T_{sc}, °C) in interaction with age (in years) during resting (left panel) and while active (right panel). Points and their error bars represent mean ± standard deviation adjusted for the other model variables (table 1). The lightly shaded points are values below the lower 0.01 or above the upper 0.99 quantiles of T_{sc}, i.e. representing less than 2% of the data.

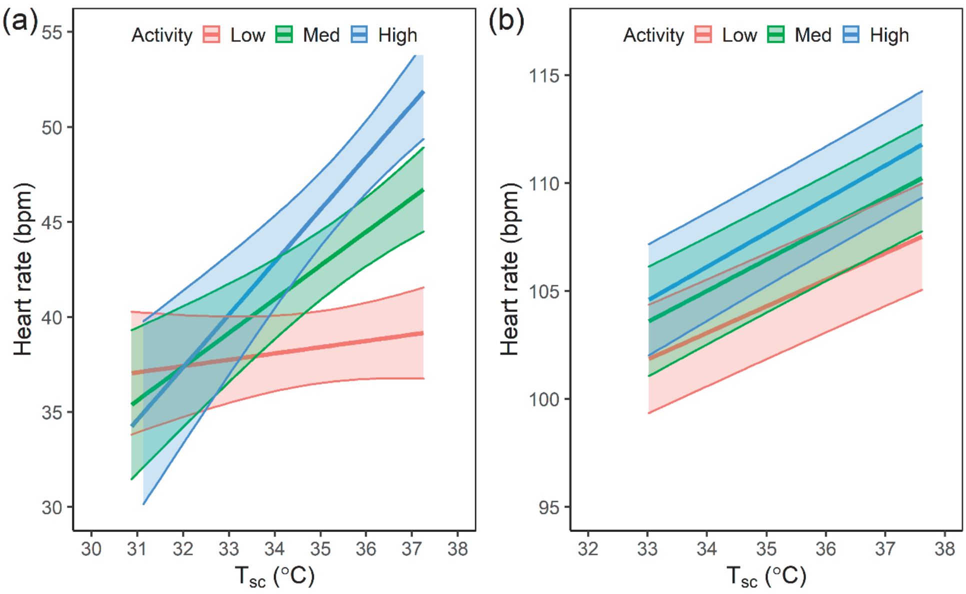

Figure S13. Predicted active heart rate (± 95% confidence intervals) of Svalbard reindeer females in winter, plotted against subcutaneous body temperature (T_{sc}, °C) in interaction with activity, grouped into “low”, “medium” and “high” intensity based on the 0.15, 0.5 and 0.85 quantiles of the distribution of activity in each season: (a) winter (N = 9), and (b) summer (N = 19).

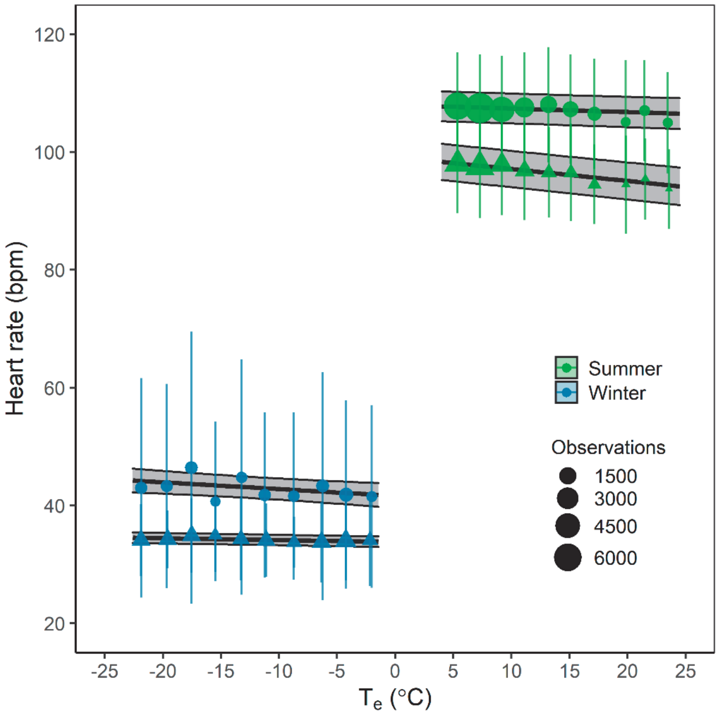

Figure S14. Predicted heart rate (± 95% confidence intervals) of Svalbard reindeer females, plotted against environmental temperature (T_e) (data binned with increments of 2.5°C) in summer (green) and winter (blue). The solid lines represent heart rate predicted from linear mixed-effects models in each season (table 1) and shaded areas represent 95% confidence intervals of the model predictions. Points and their error bars represent mean ± standard deviation adjusted for the other model variables (table 1). The size of each point represents the number of unique recordings per temperature increment.

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Paper III

Energetics of lactation in reindeer (*Rangifer tarandus*)

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ABSTRACT

The heat dissipation limit (HDL) theory predicts that reproductive output will be reduced if endotherm females are constrained in their capacity to dissipate excess body heat during reproduction. Lactation is the most energetically demanding period in the mammalian reproductive cycle. Here, we measured daily energy expenditure (DEE), milk production and milk energy output (MEO) during peak lactation, in lactating ($n = 8$) and non-lactating ($n = 6$) semi-domestic reindeer (*Rangifer tarandus*), using the doubly labelled water (DLW) technique. We combined these estimates of energy output with biologging data of heart rate (HR), subcutaneous temperature (T_{sc}), and activity to examine physiological and behavioural responses to a naturally occurring heatwave. During peak of lactation, DEE did not differ between the reproductive groups, but lactating females had on average ~50% greater total energy budget (TEB) than non-lactating individuals, due to energy exported as milk. Lactating females had higher resting HR, but neither activity levels nor T_{sc} differed significantly between the groups. Mean body mass of calves correlated with MEO of the mothers, but neither maternal body mass, which varied from 66 to 90 kg, nor their body mass loss, explained variation in MEO. During the heatwave, both groups were most active at effective ambient temperatures (T_{eff}) between 16 and 18°C, above which the activity levels declined. Resting HR and activity level declined with increasing T_{eff}, but to a greater extent in non-lactating than lactating females. T_{sc} increased with T_{eff} with no difference between groups. Lactating reindeer exhibit different responses to the heatwave than non-lactating females, indicating that lactating females may be under greater constraints during hot weather. Our results provide insight into energetics of reindeer during lactation, focusing on the physiological and behavioural responses to abnormally high ambient temperature.

List of abbreviations and units

BM	Body mass, kg
DEE	Daily energy expenditure, MJ day^{-1}
DLW	Doubly labelled water
GAMM	Generalised additive mixed-effects model
GE_m	Gross energy content of milk, MJ kg^{-1}
HDL	Heat dissipation limit
HR	Heart rate, beats per minute
MEO	Milk energy output, MJ day^{-1}
MP	Milk production, kg day^{-1}
ODBA	Overall dynamic body acceleration (*g*)
VeODBA	Vectorial overall dynamic body acceleration (*g*)
T_a	Ambient temperature, °C
TEB	Total energy budget, MJ day^{-1}
T_{eff}	Effective ambient temperature, °C
T_{sc}	Subcutaneous body temperature, °C

1. INTRODUCTION

Constraints on the maximum rates of sustained energy intake and metabolism may substantially limit reproductive performance (Hammond and Diamond 1997). The heat dissipation limit (HDL) theory posits that the maximum rates of sustained energy intake and metabolism, particularly during lactation, are constrained by the animals' ability to dissipate body heat, a by-product of metabolism (Speakman and Król 2010). The risk of hyperthermia associated with the excess of body heat may thus limit energy intake and lead to the reduced reproductive output via lower milk production and reduced offspring growth. The HDL theory has been supported by a multitude of experiments performed on laboratory mice and rats (reviewed in Speakman and Król 2010) and other small mammals (Valencak et al. 2010; Simons et al. 2011; Bao et al. 2020). In livestock, heat stress has been shown to lead to decreased reproduction rates (Dash et al. 2016) and milk production (Igono et al. 1992; Kadzere et al. 2002). Furthermore, experimental studies on wild birds have shown that the reduced capacity to dissipate heat may lead to reduced reproductive effort (Nilsson and Nord 2018), reduced offspring growth, and elevated parental body temperatures (Nord and Nilsson 2019). An animal's ability to dissipate body heat during crucial periods, such as reproduction, can therefore potentially alter reproduction rates in wild populations and livestock (Dash et al. 2016).

Lactation is considered the most energetically demanding part of reproductive cycle in female mammals (Gittleman and Thompson 1988; Speakman 2008). During this period, females need to allocate energy resources to both weaning the young and to their own maintenance and survival (Speakman 2008). In addition to the energy exported as milk, the process of milk synthesis releases thermal energy as heat, which constitutes the respiratory cost of milk production (Moe et al. 1971). Because of milk, both total energy budget (TEB, i.e. respiration and production) and often daily energy expenditure (DEE, i.e. respiration) are substantially higher in lactating females than in non-breeding females (Oftedal 1985; Mellish et al. 2000; Speakman 2008; Fletcher et al. 2012). In ungulates, the energetic costs of reproduction increase with gestational stage and peak during lactation (Oftedal 1985), a period that often coincides with mid-summer. This may suggest that lactating females are at a greater risk of developing hyperthermia during hot conditions as they have higher metabolic rates than non-lactating females. Some studies have shown that temperate ungulates respond to hot conditions via reductions in foraging activity (Ditmer et al. 2018), shifts in diurnal foraging patterns (Borowik et al. 2020; Semenzato et al. 2021) and increased use of thermal refugia (van

Beest et al. 2012; McCann et al. 2016; Williamsen et al. 2019; Brivio et al. 2019; Sarmento et al. 2019). Physiological responses to heat stress include vasodilation and increased heart rates (HR) to transfer blood to evaporative surfaces (Cain III et al. 2006; Lust et al. 2007), sweating and panting (Renecker and Hudson 1986), and lowering metabolism to reduce internal heat load (Silanikove 2000).

Reindeer (*Rangifer tarandus*) are large ungulates with circumpolar distribution in the Northern Hemisphere. They are the only cervids with a long history of domestication. Reindeer mate in autumn (September-October) and give birth to a single calf (twin births are rare) in late spring/early summer (May-June) (Paoli et al. 2018). Peak lactation occurs 3–5 weeks post-partum and lactation can last up to 26 weeks, but calves begin to feed on solid food when their rumen function develops at around one month of age (Gjøstein et al. 2004). Reindeer milk is known to be particularly energy-rich compared to other ruminants, but milk composition changes throughout the lactation period (Gjøstein et al. 2004). Reindeer typically utilise the remainder of their winter fat reserves for milk production in the early lactational stages and increase foraging intensity throughout the lactation period (Luick et al. 1974). Although milk composition, energy output (McEwan and Whitehead 1971; Luick et al. 1974; Rognmo et al. 1983; Holand et al. 2002; Gjøstein et al. 2004) and nutritional demands for reproduction (Barboza and Parker 2008; White et al. 2013; Denryter et al. 2020) are well studied in reindeer, we are not aware of any measurements of daily energy expenditure in lactating and non-lactating females simultaneously. Furthermore, no studies have combined the use of biologgers during peak lactation with the measurements of energy expenditure to identify the lactation-related activity and body temperature patterns.

In the summer of 2018, Europe experienced a record-breaking heatwave causing massive droughts and wildfires (WMO, 2018). Several areas above the Arctic circle (66°N), recorded the highest temperature anomalies since 1950. In July, the monthly average temperature in Northern Finland was on average 5.5°C higher than the climatological mean for this region, with anomalous high pressure and little precipitation (Sinclair et al. 2019). The Arctic is one of the regions where climate change is happening at the fastest rates, and heatwaves and droughts are becoming more frequent (IPCC 2019). Because reindeer are an important livestock in Arctic regions, understanding how they respond to and are affected by summer heatwaves can inform decisions on management and conservation practices.

Here, we quantified energetics, thermoregulatory physiology, and behaviour of female reindeer from a semi-domestic heard in Northern Finland, during peak lactation and during a subsequent heatwave. First, to quantify the contribution of lactation to overall energy expenditure, we measured daily energy expenditure (DEE) by the doubly labelled water (DLW) method (Speakman 1997) in lactating (n = 8) and non-lactating (n = 6) reindeer ~3–4 weeks post-partum. Due to the high metabolism and associated heat production during peak lactation, lactating females are expected to be more susceptible to hyperthermia than non-lactating females (Abdalla et al. 1993). If lactating females become limited in their ability to dissipate body heat, they are expected to reduce energy turnover, which can be manifested through lowering their DEE, and/or reducing the amount of energy exported as milk (Abdalla et al. 1993; Speakman and Król 2010). As larger females have smaller surface-to-volume ratio, their threshold for being heat stressed is expected be lower than that of smaller females. Second, we quantified lactation performance of mothers across a 35% body mass range (66–90 kg) by measuring milk production through the transfer of deuterium (^{2}H) from mother to calves (Haisma et al. 2003), and milk energy content by bomb calorimetry. We predict that larger mothers should produce more milk if not constrained by HDL. Third, we explored responses in the activity levels, HR and subcutaneous body temperature (T_{sc}) to local weather conditions during a heatwave lasting several weeks in July 2018. We predict that lactating females should display responses to a high environmental heat load at lower ambient temperatures that non-lactating females, via reductions in activity levels and/or increases in HR. Finally, we discuss our findings in the context of heat dissipation constraints in a warming climate scenario.

2. METHODS

2.1 Animals and experimental protocol

The experiment was performed on 19 semi-domestic reindeer (*Rangifer tarandus*) females at the Kutuharju Reindeer Research Facility (Kaamanen, Northern Finland, 69° 8' N, 26° 59' E), between February 2018 and February 2019. These females (Table S1) were part of a herd of ~100 animals, belonging to the Reindeer Herders' Association. The herding management included keeping reindeer in two large enclosures (~13.8 and ~15 km^2) after calving and until the rut, after which animals were moved to a winter enclosure (~15 km^2) and then in spring to a calving paddock (~0.3 km^2) to give birth. The surrounding area consists mainly of open birch (*Betula spp.)* and pine (*Pinus sylvestris*) forest with small lakes and wetlands (Paoli et al. 2020). All newborn calves were weighed, sexed, and marked with ear tags on the day of birth. The animals in the herd were weighed monthly between September and April each year (excluding summer months), with some variability in timing and capture rates between years.

In February 2018, all experimental females were instrumented with a heart rate (HR) and temperature logger (DST centi-HRT, Star-Oddi, Gardabaer, Iceland). Furthermore, the animals were fitted with collar-mounted tri-axial accelerometers (Vertex Plus Activity Sensor, Vectronic Aerospace GmbH, Berlin, Germany) to monitor their activity levels. Both types of biologgers were retrieved in February 2019.

Based on the body mass (BM) distribution in April 2018, 14 of the 19 females were allocated to the doubly labelled water (DLW) experiment performed between May 30 and June 15 (2018) on 8 lactating and 6 non-lactating animals, while 5 remaining females (all lactating) served as non-injected controls (Table S1). The non-lactating group consisted of 4 females with the calves removed on 27 May (2–22 days post-partum), and 2 barren females that did not produce young that year. The DLW technique was used to measure i) daily energy expenditure (DEE) of lactating females with simultaneous measurements of DEE in non-lactating females (based on the difference in the elimination rates between oxygen-18 and deuterium from female blood samples), and ii) milk production of lactating females (based on the deuterium transfer from mother to calf *via* milk using mother and calf blood samples). Both DEE and milk production were measured 3–4 weeks post-partum which coincides with peak lactation (Gjøstein et al. 2004). The ages of the calves on May 30 ranged from 18 to 22 days. The DEE measurements were coupled with the collection of milk samples to measure gross energy content of milk (GE$_m$, by bomb calorimetry) for evaluation of milk energy output (MEO, milk production × GE_m). The milk samples were also measured for the levels of deuterium enrichment to support the isotope-derived estimates of milk production.

The experimental reindeer were kept in the calving paddock in the period between calving (median calving date May 12) and the end of the first week of the DLW experiment (June 15), instead of being released to the larger summer enclosure 2–3 days post-partum. During handling, the reindeer were herded into a corral (~60 m^2), then individually (non-lactating females) or in mother-calf pairs guided into a smaller corral (~10 m^2) before walking into an indoor area customised for close handling of the adult animal. Calves were handled immediately after their mothers. Each reindeer first walked onto a floor scale to be weighed (± 0.5 kg), before being transferred onto a wall-mounted rack to be restrained during blood sampling (calves were restrained by hand). While restrained, animals were subjected to i) blood sampling from the left jugular vein (females and calves) and ii) milk sampling (lactating females).

2.2 Biologger implantation

The DST centi-HRT sensors (diameter × length: 15 mm × 46 mm, mass ~19 g) were sterilised with ethylene oxide gas (Anaprolene AN74i 60 L, Andersen 60 Europe, Kortrijk, Belgium) prior to implantation. The loggers were implanted subcutaneously on the left side of the animal chest (behind the elbow). During the implantation, animals were sedated with a mix of detomidine (0.039 mg kg^{-1} BM), ketamine (0.9 mg kg^{-1} BM) and butorphanol (0.051 mg kg^{-1} BM) or only detomidine (0.046 mg kg^{-1} BM) and ketamine (1.04 mg kg^{-1} BM), followed by administration of meloxicam (0.5 mg kg^{-1} BM) for post-operative analgesia. For local anaesthesia, we used 0.5–1 mL of bupivacaine (5 mg mL^{-1}). The protocol for inserting the loggers has been described elsewhere (Trondrud et al. 2021). Antisedatives were not used, and animals were kept under observation until they regained consciousness and could stand and walk on their own. Once conscious, animals were released into the calving paddock and monitored for 2 days before release into the larger winter enclosure. One year later, the loggers were retrieved using a similar procedure as described above. Only 14 out of the 19 implanted loggers were successfully retrieved. The remaining loggers were likely expelled from the animal's body and lost, as we detected scar tissue at the site where the logger was originally implanted. Furthermore, only 13 loggers had data recorded over summer 2018, 10 of which were from the DLW animals (5 lactating and 5 non-lactating), and 3 were from non-injected control animals (for details see Table S1). Ten of the 13 loggers had overlapping data with activity sensors (DLW = 8, non-injected = 2).

2.3 Heart rate (HR) and subcutaneous body temperature (T_{sc}) data

The DST centi-HRT sensors recorded HR and subcutaneous body temperature (T_{sc}) every 15 min. HR was automatically calculated from a 4-sec electrocardiogram (ECG) at a 150 Hz measurement frequency, coupled with a signal quality index from 0 to 3, where 0 was the highest quality. Additionally, a raw ECG signal was stored every 6 h alongside the HR and the signal quality index, which we used to manually validate the recorded HR following the method described in Trondrud et al. (2021). Briefly, the accuracy of the internal algorithm was assessed by the goodness-of-fit of the recorded HR to the manually validated HR, with a threshold R^2 of 0.9. This was greatly reduced at values > 175 bpm for all quality levels, and in general at quality levels 1–3. Therefore, we retained only HR < 175 bpm with the quality levels 0 for further analysis. The temperature sensors measured T_{sc} with an accuracy of 0.2°C, with the calibration performed by the manufacturer prior to implantation.

Data from the loggers were downloaded using Mercury software (StarOddi, Gardabaer, Iceland). At the time of data download, the software recorded the time in the internal clock alongside the time of the computer (in UTC). For the loggers still working until the time of download, the internal clock was on average 7 min 45 sec behind the computer time (range from 4 min 45 sec to 12 min 30 sec). For each logger, the offset in time was corrected assuming that the drift occurred linearly. For the loggers that stopped working before download, time drifts were unavailable and the average of 7 min and 45 sec was used.

2.4 Activity data

The tri-axial accelerometers recorded acceleration (*g*) in three directions representing back-forward, lateral, and dorsal-ventral movements at 8 Hz resolution. For each axis, partial dynamic body acceleration (PDBA) was calculated by subtracting the static acceleration using a 4 sec running average from the raw acceleration (Shepard et al. 2008). The sum of the absolute values of PDBA generated estimates of overall dynamic body acceleration (ODBA) (Gleiss et al. 2011). We also estimated vectorial ODBA (VeODBA) by calculating the square root of the sum of squared PDBAs (Wilson et al. 2020). While ODBA and VeODBA are similar and correlated, VeODBA can provide a better estimate than ODBA when device orientation and position vary with time and between individuals (Qasem et al. 2012). In our case, some collars were more loosely fit than others, resulting in shifts in the sensor's position that we could not account for. Because ODBA and VeODBA were highly correlated (cor = 0.99), we used only VeODBA as proxy for activity. The clock drift was assessed by recording the exact time (in sec) when a strong magnet was connected to the sensor following retrieval from the animals. For the sensors that worked until the magnet was connected (n = 10), the mean absolute offset was 5 min (range from −10 to +3 min). One sensor lagged by 2 hrs, another by 2 days, and two sensors stopped working several months prior to retrieval. The offset in time was corrected assuming a linear drift. For the loggers that stopped working, the average drift of 5 min was used. We aggregated the VeODBA data into 1-min averages due to limitations on computer processing capacity. Mean VeODBA showed a clear bimodal distribution (Figure S1). We classified the data into "inactive" and "active" bouts using a simple mixture model (function 'flexmod' in the flexmod package) that assigns each value to one of two categories based on the gaussian distributions for each category (Grün and Leisch 2008). We set the cluster thresholds for "activity" at 0.125 *g* based on the lowest point between the two peaks in the bimodal distribution (Figure S1). The maximum values in the inactive category resulting from the mixture model was 0.09 *g*. We verified these categories visually by exploring real-time plots and video observations of the animals. Specifically, the inactivity at 0.09 *g* included minor head movements and standing behaviour. We used these categories (inactive and active) to quantify the relative proportion of time spent in activity each day (daily activity levels) and the mean daily VeODBA over the DLW experiment.

2.5 Doubly label water (DLW) experiment

The DLW experiment was done by administering a dose of DLW to 8 lactating and 6 non-lactating females, and then measuring the elimination rates of oxygen-18 (^{18}O) and deuterium (^{2}H) from their body water pools over time, through regular tracing of the isotope concentrations in their blood samples. At the same time, we collected milk samples from the lactating females and blood samples from their calves to trace the transfer of ^{2}H from the mothers to young. The difference in the elimination rates between ^{18}O and ^{2}H was used to calculate DEE of lactating and non-lactating females (n = 14). The transfer of ^{2}H from the mother to her calf was used to calculate milk production of lactating females (n = 8).

The DLW experiment started when the calves of lactating females were 17–21 days old (counted from parturition on day 0) and lasted for 17 days (calves were 33–37 days old at the end of experiment). All 14 females and 8 calves were first blood sampled for background levels of ^{18}O and ^{2}H (method A in Speakman and Racey 1987) and then the females were injected with 0.31 ± 0.01 g of DLW (65 atom% ^{18}O, 35 atom% ^{2}H) per kg BM. All females and calves were sampled on day 1 post-injection to obtain an initial blood sample, following a 24 h period of isotope equilibration required for the isotopes to mix with the body water pools. Further sampling was conducted on days 2–6 (every day for lactating and every second day for non-lactating females), 13 and 16 post-injection to obtain multiple isotope samples. Milk samples for ^{2}H enrichment and gross milk energy content were collected on the same occasions (day of injection, and days 1–6, 13 and 16 post-injection), by manual milking without the use of oxytocin. During the first week of the DLW experiment, animals were kept in the calving paddock, after which they were released to the larger enclosure (~13.8 km^2).

Blood samples were collected in BD vacutainer tubes EDTA (K2) and kept for a maximum of 2 h at ~15°C before transfer to the 100 µL flame-sealed glass capillaries, which were then stored at room temperature prior to isotope analysis. Milk samples after collection were stored for a maximum of 2 h at ~5°C, after which they were subsampled to the 100 µL flame-sealed glass capillaries for the ^{2}H enrichment analysis. The remaining milk samples were stored at −20°C before analysis for gross energy content.

Samples of blood and milk were vacuum distilled into glass Pasteur pipettes (Nagy 1983). The resultant water was used for analysis of ^{18}O:^{16}O and ^{2}H:^{1}H (blood) and ^{2}H:^{1}H (milk) on a laser-based Isotopic Water Analyzer IWA-35-EP (Los Gatos Research, San Jose, CA, USA) as described previously (Berman et al. 2012). Each batch of samples was run alongside 5 lab and 3 international standards (SMOW, GISP and SLAP) to correct for day-to-day variability in the machine performance. All isotope enrichments were expressed in delta (δ) per mil (‰) relative to the working standards and converted to parts per million (ppm) using the established isotope ratios for the reference materials. Inter-sample memory was avoided by injecting water samples multiple times and ignoring the results from the first few injections (Lis et al. 2008). The measures of isotope enrichment were based on a minimum of 5 readings; all subsequent calculations were performed on the mean values.

2.6 Daily energy expenditure (DEE) by DLW technique

The applicability of DLW technique to measure DEE has been validated by indirect calorimetry in a wide range of mammals, providing an accurate measure of metabolism over periods of several days (Butler et al. 2004). In the current experiment, the blood samples collected on days 13 and 16 post-injection were too close to the background enrichments of ^{18}O and ^{2}H and therefore removed from the analysis (Table S1). As a result, DEE was estimated using the blood samples from the first week of the DLW experiment (days 2–6 post-injection). Initial dilution spaces for ^{18}O (N_o) and ^{2}H (N_d) were calculated by the intercept method (Coward and Prentice, 1985), then converted from moles to grams assuming a molecular mass of body water as 18.0153 g mol^{-1}, and finally expressed as a percentage of BM before initial blood sample. We used the intercept rather than the plateau method because estimates generated by the intercept method are typically more robust to the timing of the initial blood sample (Speakman and Król, 2005). Final dilution spaces were inferred from the BM on day 6, assuming the same percentage of BM as measured for the initial dilution spaces. The isotope elimination rates for k_o and k_d were calculated following Nagy (1983). The mean rate of CO_2 production (rCO_2 mol day^{-1}) was calculated using a two-pool model (equation A6 from (Schoeller et al. 1986), as recommended for large animals and humans (Speakman 1993).

2.7 Milk production by deuterium (^{2}H) transfer from mother to calf

We assumed that milk intake by calf equals milk production and calculated the milk intake using the dose-to-the mother deuterium-oxide turnover technique (Coward et al. 1982; Butte et al. 1988), with the source of ^{2}H being the DLW injectate. This technique is based on fitting the ^{2}H data (ppm) to i) a single exponential model for water turnover in the mother (representing the disappearance of ^{2}H), and ii) a multi-exponential model of water turnover in the young (representing the appearance of ^{2}H) (Haisma et al. 2003; International Atomic Energy Agency 2010). For the mother, the ^{2}H data from days 1–6 post-injection were fitted to

$$E_{m(t)} = E_{m(0)} e^{-K_{mm} t}$$

where $E_{m(t)}$ is isotopic enrichment above background at time t (ppm), $E_{m(0)}$ is the zero time isotope enrichment (ppm), t is time post-injection (day) and k_{mm} is water turnover in the mother (kg day^{-1}). For the calf, the ^{2}H data from days 1–6, 13 and 16 post-injection were fitted to

$$E_{c(t)} = E_{m(0)} \left(\frac{F_{cm}}{V_c}\right) \left(\frac{e^{-K_{mm} t} - e^{(F_{cc}/V_c)t}}{(F_{cc}/V_c) - K_{mm}}\right)$$

where $E_{c(t)}$ is isotopic enrichment above background at time t (ppm), F_{cm} is the transfer of water from the mother to the calf *via* milk (kg day^{-1}), V_c is the calf's total $^{2}H_2O$ distribution space (kg) and F_{cc} is total water loss in the calf (kg day^{-1}). Curve fitting was done using the 'Solver' function in Microsoft Excel to minimise the sum of the squares of the differences between observed and fitted values for the mother and calf data combined. Parameters fitted were $E_{m(0)}$, F_{cm}, k_{mm} and F_{cc}. V_c was assumed to change linearly with BM, as $V_c = 0.876 \times BM^{0.936}$ (Riek and Gerken 2009). Milk production (kg day^{-1}) was calculated from milk water intake as F_{cm}

(kg day^{-1}) × milk water content (%). The estimates of milk production were then used to calculate MEO (MJ day^{-1}) as milk production × GE_m (MJ day^{-1}). MEO was estimated for the whole two weeks (between injection and day 16 post-injection), as opposed to DEE which was only calculated for the first week (between injection and day 6 post-injection).

2.8 Milk water and energy content

Defrosted milk samples were weighed and then dried to a constant mass at 60°C for a minimum of 2 weeks, using a Gallenkamp Prime Oven (Weiss Gallenkamp, Loughborough, UK). The masses of fresh and dried milk samples were used to calculate milk water content (%). Determination of gross energy content of milk (MJ kg^{-1}) was performed on the dried milk pellets (~0.2 g each, weighted to the nearest 0.0001 g) using a 1109 Semi-micro Oxygen Bomb and 6200 Oxygen Bomb Calorimeter (Parr Instrument Company, USA). The calorimeter was calibrated daily with benzoic acid. Prior to bombing, the dried milk samples were pooled based on the collection time for each lactating female: pool A contained samples collected on day 0 (DLW injection) and days 1–3 post-injection, pool B samples from days 4–6 post-injection, and pool C samples from days 13 and 16 post-injection. The values from pools A–C for each female were averaged to match with the final estimates of the water transfer from mother to calf (F_{cm}).

2.9 Weather station data and definition of heatwave

Prior to the DLW experiment, we set up a HOBO weather station (Onset Computer Corporation, Bourne, MA, USA) mounted on a 2 m tall tripod. The placement of the station was 5 m east of the calving paddock where the animals stayed during the first week of handling. The weather station was equipped with sensors to detect wind direction (ø), wind speed (m s^{-1}), wind gust (m s^{-1}), solar radiation (W m^{-2}), air temperature (°C) and relative humidity (%). The data were recorded onto a HOBO Micro Station Data Logger (H21-002) every 15 min. The clock drift was not recorded, but the manufacturer reports a temperature-dependent clock drift of up to 21 sec per week at −20°C, so the worst-case scenario would result in a total shift by ~15 mins over the entire logging period (Onset Computer Corporation 2014). We therefore assumed a linear change in air temperature and solar radiation between the two consecutive readings (15 min apart) and calculated values for every minute, which were then matched to the VeODBA data as well as HR and T_{sc} recordings.

To define a heatwave, we compared the ambient temperature (T_a) recorded by the weather station with the 30-year average in T_a (1980–2010) recorded at the nearest weather station (~60 km, Ivalo Airport, 150 m.a.s.l, station ID 102033), provided by the Finnish Meteorological Institute (https://en.ilmatieteenlaitos.fi/). Based on the 30-year average, we selected a period which spanned from the end of the DLW experiment (June 15) to the end of August 2018, when daily T_a was greater than the 30-year average by minimum 5°C, until the last day when the 5°C anomaly was recorded. This resulted in a time period spanning from July 2 to August 1, 2018. Hereafter, this period is referred to as the "heatwave". Using the records of T_a, relative humidity and wind speed, we calculated the effective ambient temperature (T_{eff}) (Suping et al. 1992) with the function 'etv' in the '*ThermIndex*' package (Castelhano 2017).

2.10 Statistical analyses

Drivers of daily energy expenditure (DEE)

All statistical analyses were conducted in R version 4.0.5 (The R Core Team 2021). Values are presented as means ± standard deviation (s.d.) unless specified otherwise. We evaluated differences in BM, and BM loss between injection day, day 6 and day 16 post-injection, between lactating and non-lactating females, with Student's T-tests (for BM dynamics) and present test results as 95% confidence intervals (CI). Tests were considered significant if the CI did not overlap zero. We fitted linear regressions for each variable of interest against DEE. These variables were: mean BM between injection day and day 6 (hereafter 'mean BM'), difference in BM between injection and day 6, lactation status (1, lactating; 0, non-lactating), age (years), mean HR, mean daily amplitude HR (highest − lowest HR per day) in, mean T_{sc}, mean daily amplitude in T_{sc}, time spent active (%) and mean VeODBA. We also evaluated the impact of the same drivers on DEE while including mean BM as a covariate in each regression. Hereafter, this will be referred to as 'mass-adjusted DEE'. For lactating females, we investigated correlates of MEO using the same variables as that for DEE, as well as mean calf BM (between injection and day 16) and growth (daily change in BM between injection and day 16 post-injection). Regressions were considered significant if the CI of the estimates did not overlap zero. We also calculated the change in GE_m over time (days) across individuals using weighted mean values of GE_m for each pooled sample (described in 2.8) relative to lactation day. The weights were based on the relative contribution (in mass) of each daily milk sample to the pool. We then fitted a linear mixed model with GE_m as the response and day of lactation as the explanatory variable, with individual as a random term. This result is presented as slope and standard error (s.e.) of the parameter estimate.

Drivers of heart rate (HR), subcutaneous body temperature (T_{sc}) and activity

When investigating drivers of HR, T_{sc} and activity, we used the finest temporal resolution available (15 min for HR and T_{sc}, 1 min for activity). All response variables were fitted with generalised additive mixed-effects models (GAMM) with a similar base model structure, including the following smooth terms: calendar day as a thin-plate regression spline, time of day (ToD, in hours, knots [*k*] = 10) as a cubic circular regression spline and individual term as random effect. All models were fitted with restricted maximum likelihood and a penalization value (λ) of 1.4 (Wood 2017). Furthermore, in the cases were there was temporal autocorrelation in the data, we used an autoregressive structure (AR1) fitted for each individual. All numeric variables were scaled for easier comparison of effect size(s). The number of knots (*k*) was assessed for each model using the '*gam.check*' function from the 'mgcv' package. All model outputs are listed and described in the Supporting Information.

We matched each HR and T_{sc} record with mean VeODBA activity recorded in the past 1, 5 and 15 min. We also quantified the proportion of time spent active every 5 and 15 min to match with HR and T_{sc} records. To investigate the drivers of variation in HR and T_{sc}, we first assessed which parameter of activity explained the most variation in HR and T_{sc}. The candidate parameters were: i) mean VeODBA in the past 1 min, ii) mean VeODBA in the past 5 min, iii) percent activity in the past 5 min, iv) mean VeODBA in the past 15 min, and v) percent activity in the past 15 min. We generated GAMMs containing the following variables: age, BM, lactation status, T_{eff} and solar radiation, as well as interactions between lactation status and T_{eff}, and lactation status and solar radiation. For each model, we added one activity parameter and the interaction between that parameter and lactation status. We then compared the models and retained the most parsimonious one using Akaike's Information Criteria (AIC) (Burnham and Anderson 2002) (Table S4). Because the model containing mean VeODBA in the past 5 min was the best fit, we proceeded to categorise HR and T_{sc} for inactive and active categories using a mixture model approach (described in 2.4) for the 5-min mean VeODBA. We then investigated the drivers of variation in HR and T_{sc}, within the inactive and active states. Inactive HR and T_{sc} were fitted with the model structure described in the previous paragraph, while the models of active HR and T_{sc} also contained 5-min VeODBA and an interaction between VeODBA and lactation status, as well as an interaction between VeODBA and body mass.

For the activity data, we created three sets of GAMMs with different activity parameters as the response variables: i) VeODBA, ii) probability of being active (binomial, logit-link), and

iii) VeODBA when active. We used the *bam* function for very large data sets ‘mgcv’ package (Wood 2017). In all three models, we added the following explanatory (linear) variables: age (years), BM (measured in September), lactation status, effective ambient temperature (T_{eff}) and an interaction between BM and T_{eff}. Based on initial visual exploration of data, we fitted an interaction between lactation status and T_{eff} as a cubic regression with $k = 7$. We also fitted an interaction between lactation status and solar radiation with a cubic regression with $k = 5$. When presenting the results from the GAMMs, we used predicted values (shown as lines with 95% CI) and adjusted points shown as means ± standard error of the mean (s.e.m.).

3. RESULTS

3.1 Body mass dynamics

Body mass (BM) of the lactating and non-lactating females did not differ at any time during the DLW period (Figure 1a). Total BM losses between day 0 (injection) and day 16 post-injection were 10.6 ± 2.3 kg (n = 8 lactating females) and 8.5 ± 3.4 kg (n = 6 non-lactating females). Both groups gained mass between day 16 post-injection (June 15) and mid-September (average BM gain 3.8 ± 3.4 kg, n = 14) and continued to gain mass through to October and December (Figure 1a). BM of lactating and non-lactating females did not differ in September (CI −9.1, 8.1) or in the end of October (CI −9.1, 24.5), and there were no differences in BM gain from June to September (CI −4.5, 7.8) or from June to October (CI −10.1, 23).

3.2 Daily energy expenditure (DEE)

Although DEE of lactating females (27.5 ± 3.9 MJ day^{-1}) was slightly higher than that of non-lactating females (25.2 ± 2.2 MJ day^{-1}), the difference was not statistically significant (Figure 1b, Table 1). Neither BM nor BM loss explained the variation in DEE (Figure 1c, Table 1). The only significant explanatory variable of DEE was daily activity (% active), and the relationship was stronger when including BM as a covariate (Figure 1d, Table 1). In both cases (with and without the covariate), this was driven by one individual, the removal of which caused the relationship to disappear (Table 1). On the other hand, the relationship between daily mean vectorial overall dynamic body acceleration (VeODBA) and mass-adjusted DEE was significant when the same individual was removed (slope = 1.97, Adj. R^2 = 0.45, CI: 0.29, 3.65). None of the explanatory variables contributed to explain variation in DEE in the subset for lactating females (results not shown).

3.3 Lactation performance

While DEE did not differ between the lactating and non-lactating females, the total energy budget (TEB) was substantially higher in reproductive females. Mean TEB of lactating females (37.8 ± 3.6 MJ day^{-1}, DEE + MEO) was ~50% greater than that of non-lactating females (25.2 ± 2.2 MJ day^{-1}, equal to their DEE, Figure 1b). Milk production (MP) averaged 1.54 ± 0.09 kg day^{-1} (range 1.43–1.66 kg day^{-1}). Mean milk water content was 74.52 ± 0.56% and gross energy content of fresh milk (GE_m) averaged 6.62 ± 0.20 MJ kg^{-1}. Between days 18 and 28 post-partum, GE_m increased on by 0.48 ± 0.16 (s.e.) MJ kg^{-1} per day across individuals, but there was no further increase between day 28 and days 34−38 post-partum (Figure S2).

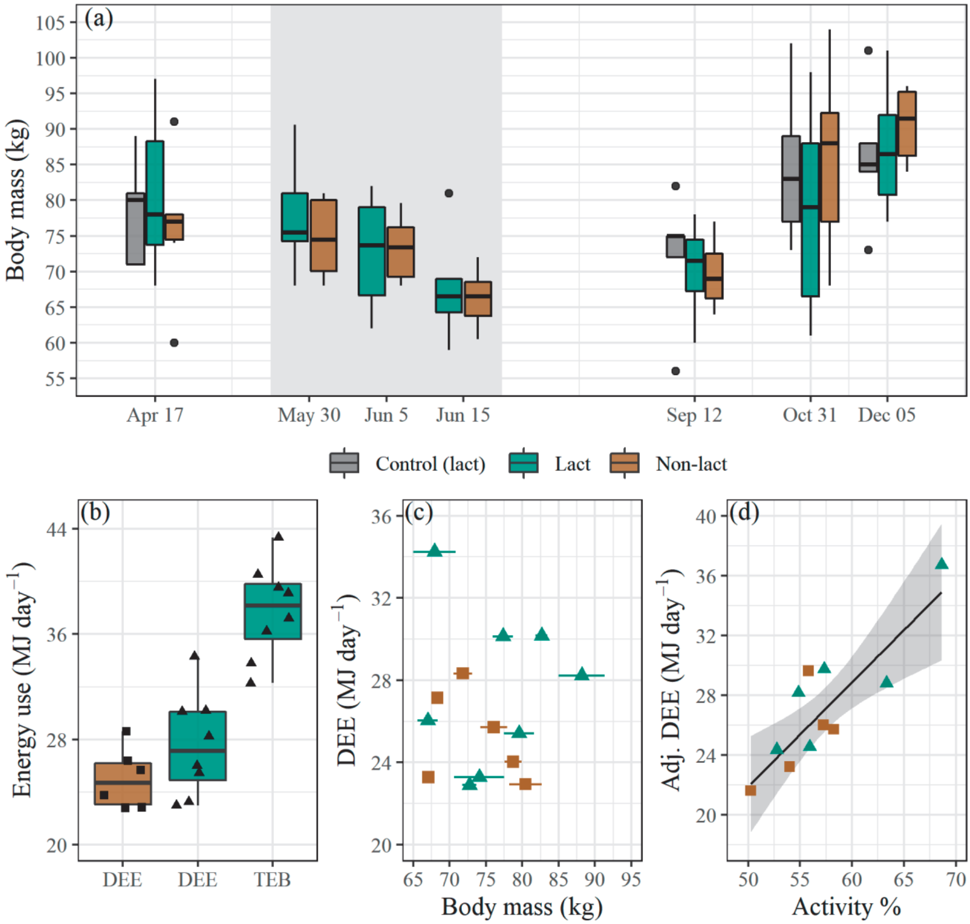

Figure 1. Body mass (BM), energy use, and correlates of daily energy expenditure (DEE) in reindeer females. Colour code refers to 8 lactating (green and/or triangles), 6 non-lactating (brown, and/or squares) and 5 non-injected lactating (grey) individuals. Panels (a) and (b) show box and whisker plots with the median (horizontal line), the 25th and 75th percentiles (box), and up to 1.5 times the percentiles (whiskers). Round points beyond this are outliers. **(a)** BM of females before calving (April), during the DEE experiment (May 30–June 15, grey shading), late lactation (September), and after completing reproduction (October–December). **(b)** Daily energy expenditure (DEE) of lactating and non-lactating females, and total energy budgets (TEB) of lactating females (calculated as sum of DEE and energy exported as milk). The points are individual values, there were no outliers. **(c)** DEE plotted against mean ± s.d. of BM recorded between injection day 6. **(d)** Mass-adjusted DEE fitted against daily activity (% of time spent active). The solid line shows prediction from a linear regression including BM as a covariate and shaded area shows the 95% confidence interval of the predictions. Individual values are adjusted for BM.

Milk energy output (MEO) ranged from 9.07 to 11.26 MJ day^{-1} with a mean of 10.22 ± 0.83 MJ day^{-1} ($n = 8$). Neither DEE nor maternal BM explained variation in MEO (Figures 2ab, Table 2). However, MEO was greater in females with heavier calves (Figure 2c, Table 2). Although the relationship between mean T_{sc} and MEO was significant (Adj. $R^2 = 0.86$; CI −1.17, –0.17; Table 2), this was driven by one individual (Figure 2d).

Mean growth rate (GR) of calves from birth to the second week of sampling (age 34–38 days) was 318 ± 22 g day^{-1} (range from 286 to 346 g day^{-1}). From birth to September (age 122−126 days), mean GR was 259 ± 20 g day^{-1}. The BM of mothers did not affect the BM of calves during the DLW period but explained 59% of variation in calf BM in September (CI 0.1, 0.6). However, this relationship disappeared when the mother-calf BM data of the 3 non-injected females were included, yielding in total 7 female and 4 male calves. With the BM data for 11 calves, the sex of calves was the only significant variable to explain variation in the calf BM observed in September (Adj. $R^2 = 0.47$, CI 1.7, 10.6), with male calves (mean of 42.8 ± 1.4) being on average 6 kg heavier than females (mean of 36.6 ± 2.3 kg).

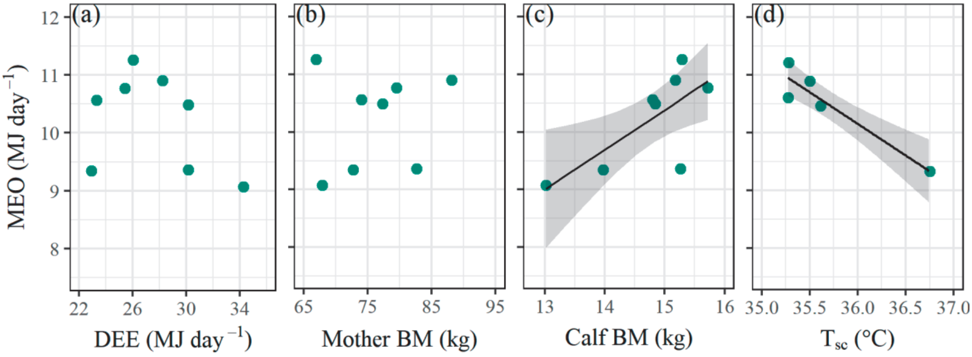

Figure 2. Milk energy output (MEO) at peak lactation (weeks 3−4 post-partum) in reindeer females plotted against **(a)** daily energy expenditure (DEE), **(b)** mean mother body mass (BM), **(c)** mean calf body mass (BM), and **(d)** mean mother subcutaneous body temperature (T_{sc}). In panel (d), the relationship is strongly leveraged by one individual (far right point). In all panels, the points show individual values, and in panels (c) and (d), the solid lines and shaded areas indicate predicted relationships and 95% confidence intervals, respectively.

3.4 Heart rate, subcutaneous body temperature and activity

During the DLW experiment, lactating females had higher heart rate (HR) when inactive (69.7 ± 4.5 bpm, n = 3) compared to non-lactating females (59.5 ± 2.4 bpm, n = 5, CI 54.8, 64.1), with the mean difference of 10.2 bpm (CI 2.64, 17.76). The relative increases in HR from inactive to active states were substantially lower in lactating females (an increase by 2 bpm) than in non-lactating females (an increase by 12 bpm) (Figure 3a, Table S5). In lactating females, the difference between inactive and active HR was not significant (Table S5). Subcutaneous body temperatures (T_{sc}) did not differ between the reproductive groups within each activity state, but on average T_{sc} decreased by 0.5°C when lactating females were active (CI −0.61, −0.42), while it increased by 0.1°C when non-lactating females were active (CI 0.05, 0.16) (Figure 3b, Table S6). Lactating females spent on average 60% of their time in activity and although they tended to be more active than non-lactating females (55%), the difference was not significant (Figure 3c, Table S5).

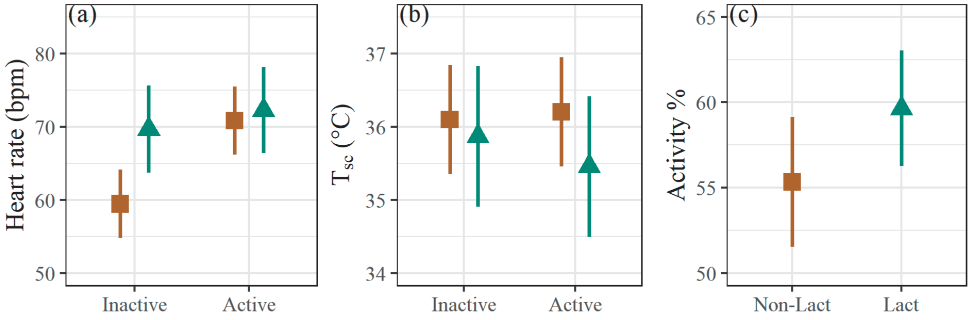

Figure 3. Heart rate (HR), subcutaneous body temperatures (T_{sc}) activity at peak lactation (weeks 3−4 post-partum) in lactating (green) and non-lactating (brown) reindeer females. The panels show predicted means ± 95% confidence intervals of **(a)** HR while inactive and active, **(b)** T_{sc} while active and inactive, and **(c)** proportion of time in activity; based on the data for 3 (HR and T_{sc}) or 6 (activity) lactating and 5 non-lactating females. All predictions were generated from generalised additive mixed-effects models.

3.5 Responses to mid-summer heatwave

Overall, our models of activity, heart rate and T_{sc} explained considerably different amounts of variation in the data. We were able to explain 9.3% of the variation in VeODBA and only 5.5% of the variation in the proportion of time in activity (Table S6). For HR, our models explained 23 and 22% of the variation in the inactive and active states, respectively (Table S6). Finally, our models of T_{sc} explained as much as 35% and 55% of the variation in the inactive and active states, respectively (Table S8).

All but four days in July 2018 had daily mean T_a above the 1980−2010 average (13.7°C; Finnish Meteorological Institute; Figure 3a). Over the mid-summer period, the highest daily mean T_a was 25.1°C recorded on July 18 and 19 (Figure 4a). Activity, HR and T_{sc} had all significant correlations with effective ambient temperature (T_{eff}), and some differences between the reproductive groups were observed. First, the probability of being active changed non-linearly with T_{eff}. Specifically, activity was the lowest at the lowest recorded T_{eff} (6−8°C) and increased with increasing T_{eff} up to a threshold around 18°C, at which it began to decline again (Figure 4b). Overall, lactating females were more active than non-lactating females by an average of 5% points, but the response to T_{eff} was similar (Figure 4b, Table S6). Second, inactive lactating females had higher HR than non-lactating females, but this difference was more pronounced at higher T_{eff}, meaning that the decline in HR was significantly steeper in non-lactating than lactating females. Over the range of T_{eff} (6–26°C), HR declined by ~10 bpm (slope = −2.3, CI −3.0, −1.6) in non-lactating females and by < 5 bpm in lactating females (difference in slopes of 1.3 with CI 0.59, 1.99) (Figure 4c, Table S7). In the active state, there was no difference in HR between reproductive groups, and the decline in HR in response to increasing T_{eff} was less pronounced (Table S7). Third, T_{sc} increased linearly with T_{eff} (slope = 0.38; CI: 0.34, 0.43; Figure 4d). While there was no difference between the reproductive groups in the mean T_{sc} during active and inactive states, lactating females had a steeper increase in T_{sc} in response to T_{eff} than non-lactating individuals when active (slope difference = 0.15; CI: 0.12, 0.17; Table S8).

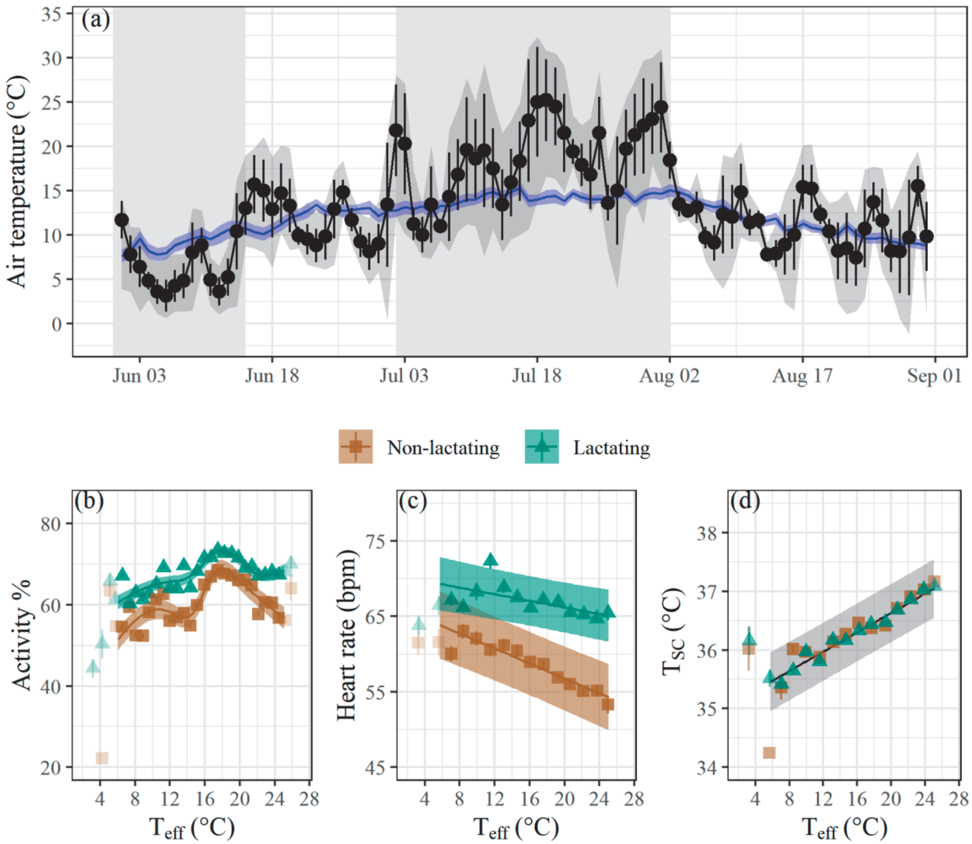

Figure 4. Air temperature (T_a), and responses in activity, heart rate (HR) and subcutaneous body temperature (T_{sc}) of reindeer females (both injected and non-injected) to a mid-summer heatwave. **(a)** Daily mean ± s.d. (circles ± error bars) of T_a from June to September 2018 recorded on-site. The grey ribbon shows the daily range of T_a. The blue line shows the daily mean ± s.d. of the 30-year (1980−2010) average of T_a recorded at the nearest weather station (~60 km, Station ID 102033). The grey shaded region on the left shows the DLW period, the shaded region in the centre marks the heatwave period. Panels b–d are from the heatwave. **(b)** activity levels (time spent active in percentages, n = 14), **(c)** heart rate while inactive and **(d)** subcutaneous temperature (T_{sc}) while inactive, all fitted against effective ambient temperature T_{eff} (n = 10) in separate models. Solid lines show the predicted relationships, with 95% confidence intervals given by the shaded areas. Points are adjusted values and error bars represent s.e.m for each adjusted value. Lightly shaded points outside the predicted lines are values that fall outside of the lower 1% and upper 99% of their distributions.

HR and T_{sc} had contrasting relationships with VeODBA (reflecting the intensity of movement). In both groups, HR increased linearly with increasing VeODBA. However, the slope was steeper for non-lactating compared to lactating females. HR increased by almost 40 bpm in non-lactating females and by 30 bpm in lactating females when VeODBA increased 10-fold (from 0.1 *g* to 1 *g*). In contrast, T_{sc} increased by less than 0.5°C in non-lactating females and decreased by less than 0.5°C in lactating females, over the same range of VeODBA (Figure S3, Table S6). At high VeODBA, T_{sc} was therefore ~1°C higher in non-lactating females than in lactating females.

In lactating females, HR declined with increasing T_{sc}, while in non-lactating females HR increased with increasing T_{sc} (Figure 5, Table S7). This pattern was most evident after correcting for T_{eff}, which impacted both HR and T_{sc} (Figures 4cd). Both reproductive groups had lower HR at high T_{eff}, but for a given T_{eff}, the difference in HR between the reproductive groups was greater when T_{sc} was low (< 35°C), and smaller at high T_{sc} (> 36°C) (Figure 5). We found no effects of age or BM on activity levels, HR or T_{sc}, but there was a significant interaction between BM and T_{eff} on VeODBA with heavier females being slightly less active at high T_{eff} than lighter females (Table S6).

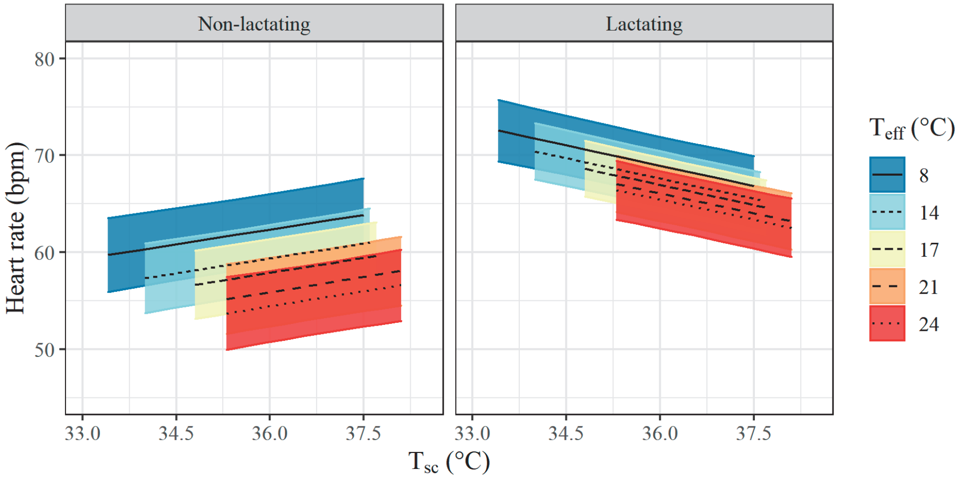

Figure 5. Resting heart rate (HR) in response to changes in subcutaneous body temperature (T_{sc}) at different effective ambient temperatures (T_{eff}) in non-lactating (left panel, n = 4) and lactating (right panel, n = 6) reindeer females, measured in mid-summer between July 2 and August 2. Each line represents the predicted HR (Table S8) for the range of T_{sc} recorded within a given range of T_{eff} (each range is ± 2°C within the 5, 25, 50, 75 and 90% quantiles of recorded T_{eff}). The shaded areas indicate the 95% confidence interval (CI) for each prediction.

4. DISCUSSION

Based on the HDL theory, we predicted that any constraint in the capacity of lactating reindeer to dissipate body heat would be manifested through their reduced rate of metabolism (measured as daily energy expenditure, DEE) and/or reduced reproductive output (measured as the amount of energy exported as milk and the growth rate of calf). We found that DEE did not differ between lactating and non-lactating females at peak lactation, although lactating females had higher total energy budgets (TEB) and higher heart rates (HR) when inactive. The lack of any increase in HR from the inactive to actives state may indicate that lactating females have compensated for the high energetic demands of lactation by downregulating other metabolic activity unrelated to reproduction (Mellish et al. 2000; Shuert et al. 2020). Yet, neither activity levels nor subcutaneous body temperature (T_{sc}) differed significantly between lactating and non-lactating females during peak lactation. The negative relationship between T_{sc} and milk energy output (MEO) could indicate that females with higher body temperature produced less milk. However, this conclusion is based on the leverage of a single individual, and therefore it lacks robustness. Because ambient temperatures (T_a) in early June 2018 were generally cool (T_a range 3–12°C), the weather was likely not suitable for detecting signs of heat stress in reindeer females.

In contrast, during the July 2018 heatwave, our results suggested that lactating reindeer were likely more susceptible to heat stress (Abdalla et al. 1993), consistent with the HDL theory. Although both groups displayed similar responses to increasing T_{eff}, lactating females were more active and had higher resting HR than non-lactating females at any effective ambient temperature (T_{eff}), suggesting that lactating individuals spent more time foraging than non-lactating ones (Denryter et al. 2020). The apparent threshold of T_{eff} at which activity levels began to decline was similar for both groups (~18°C, Figure 4b). In reindeer, feeding rates have been found to decline with increasing T_a above 14°C (Thompson and Barboza 2014). Reductions in food intake can subsequently reduce the heat increment of feeding (since heat is a by-product of metabolism), and hence reduce internal heat load (Lawler and White 2003; Thompson and Barboza 2014; Shively et al. 2019). A decline in HR with increasing ambient temperature has also been documented in moose (*Alces alces;* Thompson et al. 2020). The reduction in activity levels and resting HR at the high T_{eff} observed by us in July 2018 could reflect the reduced foraging efforts to alleviate internal heat load, as both HR (Mesteig et al. 2000) and activity (Denryter et al. 2020) are associated with feeding rates in reindeer and caribou, respectively. The observation that lactating reindeer did not reduce HR to the same extent as non-lactating reindeer (Figure 4c) may suggest that lactating females had a greater constraint on their ability to reduce energy expenditure due to the energetic demands of lactation. Alternatively, an elevated HR may reflect the increased respiration rates of lactating females to dissipate excess body heat (Parker and Robbins 1984). However, the latter explanation seems less likely, because resting HR in lactating reindeer decreased as their T_{sc} increased (Figure 5). Peripherally measured body temperatures, such as of skin, typically display more variation than core body temperature (Schmidt-Nielsen 1997; Ponganis et al. 2003; Lust et al. 2007), and also fluctuate more with variation in T_a (Arnold et al. 2004; Brinkmann et al. 2012). Here, we found a positive and linear effect of T_{eff} on T_{sc} with the predicted increase in T_{sc} of 2.5°C (from 35.5 to 37°C) across the range of observed T_{eff}. However, within a given range of T_{eff}, lactating females reduced their HR, while non-lactating females increased their HR as T_{sc} increased. Elevated T_{sc} may reflect vasodilation (Cain III et al. 2006; Lust et al. 2007) or changes in cardiac output (Thompson et al. 2020), which may facilitate heat dissipation without an increase in HR. Finally, a reduction in HR may reflect a decrease in rumination to avoid hyperthermia when T_{sc} increases (Abdalla et al. 1993; Silanikove 2000; Kadzere et al. 2002).

Both lactating and non-lactating females lost considerable amounts (~10 kg) of BM throughout the period of peak lactation when we conducted the DLW experiment. We found no differences in BM or BM loss between the reproductive groups, nor could we find any explanation for this loss. BM loss in the early stages of the lactational period has been observed in ungulates that are known to mobilise internal stores for milk production, e.g., musk oxen (*Ovibos moschatus*; Gustine et al. 2010) and reindeer (Barboza and Parker 2008). Yet, we observed similar losses in non-lactating females, including one of the two barren females. The females that had their calves removed prior to the study may have been undergoing involution of the mammary glands (Sordillo and Nickerson 1988). Cessation of milking in ewes (*Ovis aries*) in early stages of lactation led to a reduction in milk yield to 40% of the pre-cessation values already on day 4 post-cessation, but the udders did not dry completely until 4 weeks post-cessation (Fleet and Peaker 1978). Similarly, in dairy cows (*Bos taurus*), the mammary gland epithelium does not resemble the non-lactating state until 14 to 21 days following cessation of milking (Hurley 1989). It is therefore likely that the reindeer in our study who had their calves removed 4 days prior to the DLW experiment (days 2–22 post-partum), were undergoing involution, and as a result, they were physiologically distinct from the barren females.

Our estimates of DEE for lactating (27.5 ± 3.9 MJ day^{-1}) and non-lactating (25.2 ± 2.2 MJ day^{-1}) females are similar to those previously reported for semi-domestic, non-reproductive Norwegian reindeer females of similar body mass (79–91 kg) and age (> 4 years) in summer (early July), using the DLW method (mean of 25.4 MJ day, range 15.6–32.6 MJ day^{-1}, recalculated from Gotaas et al. 1997). The lack of a difference in DEE between lactating and non-lactating females was unexpected because milk synthesis is typically associated with relatively high lactogenic heat production, which is integrated in the estimate of DEE (Król and Speakman 2019). While it could be possible that the females who had their calves removed had their DEE elevated due to involution, DEE of these females did not differ from the two barren (non-reproductive) females. We are not aware of any studies quantifying the milk production efficiency of reindeer and we were not able to quantify this in our study. Using an average value for milk production efficiencies in cattle (~63%; Moe et al. 1971; Yan et al. 1997), an MEO of 10.2 MJ day^{-1} measured in our study would amount to the associated lactogenic heat production of ~6 MJ day^{-1} (calculated as (100–63)/63; Król and Speakman 2019). Milk production using internal reserves is typically more efficient (~80% in rats; Romero et al. 1976) and would cost ~2.6 MJ day^{-1}. The large variation in DEE between lactating females (23.0–34.2 MJ day^{-1}), but relatively small variation in MEO (9.1−11.3 MJ day^{-1}), could be due to variation in milk production efficiency (food vs internal reserves as source for synthesis; Romero et al. 1976; Yan et al. 1997). The low DEE in some females may also suggest the use of compensatory mechanisms to help meet the energetic costs of lactation by downregulating metabolic processes unrelated to reproduction. Compensation during lactation has been demonstrated in long-eared bats (*Plecotus auritus*; Mclean and Speakman 1999), koalas (*Phascolarctos cinereus*; Krockenberger 2003) and grey seals (*Halichoerus grypus;* Shuert et al. 2020). In the latter study, the compensation was manifested as reduced levels of activity. However, we did not find any lactation-specific differences in the activity levels that would support the use of compensatory mechanisms in our study.

Earlier studies suggested that the variation in DEE of reindeer females might have been caused by differing levels of activity between individuals (Gotaas et al. 1997), but the authors could not quantify activity levels in their study. Here, we show that activity levels explain up to 51% of variation in DEE across reproductive groups (Figure 1d), after adjusting for body mass. This relationship was mostly driven by the most active female; yet excluding this female resulted in a significant relationship between VeODBA and mass-adjusted DEE. The mass-dependent relationship between DEE and activity is supported by the findings of Byrnes et al. (2021), who demonstrated a mass-dependent relationship between oxygen consumption and dynamic body acceleration in lemon sharks (*Negaprion brevirostris*). Furthermore, Trondrud et al. (2021b) showed that activity levels correlate significantly with DEE in Svalbard reindeer (*R. t. platyrhynchus*) during winter, also after accounting for lean body mass.

The use of biologgers to quantify energy expenditure in free-ranging mammals is becoming increasingly common, and our results are similar to those found in many other species (Wilson et al. 2006; Stothart et al. 2016; Jeanniard-du-Dot et al. 2017; Pagano and Williams 2019), providing a promising avenue for long-term monitoring of energy budgets. Although the use of HR is a sound method to predict energy expenditure (Green et al. 2009), we failed to find a significant correlation between mean daily HR and DEE in our study. This could be due to the differences in how well HR corresponds to oxygen consumption (and hence energy expenditure) under various physiological and behavioural conditions (e.g., activity, resting, ruminating) and within individuals, which require detailed calibrations (Green 2011; Halsey and Bryce 2021).

The best explanation for variation in MEO was the mean body mass of young over the DLW period (Figure 2b). Milk production normally scales positively with both body mass and growth rates of young (Riek 2008). In our study, variation in MEO was not explained by calf growth rate, maternal body mass, or maternal mass loss. Yet, our estimates of milk production (1.43–1.66 kg day^{-1}) were in similar range as milk intake measured by McEwan and Whitehead (1971) in reindeer and caribou calves from birth (1.4 kg day^{-1}) to week 4 post-partum (1.7 kg day^{-1}), and greater than those previously measured in reindeer 3 weeks post-partum (range 0.6−1.2 kg day^{-1}; Gjøstein et al. 2004). Contrastingly, MEO was lower than that found in both studies (12.9−17.2 MJ day^{-1}, McEwan and Whitehead 1971; 12 MJ day^{-1}, Gjøstein et al. 2004). The gross energy content of fresh milk (GE_m) was similar to that found by Gjøstein et al. (2004) at week 3 of lactation (6.8 ± 0.4 MJ kg^{-1}), but it was rather unexpected that the milk energy content did not increase between the end of the first week and the end of the second week of sampling in our study (Figure S2), as GE_m has been shown to increase linearly with time following birth (Gjøstein et al. 2004). Using the mean values over the 2-week DLW period, we compared MEO measured in our study with the predictions of the allometric relationships between MEO and calf mass ($971 \times BM^{0.80}$; Riek 2008) and between MEO and maternal mass ($0.31\times BM^{0.74}$; Riek 2021) for ungulates. Our estimates of MEO were on average 22.1 ± 6.8% greater (range 9.0–30.3%) than the allometric prediction using the calf body mass, and 15.4 ± 12.1% greater (range 1.9–38.6%) than the allometric prediction based on the maternal body mass. These differences may be due to food supplementation used in the current study (as a part of reindeer herding management) and also due to lack of cross-validation between different methodologies employed to measure the amount of energy exported as milk.

5. CONCLUSION

As climate change progresses, heatwaves are expected to become more frequent in the Arctic (IPCC 2019). Cold-adapted ungulates may face increasingly challenging environmental conditions for effective thermoregulation in summer, especially during the periods of high metabolic intensity such as lactation. We expected that during lactation, heavier reindeer should display signs of heat stress at lower ambient temperatures than lighter reindeer, but we did not find clear evidence to support this notion, possibly because our measurements of energetics of lactation coincided with relatively cool ambient temperatures. The indication that the amount of energy exported as milk (MEO) was lower in females with higher mean T_{sc}, suggests that lactating reindeer may produce less milk when facing hyperthermia, but our sample size was relatively small, and the result hinged on a single individual. Yet, we found differential responses in lactating and non-lactating reindeer to high T_{eff} during a record-breaking heatwave. Although we did not a find consistent evidence to support the HDL theory, our results are in line with the postulation that lactating females may be more susceptible to the environmental heat stress than non-lactating conspecifics (Abdalla et al. 1993; Dash et al. 2016). Although the successful alternation between foraging habitats and thermal shelters can lead to a greater body mass gain in moose during summer (van Beest and Milner 2013), the applicability of the differential thermoregulatory behaviour and physiological responses to alter reproductive success in reindeer under climate change scenario warrants further investigations.

TABLES

Table 1. Summary outputs of linear regression models of daily energy expenditure (DEE) and mass-adjusted DEE (with body mass included as a covariate) against physiological and behavioural parameters measured in 8 lactating and 6 non-lactating reindeer females. Presented are sample size (n), estimates and 95% confidence interval (CI) for each slope (β), and Adj.R^2 of each model. All variables are scaled with a mean of zero. Significant results are given in bold text.

Model predictors	n	**DEE (MJ day^{-1})**		**Mass-adjusted DEE (MJ day^{-1})**	
		β (CI)	Adj.R^2	β (CI)	Adj. R^2
BM[a] (kg)	14	0.0 (−2.1, 2.1)	0.00	–	–
Mass loss[b] (kg)	14	−0.1 (−2.5, 1.7)	0.00	–	–
Lactation status	14	2.3 (−1.6, 6.2)	0.05	2.4 (−1.8, 6.5)	0.00
BM		–		−0.2 (−2.4, 1.9)	
Age (years)	14	−0.9 (−3.0, 1.1)	0.00	1.6 (−4.4, 1.1)	0.00
BM		–		1.1 (−1.7, 3.8)	
VeODBA[a] (*g*)	11	2.0 (−0.1, 4.1)	0.27	2.4 (0.0, 4.9)	0.24[c]
BM		–		0.8 (−1.5, 3.1)	
Activity [a] (%)	**11**	**2.2 (0.1, 4.2)**	**0.33[d]**	**3.5 (1.2, 5.9)**	**0.51[d]**
BM		–		**2.0 (−0.2, 4.1)**	
HR[a] (bpm)	10	−1.2 (−3.1, 0.7)	0.11	−1.1 (−3.5, 1.2)	0.00
BM		–		0.2 (−2.2, 2.6)	
HR amp[a] (bpm)	10	−0.6 (−2.7, 1.5)	0.00	−0.7 (−2.9, 1.5)	0.00
BM		–		0.8 (−1.4, 3.0)	
T$_{sc}$ [a](°C)	10	−0.9 (−2.9, 1.1)	0.00	−0.8 (−3.0, 1.5)	0.00
BM		–		0.5 (−1.8, 2.8)	
T$_{sc}$ amp[a] (°C)	10	1.13 (−0.8, 3.1)	0.08	1.0 (−1.1, 3.1)	0.00
BM		–		0.5 (−0.2, 2.7)	

Abbreviations: amp – amplitude (daily maximum − minimum values), BM – body mass, VeODBA – vectorial overall dynamic body acceleration, HR – heart rate, T$_{sc}$ − subcutaneous body temperature. Superscripts: [a] mean daily value between days 1 and 6 post-injection, [b] difference in BM between days 1 and 6 post-injection, [c] significant when removing one individual, [d] significant when including the same individual as in [c].

Table 2. Summary outputs of linear regression models of milk energy output (MEO) at peak lactation against physiological and behavioural parameters measured in 8 lactating reindeer females. Presented are sample size (n), estimates for each slope (β), 95% confidence intervals (CI) and adjusted R^2 of each model. All variables are scaled with a mean of zero. Significant results are given in bold text.

	Milk energy output (MJ day^{-1})			
Model predictor	n	β	CI	Adj. R^2
DEE (MJ day^{-1})	8	−0.33	−1.09, 0.44	0.01
BM^a (kg)	8	0.13	−0.69, 0.95	0.00
Calf BM^a (kg)	**8**	**0.60**	**0, 1.18**	**0.44**
Calf growth^b	8	0.57	−0.3, 1.18	0.38
Calf age (days)	8	0.43	−0.29,1.14	0.14
Age (years)	8	0.17	−0.65, 0.98	0.00
VeODBA^a (*g*)	6	0.19	−1.39, 1.01	0.00
Activity^a (%)	6	−0.47	−1.51,0.58	0.12
HR^a (bpm)	5	0.06	−1.38,1.26	0.00
HR amp^a (bpm)	5	0.15	−1.15,1.45	0.00
T_{sc} (°C)$^{a, c}$	**5**	**−0.67**	**−1.17, −0.17**	**0.82**
T_{sc} amp (°C)$^{a, c}$	**5**	**0.71**	**0.48, 0.94**	**0.96**

Abbreviations: amp – amplitude (daily maximum − minimum values), BM – body mass, VeODBA – vectorial overall dynamic body acceleration, HR – heart rate, T_{sc} − subcutaneous body temperature. Superscripts: ^{a} mean of daily values between days 1 and 16 post-injection. ^{b} difference in BM of calf between days 1 and 16 post-injection. ^{c} significant relationship driven by a single individual, see Results for details.

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Conflict of interest

We have no conflict of interest.

Author contributions

LEL, JRS, EK, ER, and AE conceived the ideas. LEL, AE, ER and LMT planned the study. JK facilitated the field work, AE and ER performed surgery, and LMT ER, JK and LEL collected the data. CH measured energy content of milk and analysed isotope data; EK analysed isotope transfer from mother to calves. LMT curated the data and did statistical analyses in collaboration with GP. LMT led the writing of the manuscript. All authors contributed to editing and approved the final version of the manuscript.

Data availability

Data from the DLW study is provided in the supplementary material; biologger data will be submitted to Dryad digital repository together with manuscript submission.

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SUPPORTING INFORMATION

Additional figures

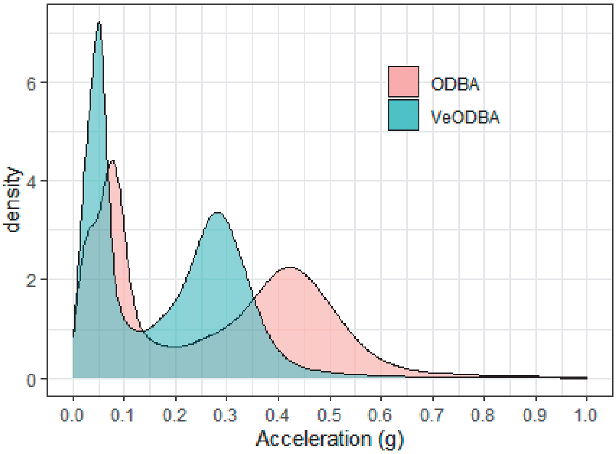

Figure S1. Density distribution of overall dynamic body acceleration (ODBA) and vectorial ODBA (VeODBA) aggregated to 1-min mean values recorded in 14 reindeer females, based on the 4- month data (May–August 2018; total no. observations = 2,536,215).

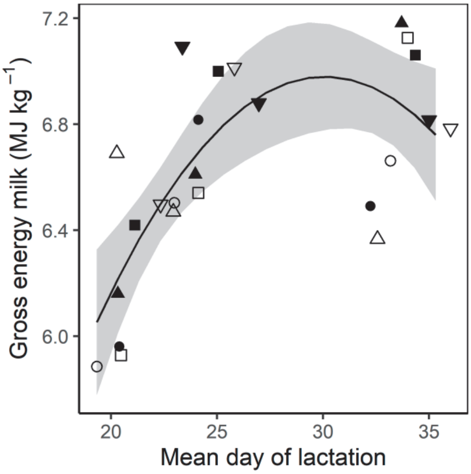

Figure S2. Gross energy content of fresh milk (GE_m) of lactating females as a function of lactation day. Each shape of relates to a specific individual. The mean day of lactation is the weighted mean across each pooled sample for each female. The solid line indicates change in GE_m as a function of day of lactation, predicted from a linear mixed-effect regression with individual fitted as a random effect. The grey area indicates the 95% confidence interval of the prediction.

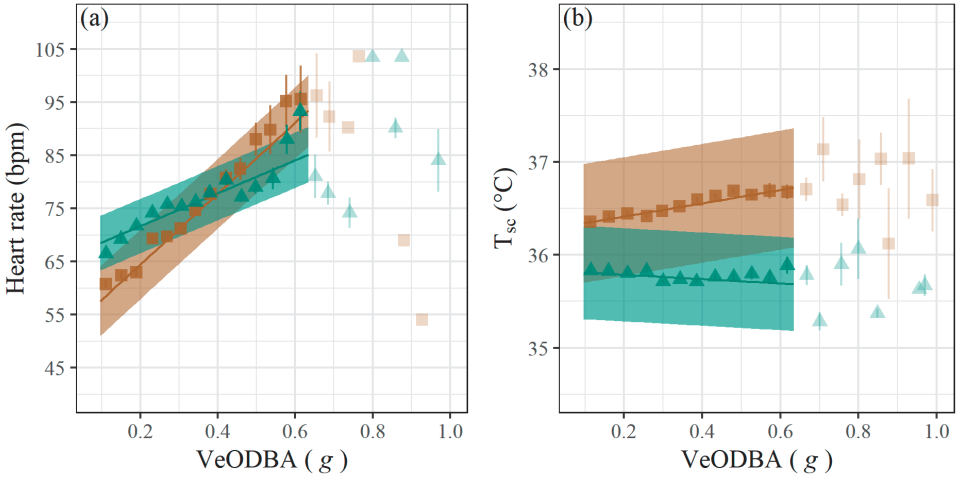

Figure S3. Changes in **(a)** heart rate and **(b)** subcutaneous body temperature (T_{sc}) with increasing activity, measured as vectorial overall dynamic body acceleration (VeODBA), measured in reindeer females (both injected and non-injected) during a mid-summer heatwave. Solid lines show the predicted relationships, with 95% confidence intervals given by the shaded areas. Points are adjusted values and error bars represent s.e.m for each adjusted value. Lightly shaded points outside the predicted lines are data points that fall outside of the lower 1% and upper 99% of their distributions.

Additional tables

Table S1. Characteristics of 19 semi-domestic reindeer females dosed with doubly labelled water (DLW) to measure daily energy expenditure (DEE) in June 2018. Provided are animal ID, age (years), reproductive group (L, lactating, N, non-lactating), manipulation group (DLW, animals injected with the isotopes; control, non-injected animals), body mass (BM) measured in April 2018 (prior to DLW study) and in September 2018, availability of biologger data (+, yes; −, no) for heart rate (HR) subcutaneous body temperature, (T_{sc}) and activity, and calf information on date of birth (DoB, as day and month in 2018), sex and body mass (BM) at birth.

ID	Age (years)	Repro group	Manipulation group	BM (kg)		HR, T_{sc}	Activity data	Calf		
				April	Sep			DoB	Sex	BM (kg)
ke61	6	L	DLW	82	76	−	+	12.05	M	5.9
ke63	6	L	Control	80	75	+	+	30.05	F	5.8
ke66	6	L	Control	89	82	−	+	15.05	F, M[b]	4.2, 5
ke71	6	L	Control	71	56	−	−	09.05	M	7.1
ke75	5	L	DLW	73	60	+	+	11.05	F	6
ke79[a]	5	N	DLW	76	64	+	+	25.05	F	5.3
ke80	5	L	Control	71	72	+	+	28.05	F	6
ke81[a]	5	N	DLW	78	67	+	+	05.05	F	5.9
ke84	5	L	DLW	68	62	−	+	13.05	F	5.2
nk30[a]	7	N	DLW	91	−	+	+	12.05	F	7
nk34[c]	7	L	Control	81	75	+	+	−	M	−
nk36	7	N	DLW	74	71	+	+	Barren	−	−
nk37	7	L	DLW	74	73	+	−	12.05	F	6.4
nk38[a]	7	N	DLW	78	77	+	+[d]	10.05	F	6.4
nk39	7	L	DLW	89	74	−	+	13.05	M	7
nk40	7	L	DLW	78	69	+	−	12.05	F	5.9
nk43	7	L	DLW	78	70	+	+	10.05	F	5.4
nk44	7	L	DLW	97	78	+	+	09.05	F	6.6
si26	2	N	DLW	60	−	−	−	Barren	−	−

Superscripts: [a] calves were removed from mothers prior to study; [b] gave birth to twins; [c] DoB and calf BM at birth unknown; [d] activity sensor stopped working in mid-June so activity data was only used for the DLW study period.

Table S2. Individual estimates of daily energy expenditure (DEE; MJ day^{-1}) and milk energy output (MEO; MJ day^{-1}) for 8 lactating and 6 non-lactating (DEE only) reindeer females, measured in June 2018. Provided are animal ID, reproductive group; (L, lactating, N, non-lactating), body mass (BM; kg) measured on days (D) 0 and 16 post-injection, DEE, milk production (MP; kg day^{-1}) measured by the dose-to-the-mother technique, mean gross energy content of fresh milk (GE$_m$; MJ kg^{-1}) ± s.d. of 3 pooled samples, MEO, and initial and final BM of calves.

ID	Repro group	Female BM		DEE	MP	GE$_m$	MEO	Calf BM	
		D0	D16					D0	D16
ke61	L	84	69	30.2	1.43	6.5 ± 0.6	9.4	13.6	18.0
ke75	L	68	59	26.0	1.65	6.8 ± 0.4	11.3	12.6	17.4
ke84	L	72	59	34.2	1.43	6.3 ± 0.4	9.1	14.0	15.0
nk37	L	75	67	22.9	1.45	6.4 ± 0.4	9.3	12.4	16.4
nk39	L	80	69	25.4	1.66	6.5 ± 0.2	10.8	13.8	18.6
nk40	L	75	66	23.3	1.59	6.7 ± 0.5	10.6	13.2	17.0
nk43	L	76	66	30.1	1.55	6.8 ± 0.3	10.5	13.4	17.8
nk44	L	91	81	28.2	1.57	6.8 ± 0.1	10.9	13.2	18.6
ke79[a]	N	72	63	28.3		–	–	–	–
ke81[a]	N	69	61	23.3	–	–	–	–	–
nk30[a]	N	81	72	24.0	–	–	–	–	–
nk36	N	81	69	22.9	–	–	–	–	–
nk38[a]	N	77	67	25.7	–	–	–	–	–
si26	N	68	66	27.1	–	–	–	–	–

Superscripts: [a] calves were removed from mothers prior to study.

Table S3. Summary of results of the doubly labelled water (DLW) experiment performed on 14 semi-domestic reindeer females in June 2018. Provided are body mass (BM) at different time points, elimination rates of ^{18}O (k_o) and ^{2}H (k_d for deuterium), oxygen (N_o) and deuterium (N_d) dilution spaces expressed as % of BM at injection, ratio of N_d to N_o, daily energy expenditure (DEE) and milk-related data (lactating females only).

Parameter	Mean ± standard deviation	
	Non-lactating	Lactating
Number of animals	6	8
BM at DLW injection (kg)	74.7 ± 5.7	77.6 ± 7.1
BM on day 1 post-injection (kg)	73.4 ± 5.7	76.8 ± 7.3
BM on day 6 post-injection (kg)	73.2 ± 4.7	72.9 ± 7.7
BM on day 16 post-injection (kg)	66.3 ± 4.1	67.0 ± 6.9
k_d (h^{-1})	0.0075 ± 0.0014	0.0088 ± 0.0010
k_o (h^{-1})	0.0093 ± 0.0015	0.0105 ± 0.0013
k_o/k_d	1.23 ± 0.03	1.19 ± 0.02
N_o (% of BM at injection)	73.4 ± 7.3	79.0 ± 4.0
N_d (% of BM at injection)	74.8 ± 7.4	80.3 ± 3.8
N_d/N_o	1.02 ± 0.01	1.02 ± 0.00
DEE (MJ day^{-1})	25.2 ± 2.2	27.5 ± 3.9
Milk water intake (kg day^{-1})	–	1.15 ± 0.07
Milk water content (%)	–	74.5 ± 0.6
Milk production (kg day^{-1})	–	1.54 ± 0.09
Gross energy content of fresh milk (MJ kg^{-1})	–	6.6 ± 0.2
Milk energy output (MJ day^{-1})	–	10.2 ± 0.8

Table S4. Summary of model comparison for five models of heart rate with different variations of activity as predictor variable. All models contained the same fixed and random effects as well as smooth terms, but the activity parameter was changed in the five different models. Model structure and details on activity parameters are described in the methods. Presented are the model rank, the name of the activity parameter changed, the difference (Δ) in Akaike's Information Criteria (AIC) score and the degrees of freedom (Df) for each model.

Model rank	Activity parameter	ΔAIC	Df
1	**VeODBA 5 min**	**0**	**35.5**
2	VeODBA 1 min	80	35.8
3	VeODBA 15 min	575	35.5
4	Activity % 5 min	842	35.5
5	Activity % 15 min	1180	35.4

Table S5. Summary of three separate generalised additive mixed models for activity state (binomial, logit link), heart rate and subcutaneous body temperature (T_{sc}) in reindeer females that were part of the DLW manipulation group using data during the DLW study. Provided are predictor variables, parameter estimates and 95% confidence intervals (CI) for each parameter in each model. The intercept represents non-lactating females in the inactive state and ‘1’ indicates either lactating females or the active state. Statistically significant effects ($p < 0.01$) are given in bold. Values for the smooth terms are presented with estimated degrees of freedom (EDF) and p-values. Time of day was fitted with a cubic circular regression and individual was fitted as random intercept.

	Activity state		**Heart rate**		**T_{sc}**	
Predictors	*Log−Odds*	*CI*	*Est.*	*CI*	*Est.*	*CI*
(Intercept)	**0.21**	**0.06, 0.37**	**59.47**	**54.80, 64.14**	**36.10**	**35.35, 36.85**
Lact [1]	0.18	−0.03, 0.38	**10.20**	**2.64, 17.76**	−0.23	−1.45, 0.98
Act state [1]			**11.38**	**9.77, 12.99**	**0.10**	**0.05, 0.16**
Lact [1] × act state [1]			**−8.77**	**−11.41, −6.13**	**−0.51**	**−0.61, −0.42**
Smooth terms	EDF	*p*	EDF	*p*	EDF	*p*
Time of day	7.98	< 0.001	6.41	< 0.001	1.33	0.09
Individual	8.51	< 0.001	5.54	< 0.001	5.84	< 0.001
Observations		94842	3264			7344
R^2		0.073	0.183			0.247

Table S6. Model parameter estimates of three separate generalised additive mixed-effects models using activity as the response variable, in adult female reindeer from July 2 to August 1, 2018. The first model contained all estimates of vectorial overall dynamic body acceleration (VeODBA), the second model contained activity states (inferred from mixture models) fitted as a binomial regression with logit-link function, and the third model contained only VeODBA values when in the active state. Provided are the model predictor names, estimates, 95% confidence intervals (CI), estimated degrees of freedom (EDF), which represents curvature of the smoothing parameter (the higher the number, the more variation), p-values for the smooth terms, total number of observations and the adjusted R^2 of the model. Significant parameter estimates are given in bold.

	VeODBA		**Activity (binomial)**		**VeODBA when active**	
Predictors	*Est.*	*CI*	*Odds-ratio*	*CI*	*Est.*	*CI*
(Intercept)	**0.21**	**0.21, 0.22**	0.65	**0.56, 0.74**	**0.30**	**0.29, 0.31**
Age	0.00	−0.00, 0.01	−0.02	−0.08, 0.04	**0.01**	**0.00, 0.02**
BM	−0.00	−0.01, 0.00	−0.02	−0.09, 0.04	−0.01	−0.01, 0.00
Lact [1]	**0.01**	**0.00, 0.02**	0.20	**0.09, 0.31**	0.00	−0.01, 0.01
T_{eff}	**0.01**	**0.01, 0.01**	0.17	**0.14, 0.19**	**−0.00**	**−0.00, −0.00**
BM × T_{eff}	**−0.00**	**−0.00, −0.00**	0.00	−0.00, 0.01	**−0.00**	**−0.00, −0.00**
Smooth terms	EDF	*p*	EDF	*p*	EDF	*p*
Time of day	8.91	<0.001	8.86	<0.001	8.69	<0.001
Calendar day	7.99	<0.001	7.99	<0.001	7.93	<0.001
SR (non-lact)	1.00	<0.001	3.91	<0.001	3.74	<0.001
SR (lact)	3.98	<0.001	3.99	<0.001	3.76	<0.001
T_{eff} (non-lact)	4.94	<0.001	5.92	<0.001	5.78	<0.001
T_{eff} (lact)	5.89	<0.001	4.91	<0.001	4.81	<0.001
ID	9.24	<0.001	9.87	<0.001	9.95	<0.001
Observations		619483		619483		417902
R^2		0.093		0.055		0.086

Abbreviations and units: BM – body mass (kg), lact – lactation status (1-lactating, 0-not lactating), T_{eff} – effective ambient temperature (°C), SR – solar radiation (Wm^{-2})

Table S7. Model parameter estimates of generalised additive mixed-effects models using heart rate as the response variable, in adult female reindeer from July 2 to August 1, 2018. Inactive and active heart rate were modelled separately as a function of age, body mass (BM), lactation status (0 = non-lactating, 1 = lactating), subcutaneous body temperature (T_{sc}), solar radiation and effective ambient temperature (T_{eff}). Calendar day was fitted with a thin-plate regression spline, time of day (ToD) was fitted with a cubic circular regression and individual was fitted as random intercept. Provided are the model predictor names, estimates, 95% confidence intervals (CI), estimated degrees of freedom (EDF), which represents curvature of the smoothing parameter (the higher the number, the more variation), p-values for the smooth terms, total number of observations and the adjusted R^2 of the model. Significant parameter estimates are given in bold.

	HR inactive		**HR active**	
Predictors	*Estimates*	*CI*	*Estimates*	*CI*
(Intercept)	**58.42**	**54.23, 62.62**	**66.26**	**59.73, 72.78**
Age	0.37	−2.70, 3.45	−1.29	−6.08, 3.50
BM	−0.77	−3.54, 1.99	0.19	−4.10, 4.48
T_{sc}	**1.23**	**0.71, 1.76**	**1.99**	**1.08, 2.89**
Lact [1]	**8.51**	**3.49, 13.52**	5.95	−1.86, 13.75
SR	−0.15	−0.86, 0.56	**−2.19**	**−2.91, −1.48**
T_{eff}	**−2.31**	**−3.01, −1.61**	**−2.01**	**−2.73, −1.29**
Lact [1] × T_{sc}	**−2.92**	**−3.59, −2.25**	**−4.03**	**−5.03, −3.04**
Lact [1] × SR	−0.42	−1.09, 0.26	0.67	−0.02, 1.35
Lact [1] × T_{eff}	**1.29**	**0.59, 1.99**	**0.88**	**0.16, 1.60**
VeODBA			**9.71**	**9.03, 10.38**
Lact [1] × VeODBA			**−5.20**	**−6.01, −4.38**
Smooth terms	**EDF**	***p***	**EDF**	***p***
Time of day	5.63	**<0.001**	5.02	**<0.001**
Calendar day	8.68	**<0.001**	8.80	**<0.001**
ID	5.90	**<0.001**	5.96	**<0.001**
Observations		7721		15600
R^2		0.233		0.218

Abbreviations and units: BM – body mass (kg), T_{sc} – subcutaneous body temperature (°C), lact – lactation status (1-lactating, 0-not lactating), T_{eff} – effective ambient temperature (°C), SR – solar radiation (Wm^{-2})

Table S8. Model parameter estimates of generalised additive mixed-effects models using subcutaneous body temperature (T_{sc}) as the response variable, in adult female reindeer from July 2 to August 1, 2018. Inactive and active T_{sc} were modelled separately as a function of age, body mass (BM), lactation status (0 = non-lactating, 1 = lactating), solar radiation and effective ambient temperature (T_{eff}). Time of day was fitted with a cubic circular smoothing term and calendar day with thin-plate regression spline. Individual was fitted as random intercept. Provided are the model predictor names, estimates, 95% confidence intervals (CI), estimated degrees of freedom (EDF), which represents curvature of the smoothing parameter (the higher the number, the more variation), p-values for the smooth terms, total number of observations and the adjusted R^2 of the model. Significant parameter estimates are given in bold.

	T_{sc} inactive		**T_{sc} active**	
Predictors	*Estimates*	*CI*	*Estimates*	*CI*
(Intercept)	**36.39**	**35.76, 37.02**	**36.44**	**35.80, 37.08**
Age	−0.13	−0.60, 0.33	−0.08	−0.55, 0.39
BM	0.01	−0.41, 0.42	−0.09	−0.51, 0.33
Lact [1]	−0.03	−0.78, 0.73	−0.65	−1.41, 0.11
SR	0.12	**0.07, 0.16**	0.25	**0.22, 0.28**
T_{eff}	**0.38**	**0.34, 0.43**	**0.37**	**0.34, 0.39**
Lact [1] × SR	**0.20**	**0.15, 0.24**	−0.00	−0.03, 0.02
Lact [1] × T_{eff}	0.00	−0.04, 0.05	**0.15**	**0.12, 0.17**
VeODBA			**0.10**	**0.08, 0.13**
Lact [1] × VeODBA			**−0.14**	**−0.17, −0.11**
Smooth terms	**EDF**	***p***	**EDF**	***p***
Time of day	7.57	<0.001	7.63	<0.001
Calendar day	7.70	<0.001	8.74	<0.001
ID	5.98	<0.001	6.00	<0.001
Observations		11673		26235
R^2		0.346		0.547

Abbreviations and units: BM – body mass (kg), lact – lactation status (1-lactating, 0-not lactating), T_{eff} – effective ambient temperature (°C), SR – solar radiation (Wm^{-2})

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